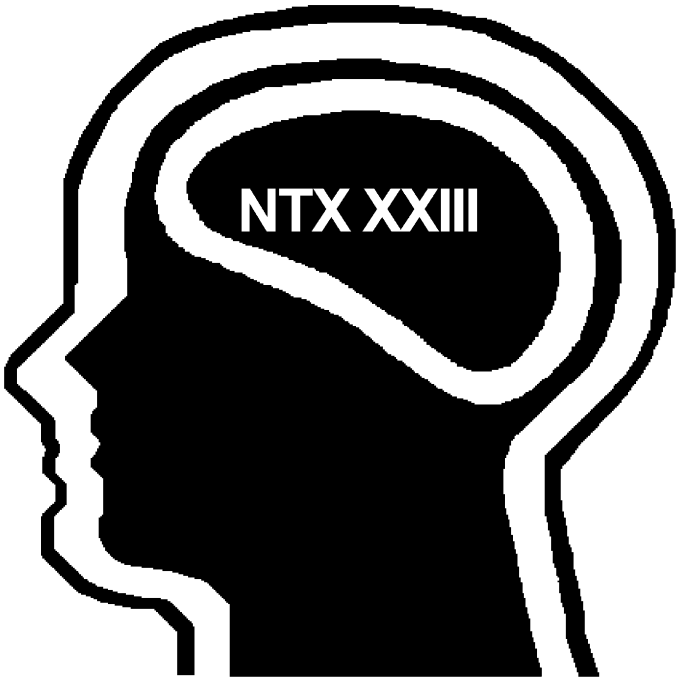
TWENTY-THIRD INTERNATIONAL NEUROTOXICOLOGY CONFERENCE

Neurotoxicity in Development and Aging



Abstracts

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Opening

SESSION I. OPENING OF THE 23RD CONFERENCE, RECOGNITION OF SPONSORS AND ORGANIZERS

Conference Chair: Joan Marie Cranmer, PhD

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TWENTY-THIRD INTERNATIONAL NEUROTOXICOLOGY CONFERENCE: "NEUROTOXICITY IN DEVELOPMENT AND AGING." Joan M. Cranmer, *Department of Pediatrics and Department of Pharmacology and Toxicology, College of Medicine, University of Arkansas for Medical Sciences and Arkansas Children's Hospital, Little Rock, AR, USA.*

The twenty-third annual meeting in this *International Neurotoxicology Conference Series* (NTX XXIII) convenes September 17-21, 2006 at the Doubletree Hotel Conference Center in Little Rock, Arkansas. The theme of this year's conference is *Neurotoxicity in Development and Aging*.

Scientists from around the world will present state-of-the-science research on hot topics in a variety of venues including: Symposia, Workshops, Platform Sessions, Poster Session, Pre- and Post-Doctoral Student Award Competitions, and focused Panel Discussions on controversial issues. Presentations will provide data to apply to important scientific, environmental, epidemiological, clinical, methodological, policy, regulatory, risk assessment and future funding issues – especially as related to the NIH Roadmap for translational research.

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.

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.

Peer-reviewed papers, the meeting report, program, abstracts, session summaries, research needs, and results of the student award competition will be published in *NeuroToxicology* Volume 28, 2007.

NTX XXIII Symposia, Workshop, Platform Session, Poster Session and Panel Discussion Topics to be addressed during this 4-day meeting include:

Sunday Afternoon Opening Symposium
SESSION 2: Consequences of Chemical Exposure on Cognition in Children: Importance of Effect Modifiers

Sunday Evening Informal Workshop & Think Tank
SESSION 3: The Epidemic of Childhood Developmental Disorders (e.g., Autism):

"Why are so many children sick . . . and what can be done to help them?"

Monday Morning Plenary Session
SESSION 4. Environment and Neurodevelopmental Disorders: Cross-Cutting Issues & Translational Research

Monday Afternoon Symposium
SESSION 5-A. Environmental Modulation of Neurotoxicants in Military-Relevant operational Environments

Monday Afternoon Platform Session
SESSION 5-B. Advancing the Science of Autism Spectrum Disorders

Tuesday Morning Plenary Session
SESSION 6. Neurotoxicity and the Path from Early Brain Development to Aging

Tuesday Afternoon Platform Session
SESSION 7-A-1: Developmental Neurotoxicity I

Tuesday Afternoon Symposium
SESSION 7-A-2: Role of Environmental Contaminants in the Etiology of ADHD-Like Behaviors

Tuesday Afternoon Symposium
SESSION 7-B: Neuroprotection by Alzheimer's Drugs

Tuesday Evening Poster Session
SESSION 8: General Poster Session

Pre- and Post-Doctoral Student Award Competition

Wednesday Morning Symposium
SESSION 9-A. Health Effects of Manganese Exposure: Humans & Animal Models I

Wednesday Morning Platform Session:
SESSION 9-B: Developmental Neurotoxicity II

Wednesday Afternoon Platform Session
SESSION 10-A: Health Effects of Manganese Exposure: Humans and Models II

Wednesday Afternoon Symposium
SESSION 10-B. The Seychelles Child Development Study of Methylmercury Exposure from Fish Consumption: New Results and Conclusions

Wednesday Evening Symposium
SESSION 11-A: The Fetal Brain on Alcohol

Wednesday Evening Platform Session
SESSION 11-B: Mercury Toxicity, Dithiocarbamates and Oxidative Stress,

Thursday Morning Plenary Session
SESSION 12. Neurotoxicity in Development and Aging: Translational Research

On Wednesday afternoon a tour of FDA's National Center for Toxicological Research will be arranged for all who wish to see this unique world-class laboratory. Post-conference tours of the University of Arkansas for Medical Sciences and Arkansas Children's Hospital will be arranged on site.

A Sunday evening "Meet & Greet" Reception and Monday social evening featuring a *Tour and Dinner at the William Jefferson Clinton Presidential Library"* will round out the 23rd conference.

Symposium

SESSION 2: CONSEQUENCES OF CHEMICAL EXPOSURE ON COGNITION IN CHILDREN: IMPORTANCE OF EFFECT MODIFIERS

Session Chair: Deborah Rice, PhD
Co-Chair: David Bellinger, PhD

Theme: *This session will examine interactions between chemical exposure and endogenous and environmental factors on neuropsychological function. Factors include the socioeconomic environment of the child, the influence of individual genes, and the modification of response to environmental chemicals by the brain structure of the child.*

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IMPORTANCE OF *EFFECT MODIFIERS* FOR THE CONSEQUENCES OF CHEMICAL EXPOSURE ON COGNITION IN CHILDREN. Deborah Rice, PhD. *Maine Center for Disease Control and Prevention, Augusta, Maine.*

Dr. Rice will provide an overview of factors that may influence the effects of exposure to a chemical in the human population. These include the *genetic make-up* of the individual, the *expressed phenotype*, and *environmental factors* such as social milieu.

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IMPULSIVITY IN PCB, MeHg and Pb EXPOSED CHILDREN REVEALED THROUGH RESPONSE INHIBITION PARADIGMS: CURRENT BEHAVIORAL FINDINGS AND FUTURE DIRECTIONS IN BRAIN IMAGING. PW Stewart, J Reihman, E Lonky, B Gump, T Darvill & J Pagano. *Psychology Department, State University of New York at Oswego. Oswego, NY. USA.*

Tests of global cognitive ability have historically dominated the assessment of neurobehavioral toxicity in children exposed contaminants including PCBs, MeHg and Pb. These tests rely heavily on language for proper performance and are insensitive to domain specific deficits which have often been demonstrated in research with animals. Recently, data from the Oswego Children's study has repeatedly demonstrated impulsivity and response inhibition deficits associated with 3 major contaminants: PCBs, MeHg and Pb. These deficits, each independent of the other, have been demonstrated using a battery of signal detection tests designed to target response inhibition, as well as operant tests designed to target delay-of-gratification and response control. These findings have been robust in a multivariate environment where more than 50 potentially confounding variables have been assessed. These data have been augmented by brain magnetic resonance imaging (MRI) scans in least and most highly exposed children. Volumetric analysis has demonstrated that the posterior region of the corpus callosum (splenium) is a powerful predictor of performance on the signal detection tasks. Moreover, children with suboptimal development of this region appear most sensitive to the putative effects of PCBs. PCB-exposed children with the smallest spleniums show deficits which are 8-fold larger than the deficits seen in the whole sample of PCB-exposed children. Beyond these findings, deficits in response inhibition are associated with a rich literature in functional magnetic resonance imaging (fMRI). Impaired frontal and striatal processing have long been associated with such deficits. The relevance of the current findings to both neurobehavioral assessment and both structural and functional MRI are discussed.

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USING THE TOOLS OF GENETICS AND NEUROIMAGING IN ASSESSSING DEVELOPMENTAL VULNERABILITY. K.N. Dietrich. *Department of Environmental Health, the University of Cincinnati College of Medicine and the Cincinnati Children's Environmental Health Center, Cincinnati, Ohio, USA.*

Our group has been examining the interaction between genetic polymorphisms associated with neurotransmitter metabolism and lead exposure on intellectual abilities and behavioral problems in two cohorts of lead-exposed children in Cincinnati, Ohio and Rochester, New York. We are also using Magnetic Resonance Imaging (MRI) to assess alterations in brain structure, function and physiology in adults who were lead poisoned as infants and young children. We have hypothesized that the association between early exposure to lead and ADHD in children and ADHD and criminality in adults will be stronger in subjects with high risk alleles previously associated with attentional disorders and violent behavior including DRD4, DAT1, SLCA4, and MAOA. Following adjustment for numerous important developmental cofactors, we have found early exposure to lead to be associated with ADHD in children and criminality in adults with previous histories of lead poisoning. We have also observed significant interactions between genetic polymorphisms associated with the dopaminergic pathway and lead with ADHD in children and adult criminality. Using the methods of functional MRI, we have observed lead-related diminished activation in the left frontal cortex, adjacent to Broca's area and left middle temporal gyrus, including Wernicke's area in association with language function in adult subjects with a history of lead poisoning in early childhood. In these same subjects, we have observed significant gray matter reductions in frontal, temporal, parietal and occipital lobes, the basal ganglia and the cerebellum in association with higher blood lead levels in early childhood. Significant lead-related reductions in orbital frontal white matter were also observed. The promise of engaging the methods of genetic epidemiology and neuroimaging to better understand the full spectrum of effects of environmental chemical exposures on human neurodevelopment will be discussed. Keywords: Genetics, Neuroimaging, Lead.

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INFLUENCE OF *TOTAL SOCIAL ENVIRONMENT* ON THE OUTCOME OF CHEMICAL EXPOSURE. David Bellinger, PhD. *Harvard Medical School, Boston, Massachusetts.*

As investigators, we recognize that the outcomes of exposure to neurotoxic chemicals early in life depend upon the properties of both the chemical and of the host's environment. When our questions focus on the toxicant,

the environmental properties tend to be regarded as marginal and swept into designations such as covariates or confounders. They become variables in our analyses to be compensated for statistically. Another perspective, based on decades of biopsychological research on animals, shows that the early, even prenatal, environment implants permanent changes in brain structure and chemistry and the resulting behavior. Aspects of the early environment, encompassing enrichment, deprivation, and maternal and neonatal stress, all help to determine the functional responses later in life that derive from the biological substrate imparted by that environment. Their effects then become biologically embedded. Human data, particularly those connected to economically disadvantaged populations, yield equivalent conclusions. This commentary argues that treating such environmental conditions as confounders is equivalent to defining genetic differences as confounders, a tactic that laboratory research, such as that based on transgenic manipulations, clearly rejects. The biological properties implanted by the early social environment should be regarded as crucial elements of the translation from laboratory research to human health.

Informal Workshop & Think Tank

SESSION 3: THE EPIDEMIC OF CHILDHOOD DEVELOPMENTAL DISORDERS: *"Why are so many children sick . . . and what can be done to help them?"*

Moderator: Martha Herbert, MD, PhD

Theme: *Over the past several years there has been a growing body of research which documents the existence of distinct medical abnormalities in children diagnosed with neurodevelopmental disorders, specifically autism. These abnormalities include thiol deficiencies, impaired methylation, oxidative stress, inflammatory bowel disease, immune system abnormalities, mitochondrial deficiencies, decreased cerebral perfusion and increased body burdens of heavy metals with corresponding low levels of excretion.*

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WHY ARE SO MANY CHILDREN SICK? A REVIEW OF THE TIME TREND EVIDENCE AND RELATED CONTROVERSIES IN AUTISM. Mark F. Blaxill, *Vice President, SafeMinds*

When Leo Kanner, one of America's leading child psychiatrists, first characterized the syndrome he named autism, he remarked that it seemed rare. Twenty years after seeing his first child, he wrote that he had seen less than eight children a year, despite serving as a diagnostic clearinghouse for North America and also the world. Until quite recently, reviews of epidemiology surveys estimated autism rates at 4 per 10,000. But starting around 1990, recorded U.S. autism rates surged and by the turn of the century had reached numbers at least ten times that high. This explosion in rates strongly implicates new environmental exposures and suggests a wholesale reframing of the disorder: its pathogenesis, its mechanisms and its prognosis. Surprisingly, official statements describing these new high rates usually fail to comment on the change in incidence and when they do tend to suggest that changes in definition, awareness and improved diagnostic practices explain much of the increases. Unfortunately, these statements are not based on evidence, and reflect testable hypotheses regarding diagnostic substitution, expansion and oversight that have already been falsified. The public policy consequences of the time trends debate are important. To address the rising needs of autistic children and their families and to restore confidence in our public health leadership, autism should be declared a national public health emergency and managed accordingly.

WHAT CAN BE DONE TO HELP THEM?

Clinicians will discuss how to appropriately work up a child with developmental disorders and review some of the cutting edge therapies which are resulting in marked improvement in both behavior and overall general health.

Panelists:

Nancy O'Hara, MD, Steve Kahler, MD, Jerry Kartzinel, MD, Elizabeth Mumper, MD

The panelists are physicians whose primary specialty is the treatment of children with Autism.

Nancy O'Hara, MD, MPH is a board certified pediatrician. Prior to her medical career she taught children with autism. Dr. O'Hara's practice is a consultative, integrative model that looks at the biochemical, immunologic, gastroenterologic and neurologic problems of each child. Each initial consultation integrates the information from a detailed history, physical exam, laboratory investigation, and dietary and behavioral evaluations to provide an individualized approach for each child. Dr. O''Hara is also the Assistant Mecial Director for physician training for DAN! and the coordinator of the practitioner mentoring program.

Steve Kahler, MD received his MD degree from Duke, trained in Pediatrics at UCSD, and in clinical and biochemical genetics at UNC-Chapel Hill. His major interests include inborn metabolic errors. He worked at Duke for 14 years where he was part of the team that developed expanded newborn screening. He worked in Melbourne, Australia for five years, returned to the US (Johns Hopkins) in 2003, and moved to Arkansas in 2005. He has been interested in autism for many years, particularly the children who are responsive to diet changes, as they are similar in this way to children with defined metabolic errors. He has been part of the Defeat Autism Now! (DAN!) network since the first meeting in 1995. In Arkansas he is working closely with Jill James, PhD, on biochemical aspects of autism.

Jerry Kartzinel, MD is the Director of Pediatric Medicine at The Thoughtful House Center for Children. Dr. Kartzinel practiced general pediatrics in private practice for 10 years until his fourth boy was diagnosed with autism. He has since dedicated his practice of medicine to those with

autism and neurodevelopmental delays. His practice is solely devoted to the research and treatment of Autism and other neurodegenerative disorders. His approach includes a comprehensive history and physical exam, and laboratory investigations that seek to find what is biologically different in a child. Once found, he implements therapeutic interventions and monitors closely how they affect restoration of health and behaviors. His current research interests include quelling chronic inflammation and the augmentation of the methylation pathway.

Elizabeth Mumper, MD is Medical Director for physician training for Defeat Autism Now! (DAN!). She is president and CEO for Advocates for Children in Lynchburg Virginia. She is also associate professor of Medical Specialties, Pediatrics at Virginia College of Osteopathic Medicine. Dr Mumper attended medical school at the Medical College of Virginia and completed her pediatric residency at the University of Virginia where she was invited to spend an additional year as Chief Resident. Her current research interests include: intestinal biopsies, natural killer cells and glutathione dysfunction, hyperbaric oxygen therapies, and methylation abnormalities in children with Autism. Dr Mumper serves on the DAN! Europe Board and also the MINDD Foundation Board in Australia.

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DIETARY AND INTESTINAL ISSUES IN AUTISM. Kahler SG. *University of Arkansas for Medical Sciences, Little Rock, AR.*

Autism is a description of unusual behavior, not a specific diagnosis or a discrete entity. The behavioral aspects must have their origin in neurochemical and neuroanatomical changes, which appear to be genetically and environmentally influenced. There has been growing awareness that these children have significant physiological derangements outside the nervous system, especially in the gut and immune system. These derangements appear to be signs of a systemic problem. Eliminating dietary wheat and dairy products can lead to significant improvement in gut and behavioral symptoms in patients with autism, especially when coupled with other measures to restore normal bowel function and mucosal integrity. There is reported to be an excessive peptiduria derived from gluten and casein, and associated with grain and milk intake. Empiric observations over the past twenty-five years on dietary interventions in autism are now being tested formally in a variety of settings. The origin of the bowel and immune system dysfunctions are not known. A large number of causes have been suggested—heavy metal toxicity, cryptic microbial agents or groups of organisms (including herpesviruses, vaccine viruses alone or in combination, Gram-negative bacteria, Chlamydia and other intracellular organisms)—on a background of genetic/environmental vulnerability, particularly including poor anti-oxidant capability. The vulnerability of the gut and immune system might be interpreted as a surrogate for brain vulnerability as well. I will briefly review these aspects of the emerging autism story, ways to bridge the gap between academic knowledge and clinician/parental observations, and I will show videos of autistic children with co-existing non-neurological problems who have responded convincingly to biomedical interventions.

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EVIDENCE IMPLICATING THIMEROSAL IN THE EPIDEMIC OF NEURODEVELOPMENTAL DISORDERS IN CHILDHOOD. Elizabeth Mumper, MD and Mark Geier, MD. *Advocates for Children, Lynchburg, VA, USA. The Genetics Centers of America, Silver Spring, MD, USA.*

Many formulations of Thimerosal (49.55% mercury by weight)-containing Rho(D) immune globulins (TCRs) were routinely administered at 28 weeks gestation to Rh-negative mothers in the US prior to 2002. Maternal Rh-negativity was assessed among 298 Caucasian children with neurodevelopmental disorders (NDs). At Clinic A (Lynchburg, VA) a total of 196 patients with NDs (including 88 patients diagnosed with autistic spectrum disorders (ASDs) and 95 patients diagnosed with attention-deficit-disorder (ADD) / attention-deficit-hyperactivity-disorder (ADHD)), and at Clinic B (Rockville, MD and Baltimore, MD) 87 ASD patients were examined. As controls, the frequency of maternal Rh-negativity was determined from 124 Caucasian children born from 1987 through 2001 without NDs at Clinic A, and the frequency of Rh-negativity was determined from 1,021 Caucasian pregnant mothers that presented for prenatal genetic care at Clinic B from 1980 through 1989. Additionally, 14 Caucasian patients with NDs born from 2002 onwards from Clinic A were assessed for maternal Rh-negativity. There were significant increases in maternal Rh-negativity among children with NDs (Clinic: A=24.2%), ASDs (Clinic: A=28.3%, B=25.3%), and ADD/ADHD (Clinic: A=26.3%) observed at both clinics compared to both control groups (Clinic: A=12.1%, B=13.9%). Children with NDs born from 2002 had a maternal Rh-negativity frequency (14.3%) similar to controls. In light of the significant increase in maternal Rh-negativity in patients with NDs observed and the apparent decrease in maternal Rh-negativity in children with NDs born from 2002, the results from this multi-center prospective study associate thimerosal containing Rhogam with some neurodevelopmental disorders.

Plenary Session

SESSION 4. ENVIRONMENT AND NEURODEVELOPMENTAL DISORDERS: CROSS-CUTTING ISSUES & TRANSLATIONAL RESEARCH

Session Co-Chairs: Deborah Cory-Slechta, PhD
Don Schmechel, MD

This session will consist of "Anchor Talks" to raise our awareness of the complexities and cross-cutting issues to be taken into account in translational research efforts to cure, conquer or alleviate childhood neurotoxic disorders, abnormal aging and neurodegenerative disorders.

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BIONETWORKS OF RISK FACTORS IN NEUROTOXICOLOGY: IMPLICATIONS FOR RISK ASSESSMENT AND TRANSLATIONAL RESEARCH. D.A. Cory-Slechta, *Environmental and Occupational Health Sciences Institute, Robert Wood Johnson Medical School, UMDNJ, and Rutgers University, Piscataway, NJ 08854.*

It is increasingly recognized that many neurodegenerative and neurodevelopmental diseases and disorders arise not from unitary causes, but from the collective interactions of various risk factors, both risk-increasing and risk-mitigating. Contributing factors include environmental, behavioral, genetic and host-based risks, as well as intercurrent disease states inherent to human populations. It is likely that progression, severity and prognosis for any person reflects their individual collection of risk factors, and that the broad array of phenotypes observed for many nervous system diseases and disorders reflects the population variance in risk factors. Experimental studies reveal evidence of cumulative neurotoxicity from intermittent contacts with risk factors across the lifespan, as well as permanent effects in response to exposures restricted to periods of early development. Yet risk assessment methods continue to determine perils associated with environmental chemical exposures using paradigms that fail to match these environmental realities. Instead, data related to nervous system hazards are generally derived from studies typically undertaken in healthy young adult subjects, in the absence of other risk factors, and in isolation from other extant environmental chemical exposures. The nervous system, by virtue of its network of system-based operations, may be particularly vulnerable to interactions when multiple sites of a given system are concurrently targeted by risk factors, compromising the homeostatic repair capacities of the system. The artificiality of current approaches raises serious questions about the adequacy and sensitivity of risk assessments as currently conducted to define hazards for the protection of human health. Critical to improving this process is the development of more realistic experimental models of risk factor interactions. The broad array and diversity of potential risk factor interactions and environmental chemical exposures means that critical thought must first be given to the relevant combinations of risk factors and chemical exposures to evaluate. Finally, new methods need to be developed for evaluating risk modification and interactions in epidemiological and population studies, and for accommodating risk modification in risk assessments by regulatory agencies.

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OF MERCURY, MICROBES, MICE AND MEN: HOW TRANSLATIONAL THINKING CAN ACCELERATE DISCOVERY OF THE ROLE OF ENVIRONMENTAL FACTORS IN NEUROPSYCHIATRIC DISORDER PATHOGENESIS. Mady Hornig, MD, MA. *Columbia University, New York, NY.*

Translation is a continuous and bidirectional process: epidemiologic and clinical research findings ideally inform the design of studies in animals and in tissue culture, just as findings from animal and tissue culture experiments provide clues to strategies for risk identification, prevention, and intervention in humans. Developmental neurotoxicity poses unusual challenges for translational research. It is difficult to determine the degree to which equivalence may be established for the timing of pre- and/or postnatal exposures with respect to outcomes across species; this is even more complex for agents or doses of agents that involve gene-environment-timing interactions, or where immune or oxidative stress mediators may be important in pathogenesis. Establishing equivalence in timing for one neural cell type may lead to important differences in maturation of other cell types; equivalence for neural components may lead to deviation across species in immune system or other organ maturation that may be important in pathogenesis. Furthermore, neuropsychiatric outcomes may not only be difficult to model in animals due to the differences in repertoire but superficially similar behaviors may rely upon different circuitry or involve different constraints or maturational timetables. **Specialized** strategies expedite integration of data from animal models and epidemiologic studies and the discovery of the mechanisms by which toxins or infectious agents may trigger or amplify adverse host responses and lead to disturbances of neuropsychiatric functioning.

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ART, CREATIVE ENERGY, MEMORY, MOOD AND INFLAMMATION: LESSONS FOR CNS DEVELOPMENT AND VULNERABILITY. Don Schmechel, MD. *Duke University, Durham, North Carolina.*

Complex genetic and environmental interactions contribute to development, aging and neurodegenerative disorders (AD, vascular dementia, and related disorders). We will focus on the paradigm of alpha-1-antitrypsin (AAT) – an inflammatory response gene whose polymorphisms alter vulnerability to toxic exposures affecting liver and lung, but also CNS neurotoxicity. From a series of 1200+ patients presenting with mood and memory complaints, we demonstrate that AAT polymorphisms may also modulate CNS development affecting artistic vocation, creative energy, and mood. Gene-gene interactions with APOE and hemochromatosis will be discussed in AAT context. Proposed mechanisms are through effects on inflammation, lipids and metal homeostasis.

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AUTISM: A BRAIN DISORDER OR A DISORDER THAT AFFECTS THE BRAIN? Martha Herbert, MD, PhD ~ *Massachusetts General Hospital; Harvard Medical School, Boston, Massachusetts*

Autism is defined behaviorally, as a syndrome of abnormalities involving language, social reciprocity and hyper focus or reduced behavioral flexibility. It is clearly heterogeneous, and it can be accompanied by unusual talents as well as by impairments, but its underlying biological and genetic basis is unknown. Autism has been modeled as a brain-based, strongly genetic disorder, but emerging findings and hypotheses support a broader model of the condition as genetically influenced and systemic. These include imaging, neuropathology and psychological evidence of pervasive (and not just specific) brain and phenotypic features; postnatal evolution and chronic persistence of brain, behavior, and tissue changes

(e.g. inflammation) and physical illness symptomatology (e.g. gastrointestinal, immune, recurrent infection); overlap with other disorders; and reports of rate increases and improvement or recovery that support a role for modulation of the condition by environmental factors (e.g. exacerbation or triggering by toxins, infectious agents, or other stressors, or improvement by treatment). Modeling autism more broadly encompasses previous work, but also encourages the expansion of research and treatment to include intermediary domains of molecular and cellular mechanisms, as well as chronic tissue, metabolic and somatic changes previously addressed only to a limited degree. The heterogeneous biologies underlying autism may conceivably converge onto the autism profile via multiple mechanisms that all somehow perturb brain connectivity. Studying the interplay between the biology of intermediary mechanisms on the one hand and processing and connectivity abnormalities on the other may illuminate relevant final common pathways and contribute to focusing the search for treatment targets in this biologically and etiologically heterogeneous behavioral syndrome. Keywords: Autism, Brain, Complex Systems, Connectivity, Gene-Environment Interaction.

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TOWARD UNDERSTANDING AUTISM'S COMPLEXITIES. Isaac Pessah, PhD. *University of California, Davis, California.*

Autism is a neurodevelopmental disorder that presents in early childhood with deficits in social reciprocity and communication, and by unusual repetitive behaviors. Autism is a complex underlying genetic predisposition that may involve >10 defective loci, each insufficient to account for autism. Thus the etiology of autism is currently unknown. The persistent increase in prevalence of autism over the last 25 years suggests that the complex set of genetic factors may enhance the susceptibility of autistic children to the harmful effects of environmental exposure. Thus genetic and environmental factors are likely to contribute to the progression and severity of autism.

Symposium

SESSION 5-A. ENVIRONMENTAL MODULATION OF NEUROTOXICANTS IN MILITARY-RELEVANT OPERATIONAL ENVIRONMENTS

Session Co-Chairs: Susan P. Proctor, DSc
COL Karl E. Friedl, PhD

Theme: *Topics presented in this session will include current research efforts examining the role of ambient temperature, metabolic interactions, and co-exposures and susceptibility on neurotoxicant exposure and effects and describing newer methodologies to measure persistent biomarkers of neurotoxicant exposures. This session will include 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).

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ENVIRONMENTAL MODULATION OF NEUROTOXICANTS IN MILITARY-RELEVANT ENVIRONMENTS. Susan P. Proctor, D.Sc. and Karl E. Friedl, Ph.D. *U.S. Army Research Institute of Environmental Medicine, Natick, Massachusetts 01760-5007, USA*

Military deployments typically expose soldiers to multiple stressors from the environment and from manmade hazards. Toxic chemicals represent an important component of these potential hazards, and concurrent stressors may modulate both exposure levels and effects. Understanding these interactions has become an important topic of investigation since the 1991 Gulf War when neurological symptoms were reported by soldiers who had been provided with chemical prophylactic drugs and insect repellents while being exposed to petroleum products, sustained exertion, heat, traumatic stress, and other operational exposures. The nature of neurotoxic modulation ranges, for example, from alterations in exposures produced by factors such as changes in blood flow and dermal absorption in humid conditions and increased ventilatory inhalation rates during hard work or at altitude, to altered systemic effects mediated through common mechanisms of xenobiotic clearance, inflammatory and oxidative stress, and neurophysiological mechanisms involving interleukins and other stress-sensitive regulators of host defense responses that affect neurologically- mediated health and performance outcomes. Seemingly benign stressors such as exercise may exert substantial influence on predictions of neurotoxicity, for example: acute exercise may increase chemical exposure rates but chronic exercise has neuroprotective benefits. Even commonly used markers of effect may be altered by other stressor effects on metabolism; thus current efforts are directed at expanding understanding of the principles underlying interactions of key stressors. This session highlights current research advances in this area.

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ROLE OF ENVIRONMENTAL HEAT AND COLD STRESS ON THE PHYSIOLOGICAL RESPONSE TO ORGANOPHOSPHATES AND OTHER TOXICANTS. Christopher J. Gordon, Ph.D. *Neurotoxicology Division U.S. Environmental Protection Agency Research Triangle Park, North Carolina USA*

Most toxicological and pharmacological studies are performed in laboratory rodents maintained under comfortable environmental conditions. However, exposure to toxicants as well as some drugs can occur under stressful conditions during rest or while exercising. Heat stress can exacerbate the biological efficacy and uptake of toxicants and drugs in several ways: The increase in pulmonary ventilation when exposed to a hot environment results in an increase in uptake of airborne toxicants. Transcutaneous absorption of organophosphates and some other pesticides on the skin as well as drugs

delivered by skin patches can be markedly accelerated during heat stress. This increased uptake is a result of the combined elevation in skin blood flow coupled with a moist skin from sweating. The toxicity of most insecticides and most other classes of toxicants is proportional to tissue temperature. Small rodents are capable of eliciting a hypothermic response following acute toxicant exposure, a response shown to improve survival to the toxicants. However, a rapid hypothermic response is attenuated with an increase in body mass. Thus, adult humans exposed the organophosphates in the heat are at greater risk because of (i) increased uptake of the toxicant through the skin and lungs and (ii) greater toxicity due to higher tissue temperatures. Overall, environmental heat and cold stress can influence the thermoregulatory responses to environmental toxicants which impacts on their pathophysiological effects. *This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.*

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HUMAN METABOLIC INTERACTIONS OF DEPLOYMENT-RELATED AND OTHER ENVIRONMENTAL CHEMICALS. Ernest Hodgson PhD *Department of Environmental and Molecular Toxicology, North Carolina State University, Raleigh North Carolina, USA.*

Recent studies in our laboratory utilizing human hepatocytes, human liver cell fractions and recombinant human xenobiotic-metabolizing enzymes (XMEs) have focused on the enzymes involved as well as their polymorphic variants. These studies, those involving human variation, and those on metabolic interactions between different xenobiotics are particularly relevant to human health risk analysis. Chemicals investigated include organophosphorus insecticides, the pyrethroid insecticide, permethrin and other pesticides (carbaryl, fipronil, etc.), the repellent DEET and the jet fuel components, nonane and naphthalene. In general the results reveal that CYP2B6 and CYP3A4 are most important in metabolism of these chemicals although other CYPs and FMOs are also involved. Studies of phase I and phase II metabolism reveal that activation to the neurotoxic oxon in the liver may not be as important in neurotoxicity as activation in the nervous system. Variations between different individuals, for example in the activation of chlorpyrifos to the cholinesterase-inhibiting metabolite chlorpyrifos oxon, may be 10-fold or higher and polymorphic variants play a significant role in human variation. We are currently genotyping a larger population sample for SNPs in the CYP 2B6, 2C19 and 3A4 genes and in the Ah receptor gene. Metabolic interactions in humans include a number based on CYP inhibition such as the inhibition of steroid hormone metabolism by thiophosphate insecticides, or the inhibition of the carbaryl metabolism. Furthermore, studies utilizing human hepatocytes indicate that some pesticides may be potent inducers of CYP isoforms and /or cytotoxic. For example, the insecticide fipronil is an inducer of CYP isoforms at low doses and is cytotoxic at slightly higher doses.

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THE EFFECTS OF DIESEL EXHAUST AND STRESS ON THE ACUTE PHASE RESPONSE AND SYMPTOMS IN THE CHEMICALLY INTOLERANT. Nancy Fiedler, Howard Kipen, Debra Laskin, Junfeng Zhang, Paul Lioy, Kathie McNeil, and Robert Laumbach. *EOHSI - Robert Wood Johnson Medical School Piscataway, NJ 08854 USA*

Exposures to diesel exhaust (DE) and other petrochemical combustion products were the exposures reported by the greatest percentage of all Gulf War veterans (GWV). Along with diesel exhaust and other chemical exposures, psychological stress has been implicated in the onset of unexplained symptoms such as chemical sensitivity among GWV. The purpose of our study is to test a model for chemical sensitivity in GWV, in which simultaneous acute exposures to DE and psychological stress cause increased symptoms via the acute phase response (APR), in susceptible individuals. Individuals who are low or high in the susceptibility factor of chemical intolerance (CI) were exposed to DE either with or without a public speaking task, an acute psychological stressor. In this 2 (DE exposure) X 2 (high vs. low CI) X 2 (stress vs. no stress) design, 50 healthy men and women, ages 18 to 45, were exposed, during two one-hour exposure sessions, to DE standardized to 300 $\mu g/m^3$ PM_{10} and to filtered air. Symptoms, end-tidal CO_2, markers of the APR in peripheral blood and markers of inflammation in the lungs and nasal mucosa were assessed at baseline and up to 24 hours post exposure. Overall preliminary results show an increase in symptoms with diesel exposure. As expected, CD4/CD8 ratio was significantly decreased and natural killer cell counts were significantly increased as a main effect of psychological stress. Thus far, DE exposure did not significantly change peripheral blood leukocyte counts immediately following exposure. However, further analyses will be reported assessing six and 24 hour post-DE main effects and DE X stress interaction effects on indicators of the APR and on lung and nasal mucosa inflammation. In addition, cortisol response to stress will be used to select individuals most susceptible to the effects of stress and to assess the interaction of DE and stress between susceptible and less susceptible subjects.

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PERSISTENT BIOMARKERS OF EXPOSURE OF POTENTIALLY NEUROTOXIC COMPOUNDS. D Noort, M. van der Schans. *Business Unit Biological and Chemical Protection, TNO Defense, Security and Safety, Rijswijk, The Netherlands*

During the last decade sensitive mass spectrometric methods have become available to determine exposure to a wide array of xenobiotics, including neurotoxic compounds. These methods can roughly be classified into two categories:

1. Methods that are based on the analysis of short-living metabolites in urine or blood
2. Methods that are based on the analysis of persistent covalent adducts with macromolecules

The focus in this presentation will lie on category 2, i.e., the more persistent biomarkers of exposure. Representative examples of agent-specific and more generic methods will

be discussed that are under development at TNO Defense, Security and Safety for biomonitoring exposure to potentially neurotoxic compounds, such as chemical warfare agents (nerve agents), pesticides (organophosphates, permethrin) and alkylating agents (e.g., metabolites from jet fuel components). The use of the described methods will be exemplified by actual incidents of exposure. Future directions of research in this field will also be addressed.

Platform Session

SESSION 5-B. ADVANCING THE SCIENCE OF AUTISM SPECTRUM DISORDERS

Session Co-Chairs: Martha Herbert, MD, PhD
Isaac Pessah, PhD

Theme: *Autism is defined behaviorally, as a syndrome of abnormalities involving language, social reciprocity and hyperfocus or reduced behavioral flexibility. It is clearly heterogeneous, and it can be accompanied by unusual talents as well as by impairments, but its underlying biological and genetic basis is unknown.*

The reframing of autism from a genetically determined neuropsychiatric disorder to a set of treatable, whole-body illnesses that have environmental influences is being driven by increases in diagnosis, the common presence of physical symptoms (especially gastrointestinal and immune) and growing reports of substantial clinical improvement. Recognizing autism as a multisystem disorder, most likely with common underlying mechanisms driving the panoply of symptoms, opens horizons for including prevention and treatment in research, clinical practice and policy.

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ADVANCING THE SCIENCE OF AUTISM SPECTRUM DISORDERS: INTRODUCTION AND OVERVIEW. Martha Herbert, MD, PhD[1] and Isaac Pessah, PhD[2]. [1] *Massachusetts General Hospital; Harvard Medical School, Boston, Massachussetts* [2] *University of California, Davis.*

Widespread volumetric, oxidative stress and inflammatory neuroanatomical changes as well as compromised brain coordination in autism are arguably consistent with an environmental etiology; they may be final common pathways resulting from mechanisms with heterogeneity of triggers, timing and vulnerabilities. Clinical interventions aimed at supporting environmentally responsive pathways have been combined in various treatment algorithms whose differential effectiveness in the autism population may indicate inter-individual differences in both vulnerability and immunotoxicological history. Since many of these treatments are GRAS ("generally recognized as safe") they may provide valuable research probes for uncovering mechanisms of disease, identifying subgroups, and optimizing and innovating treatment targets.

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AUTISM: THE CLINICAL PICTURE. Jeff Bradstreet MD, Director, *International Child Development Resource Center, Florida Hospital Celebration and Melbourne, Florida*

There is a confluence of data surrounding the central theme of oxidative stress and immunotoxicological disruption in autism spectrum disorders. Researchers from numerous different disciplines and academic centers are finding similar and supporting abnormalities in critical pathways involved in oxidative protection and mitochondrial function. Simultaneously, immunologists are defining a dysregulated immune system with evidence of autoimmunity, allergy and specific deficiencies. Researchers at Johns Hopkins identified neuroglial activation in the brain of children with autism which was in many cases quite dramatic; demonstrating evidence of a perivascualitis. Various authors have reported evidence of poor heavy metal excretion, particularly for mercury. We previously noted elevated relative body burden of mercury in the autism population compared to controls, and most recently Nataf et al noted specific mercury related precoproporphyrin elevations in autism which were even higher when seizures were present. Two separate groups, one in Texas and one in California, found an association of increased risk of autism based on air related mercury variables. Neither state has especially high levels of mercury when compared to most states east of the Mississippi. This again speaks to an extreme degree of vulnerability to the toxic effects of mercury, as would be expected in the presence of oxidative stress and thiol deficiencies.

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EVIDENCE AND IMPLICATION OF REDOX IMBALANCE IN AUTISTIC CHILDREN. S. Jill James, Stepan Melnyk, Alena Savenka, and Stefanie Jernigan. *Arkansas Children's Hospital Research Institute, University of Arkansas for Medical Sciences, Little Rock, AR*

Autism is a complex behaviorally-defined neurodevelopmental disorder with a CDC-reported prevalence of ~1 in 166 children in the US. Although both genetic and environmental factors are thought to be involved, none as yet have been reproducibly identified. The metabolic basis for autism has received much less research attention despite the fact that chronic biochemical imbalance is often a primary factor in the development of complex disease. Abnormal methionine and glutathione metabolism have been associated with several other neurologic disorders; however, these pathways have not been evaluated in autism. We measured fasting plasma levels of metabolites in methionine transmethylation and transsulfuration pathways in 80 autistic and 75 control children. The abnormal metabolic endophenotype was used as a guide for the selection of relevant candidate genes. Relative to age-matched control children, mean levels of methionine, S-adenosylmethionine (SAM), cysteine, total and free glutathione were significantly decreased, whereas S-adenosylhomocysteine (SAH), adenosine and oxidized glutathione disulfide were increased in the autistic children. Both the methylation ratio (SAM/SAH) and the redox ratio (GSH/GSSG) were significantly reduced. In the genetic analysis, the

frequencies of common allelic variants in genes coding for the reduced folate carrier (RFC-1), transcobalamin II (TCN2), and catechol-O-methyltransferase (COMT) were increased in 360 autistic children relative to 203 controls. Significant gene-gene interactions were present for RFC1/MTHFR, TCN2/COMT and RFC1/GSTM1. In cell culture experiments, lymphoblastoid cell lines derived from autistic children exhibited significant increases intracellular free radical production, decreased GSH/GSSG ratio, and increased caspase-3 activation at baseline and after nanomolar Thimerosal exposure. The observed metabolic imbalance and increased sensitivity to oxidative stress may reflect a cumulative interaction among several genes affecting a common pathway important for detoxification and cellular redox balance. An increased vulnerability to oxidative stress (environmental and/or intracellular) may contribute to the development and clinical manifestations of autism.

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GLUTATHIONE-DEPENDENT METHIONINE SYNTHASE ACTIVITY: A SENSITIVE TARGET FOR NEURODEVELOPMENTAL TOXINS. M. Waly, C. Muratore, J. Bojkovic, V.-A. Power and R. Deth. *Department of Pharmaceutical Sciences, Northeastern University, Boston, Massachusetts, USA*

By virtue of its location at a key metabolic intersection, methionine synthase (MS) activity influences both methylation and redox buffering. Oxidation of its cobalamin (Cob) co-factor halts the methionine cycle and diverts homocysteine (HCY) towards cysteine and glutathione (GSH) synthesis, particularly during oxidative stress. We earlier showed that a number of neurodevelopment toxins, including ethanol and the vaccine preservative thimerosal, along with inhibitors of PI3 kinase and MAP kinase signaling pathways, potently inhibit MS activity in human SH-SY5Y neuronal cells. This inhibition is due to reduced levels of GSH and MS activity can be fully restored by GSH supplementation. Methylcobalamin is required for MS activity in SH-SY5Y cells, and GSH is essential for MeCob synthesis from hydroxocobalamin. A comparison of MS activity in rat liver *vs.* different brain regions showed that the cerebral cortex has a particular dependence upon MeCob. Nitrous oxide, which promotes Cob oxidation, inhibits MS in both liver and cortex when assayed with hydroxocobalamin, but activity in cortex is completely restored by MeCob, whereas activity in liver is not. Thus MS activity requires MeCob in neuronal cells and cortex, and neurodevelopmental toxins impair methylation by lowering the cellular level of GSH. Keywords: Oxidative Stress, Methylation, Heavy Metal Toxicity.

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HOW GENETIC AND TEMPORAL FACTORS CONSTRAIN OUTCOMES AFTER ENVIRONMENTAL EXPOSURES: IMMUNE-MEDIATED MOUSE MODELS OF NEURODEVELOPMENTAL DISORDERS. Mady Hornig, MD, MA. *Columbia University, New York, NY.*

Dr. Hornig will present her work on genetic and maturational factors in mouse models of immune-mediated neurodevelopmental damage following exposure to low level toxicants such as thimerosal and other environmental immune disrupters. She found that low dose exposure to thimerosal during critical windows of postnatal development in a mouse strain-dependent model resulted in enlarged brains and abnormal social and learning behavior in conjunction with thimerosal-induced autoimmune disturbances. She did not find these same abnormalities in mice exposed to the same exposure but without the genetic susceptibility factors. Through microarray analyses, she is identifying key pathways associated with the alterations in developmental brain circuitry and function in the model, and laying the foundation for elucidation of the pathogenesis of autism and discovery of predictive biomarkers and novel interventional strategies.

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CRITICAL MECHANISMS, MOVING TARGETS: UNDERSTANDING IMMUNE SYSTEM MODULATION IN AUTISM RISK. Isaac Pessah, PhD. *University of California, Davis.*

The complexity of autism presents unique opportunities and challenges to study interactions among multiple susceptibility genes, and how epigenetic factors and exposure to environmental modifiers may contribute to variable expression of autism, and autism-related traits. The major goals of this presentation are to familiarize attendees with (1) genetic and epigenetic mechanisms implicated in altering the ratio of excitation/inhibition within central processing circuits of the autistic brain; including nicotinic, glutamatergic, gabaergic, and calcium signaling systems; (2) early immunologic differences in humoral and cellular immunity identified in autistic children that may impact brain development and behavior; and (3) approaches that toxicologist can develop in mouse models to better understanding how specific genetic or epigenetic defects alter sensitivity to chemical exposure(s) of current concern to children's health and autism.

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HYPERBARIC OXYGEN THERAPY IN AUTISTIC CHILDREN IMPROVES SYMPTOMOLOGY, DECREASES MARKERS OF INFLAMMATION, AND HAS NEUTRAL EFFECTS ON OXIDATIVE STRESS. Dan Rossignol*, Elizabeth Mumper, Jill James, Stepan Melnyk, Lanier Rossignol. **Department of Family Medicine, University of Virginia, Charlottesville, VA.*

Autism is a neurodevelopmental disorder currently affecting as many as 1 out of 166 children. Multiple studies have found that autism is characterized by cerebral hypoperfusion which correlates with many core features including repetitive, self-stimulatory and stereotypical behaviors, and impairments in communication, sensory perception, and social interaction. Autistic individuals also evidence gastrointestinal and neuroinflammation, increased markers of oxidative stress, and relative mitochondrial dysfunction. Hyperbaric oxygen therapy (HBOT) has been used to treat conditions marked by diminished cerebral blood flow to overcome hypoperfusion through the increased delivery of oxygen. HBOT also demonstrates strong anti-inflammatory properties, might decrease oxidative stress, and can increase the production of mitochondria and circulating stem cells. Based upon these research findings, we hypothesized that HBOT would

improve symptoms in autistic children. *Methods*: 18 autistic children underwent 40 1-hour HBOT sessions at either 1.5 atmosphere absolute (ATA) and 100% oxygen (6 children), or 1.3 ATA and 24% oxygen (12 children). Results were calculated before and after HBOT using parent-rated Aberrant Behavior Checklist, Social Responsiveness Scale, Childhood Autism Rating Scale, Autism Treatment Evaluation Checklist, and a modified GI scale. Blood was drawn before and after HBOT for inflammatory and oxidative stress profiles. *Results*: For the 1.5 ATA group, parents reported significant improvements in lethargy (49%, p=0.008), motivation (34%, p=0.018), sensory and cognitive awareness (24%, p=0.013), and speech (17%, p=0.04). For the 1.3 ATA group, parents reported significant improvements in lethargy (27%, p=0.06), motivation (26%, p=0.011), physical health (21%, p=0.012), mannerisms (21%, p=0.011), sensory and cognitive awareness (14%, p=0.026), speech (10%, p=0.033), and communication (7%, p=0.035). C-reactive protein improved in both the 1.5 ATA (61.4%, p=0.099) and 1.3 ATA groups (89.5%, p=0.100), and there was no statistically significant change in plasma oxidized glutathione levels in either group after HBOT. *Conclusions*: HBOT ameliorates some symptoms in autistic children in this prospective open label study. Further evaluation with a larger double-blind placebo-controlled study to verify these findings is indicated. Keywords: Autism, Hyperbaric Oxygen Therapy, Inflammation.

Roundtable Discussion:

Topic:

"Advancing the Science of Autism Spectrum Disorders"

Discussion Leaders: Isaac Pessah, PhD
Martha Herbert, PhD

Identify the evidentiary gaps in knowledge and brainstorm necessary next steps.

Questions to be Addressed:

1. Can we be more systematic about identifying environmentally vulnerable pathways in autism?
2. Do environmental factors affect brain function in labile ways that can be affected or reversed by treatment?
3. How can we encourage researchers to study the physiology (biochemistry, toxicology, immunology) of autism?
4. In order to get biological data on a large number of autistic individuals and correlate it with other findings, would it be feasible to develop a basic biomarker panel that brain and psychology oriented researchers could use when they are drawing blood for genetic samples that they commonly collect?
5. What brain biomarkers would be most sensitive to toxic insult and to treatment response?
6. How do we rigorously address the great biological and phenotypic heterogeneity in autism?
7. How can we integrate research on cause and mechanism with research on treatment?

Plenary Session

SESSION 6. NEUROTOXICITY AND THE PATH FROM EARLY BRAIN DEVELOPMENT TO AGING

Session Chair: Bernard Weiss, PhD

Theme: *In the past, we often tended to distinguish two realms of neurotoxicity based on chronological age. One centered on early development, the other on senescence. It is now profoundly clear that his distinction is arbitrary. The two realms are simply two narrow sectors from the arc of the lifespan, which itself is fundamentally a developmental journey. The arbitrary nature of this distinction becomes apparent when what are labeled as developmental disorders, such as autism, are seen to share biological mechanisms with neurodegenerative disorders such as Parkinson's disease. In parallel, early life events, whose influence is not evident at the time, may leave traces that only emerge decades later, when the patterns of vulnerability have changed. But the connection is not direct. When speaking of the aging brain we should recognize that it represents a mosaic of possibilities rather than a hapless monolith; some regions diminish, some even prosper. Finally, the legacy of earlier events is inevitably transformed by a cascade of intervening circumstances, such as stress, acting on this regrettable trek. This plenary session offers further perspectives on the principle that neurotoxicity is not simply a property of a designated chemical exposure, but is also a property dependent on the history and state of the host.*

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NEUROTOXIC BYWAYS ON THE PATH TO AGING. Bernard Weiss. *Department of Environmental Medicine, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642.*

The brain's journey from childhood to senescence may appear continuous, but it surely is not smooth. In accompanying this journey, environmental neurotoxicants exert their influence in differing ways and with differing potencies at different times.

Because of dramatic consequences such as those exemplified by fetal alcohol syndrome and congenital Minamata disease, this early segment of the lifespan has received the greatest and most intense scrutiny. Neurotoxicity is a lifetime issue, however, and our appreciation of its scope will suffer without extending our purview across the entire lifespan. One reason is the recognized phenomenon of delayed or latent toxicity, which describes the gap in time that separates exposure from consequences that may become visible only much later in life, as when the compensatory capacity of the brain has receded with age. But we have to accord recognition to other life stages because both brain structure and its functional expression continue to change during those times. Adolescence, for example, is a period during which the brain undergoes a precipitous period of development and vulnerability analogous to early childhood. Senescence offers its own contours of vulnerability and

epitomizes a life stage not yet accorded its proper role in neurotoxicology's domain. Across the lifespan, two modulators of nervous system function, the endocrine and immune systems, also exert profound influences and are themselves shaped by both life stage and environmental toxicants. Although It is convenient to refer to different life periods as if they were tangible entities, we basically are viewing the same river from different vantage points. (Supported in part by grant ES013247.) Keywords: Aging, Life Stage, Delayed Toxicity.

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INFLAMMATION AND REPAIR IN THE AGING BRAIN: WHAT A DIFFERENCE A DECADE MAKES. G. Jean Harry, *Neurotoxicology Group, Dept. of Health and Human Services, National Institutes of Health/NIEHS, Research Triangle Park, North Carolina.*

In the immature brain, activation of an inflammatory response is often associated with the stimulation of neural progenitor cells as a repair process. With aging, both microglia and progenitor cell activities decline, resulting in diminished capability for repair, suggesting an association between successful injury and successful repair.

Recent studies have shown that the brain is capable of generating new cells that can mature to either glia or neurons. This process occurs in the normal brain and is stimulated in distinct neurogenic regions upon brain injury. In the immature brain, this repair process is often sufficient to replace lost neurons and myelinating glia. With aging, the capacity of the brain to initiate a repair process is diminished with regard to cell survival and integration into the neural network. The decreased repair process in the aged brain may be due to a change in the progenitor cell population; an alteration in the neurogenetic environment of these cells; or the impact of microglia cells and neuroinflammation. Understanding the molecular and cellular profile of a specific progenitor cell population and the neurogenic environment in the young versus the aged brain may allow for the identification of critical targets for promotion or disruption of this critical repair process.

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CUMULATIVE IMPACT OF LEAD EXPOSURE AND STRESS OVER THE LIFESPAN. D.A. Cory-Slechta, *Environmental and Occupational Health Sciences Institute, Robert Wood Johnson Medical School, UMDNJ, and Rutgers, the State University of New Jersey, Piscataway, NJ 08854.*

Elevated lead (Pb) exposures preferentially impact low socioeconomic status (SES) populations, the same groups thought to sustain the highest levels of environmental stress. Low SES itself is a known risk factor for various disease processes and behavioral disorders, presumably through its association with chronic stress and associated elevation of glucocorticoids. As co-occurring risk factors, therefore, Pb and stress could interact, such that combined exposures could produce greater neurotoxicity than either risk factor alone, and/or that potentiated effects could occur. Such a possibility is further supported by the fact that both Pb and stress act on mesocorticolimbic dopamine and hippocampal systems of the brain as well as on the hypothalamic-pituitary-adrenal (HPA) axis, and both can permanently alter nervous system function following developmental exposures. To assess this possibility, we have examined consequences in rats of: 1) maternal Pb, maternal stress or the combination on offspring, including stress challenges; and 2) chronic postweaning Pb exposures combined with intermittent stress challenges. Outcomes confirm the importance of Pb-stress interactions as manifest in neurochemical and behavioral alterations, with female offspring evidencing greater vulnerability. The results also demonstrate permanent effects of Pb itself on HPA axis function and stress responsivity that depend upon Pb exposure parameters and the nature of the stress challenge. Finally, the profile of effects of Pb alone differs notably from that produced by Pb and stress. Collectively, these findings suggest new mechanisms for Pb-induced cognitive deficits and for Pb-induced hypertension. Permanent changes in HPA axis function in response to developmental Pb exposure raise new questions about a potential contribution of Pb exposure to metabolic disease, obesity and diabetes. These findings raise questions about the ability of current risk assessment approaches, based on studies of chemicals in isolation, to adequately identify neurotoxic risk. Finally, they suggest a critical need for Pb screening during pregnancy to avoid maternally-mediated Pb effects.

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TRANSLATIONAL RESEARCH IN ALZHEIMER'S DISEASE AND PARKINSON'S DISEASE. Don Schmechel, MD. *Duke University, Durham, North Carolina.*

Translational research in Alzheimer Disease and Parkinson Disease must address the interplay of complex genetic and environmental factors and how these may result in both regional selectivity of injury and sparing. AD and PD may share some common genes affecting age of onset and cell repair as well as having unique factors specific to each illness. Both involve oxidative stress mechanisms, exaggeration of normal aging changes, and possible problems in disposal of incorrectly folded or processed peptides and oligomeric arrays. Translational research must result in tangible answers for the detection and treatment of these chronic human illnesses whose biology of onset may differ from that of progression. The challenge is great and will needs involve pathology, cell system models, animal models, and recursive information from the study and treatment of humans at risk and with disease.

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AGING AND ENVIRONMENTAL HEALTH: EPA'S PLAN FOR EXPOSURE-DOSE-RESPONSE RESEARCH. Andrew M. Geller, *NHEERL, US EPA, Research Triangle Park, NC*

The rapid growth in the number of older Americans has many implications for public health, including the need to better understand the risks posed by environmental exposures to older adults. Biological capacity declines with normal aging; this may be exacerbated in individuals with pre-existing health conditions. In recognition of this issue, the U.S. EPA has developed a research program to better understand the relationships between external pollution sources → human exposures → internal dose → early biological effect → and adverse health effects for older

adults. The EPA's research program addresses 1) behavior/activity patterns and exposure to the pollutants in the microenvironments of older adults; 2) changes in absorption, distribution, metabolism, and excretion with aging; and 3) alterations in reserve capacity that alter the body's ability to compensate for the effects of environmental exposures. This presentation will discuss EPA's evolving strategy to understand the potential susceptibility of older adults to environmental insult and promote environmental health in older adults, and focus on issues specific to older adults in modeling exposure and the health consequences of exposure to pollutants in healthy older adults and in individuals with pre-existing health conditions. *This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.*

Roundtable Discussion:

Topic: *"Aging and Environmental Health"*

Moderator: Deborah Rice, PhD

Theme: *Despite our acknowledgement that development--and aging--are basically identical and continuous processes across the total lifespan, it would be deceptive to model them as smooth curves. Although they might not reveal sharp discontinuities, the road, so to speak, is bumpy and pitted (the seven ages of man--and woman?). It traverses periods of both diminished and aggravated vulnerabilities. Adolescence is one such period. Menopause is another. How many such periods can be distinguished? How can they be modeled in our usual laboratory rodents? And are calendar years a proper definition of age? Do such questions deserve more attention than we usually give them?*

Platform Session

SESSION 7-A-1: DEVELOPMENTAL NEUROTOXICITY - I

Session Chair: Didima de Groot, PhD
Co-Chair: Darryl B Hood, PhD

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PRINCIPLES FOR EVALUATING HEALTH RISKS IN CHILDREN ASSOCIATED WITH EXPOSURE TO CHEMICALS: THE NEED FOR A LIFE-STAGE APPROACH. Terri Damstra. *World Health Organization, International Programme on Chemical Safety, Research Triangle Park, North Carolina, USA*

Environmental factors play a major role in determining the health and well-being of children. Accumulating evidence indicates that children (who comprise over one-third of the world's population) are among the most vulnerable and that environmental factors can affect children's health quite differently than adults. Poor, neglected, and malnourished children suffer the most. These children often live in unhealthy housing, lack clean water and sanitation services, and have limited access to health care and education. One in five children in the poorest parts of the world will not live to their fifth birthday, mainly because of environment-related diseases. The World Health Organization (WHO) estimates that over 30% of the global burden of disease in children can be attributed to environmental factors.

Health is determined by a variety of factors. In addition to the physical environment, genetics, biology, social, economic, and cultural factors play a major role. It is critical to understand the various driving forces that shape health and behaviour throughout life. Children have different susceptibilities during life-stages (from conception to adolescence) due to their dynamic growth and developmental processes, as well as physiological, metabolic, behavioural differences, and unique exposure pathways. This recognition has raised international concern and identified the need to develop risk assessment approaches that take into account the special vulnerabilities of children.

WHO/IPCS has just recently developed an Environmental Health Criteria (EHC) document that addresses the scientific principles that need to be considered when assessing the potential health risks in children from exposure to environmental agents during distinct developmental stages. This presentation will summarize the key findings and conclusions of the Environmental Health Criteria document.

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LINKAGES BETWEEN EARLY CHILDHOOD LEAD EXPOSURE AND PERFORMANCE ON END OF GRADE EXAMS. ML Miranda, D Kim, MA Overstreet, AP Hull, and P Morgan. *Children's Environmental Health Initiative, Nicholas School of the Environment and Earth Sciences, Duke University, Durham, North Carolina, USA.*

The Children's Environmental Health Initiative (CEHI) at Duke University has partnered with the North Carolina Education Research Data Center (NCERDC) to explore the relationship between lead exposure and educational outcomes by linking end-of-grade test results and associated student data with early childhood blood lead screening results for nine North Carolina Counties. The study analyzes the contribution of early childhood lead exposure on educational performance later in life. After controlling for a variety of sociodemographic variables, including parental education, household income (as measured by participation in the free/reduced lunch program), sex, and race and ethnicity, as well as county-level differences, we find that early lead exposure is significantly and negatively related to performance on end of grade exams. The detrimental effects of lead are more pronounced on reading, as opposed to math, scale scores, but are significant for both. Incorporating lead exposure attenuates some of the explanatory power typically attributed to race, income, and parental education variables. The study also demonstrates significant, negative impacts of early childhood lead exposure on educational outcomes, at levels well below the CDC lead threshold for health effects. In addition, the study provides insights regarding the ability to link two large databases generated by two different offices of the State of North Carolina in the same populations but at different time periods. Lead surveillance is undertaken before age six, whereas end of grade testing in North Carolina begins in grade three (~ age 8-9). Keywords: Lead Exposure, Educational Outcomes, Database Linking.

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GESTATIONAL ENVIRONMENTAL CONTAMINANT EXPOSURE EFFECTS ON NMDA/ AMPA-DEPENDENT PLASTICITY MECHANISMS IN ADULT RAT HIPPOCAMPUS AND SENSORY CORTEX. DB Hood[1]; L Woods[2]; LA Brown[1]; SK Johnson[1]; and FF Ebner[2] [1]*Department of Biomedical Sciences; Division of Neurobiology and Neurotoxicology; Center for Molecular and Behavioral Neuroscience, Meharry Medical College, Nashville, TN, USA.* [2] *Department of Psychology, Center for Cognitive & Integrative Neuroscience, Vanderbilt University, Nashville, TN, USA.*

Gestational exposure to environmental contaminants such as Benzo(a)Pyrene [B(a)P] and 2,3,7,8,-tetrachlorodibenzo-p-dioxin (TCDD) pose a significant threat to normal growth and differentiation processes in the developing brain. To characterize the impact of gestational exposure to environmental contaminants on hippocampal and cortical plasticity and function, pregnant Long Evans rats were exposed to either sub-acute doses of B(a)P (100 $\mu g/m^3$) on gestation day 14-17 (GD) or to a single oral dose of TCDD (700ng/kg BW) on GD15. When F1 generation animals reached maturity, long-term potentiation (LTP) was measured from the hippocampus and neuronal activity was recorded from neurons in the whisker representation of SI (barrel) cortex. LTP was significantly reduced as was spontaneous/evoked activity of cortical neurons in environmental contaminant-exposed F1 generation animals when compared to controls. To mechanistically ascertain the basis of environmental contaminant induced plasticity perturbations, total RNA from F1 animals was analyzed to determine mRNA profiles for molecules that are important for synaptic plasticity. Ionotropic glutamate receptor subunits were profiled for developmental mRNA and protein expression levels in F1 control and exposed rats using semi-quantitative PCR and western analysis. The results of the molecular studies revealed a significant down-regulation in the temporal developmental expression of glutamate receptor (NMDA and AMPA) subunits in the hippocampus and S1 cortex. The results also demonstrate that F1 generation exposed animals present with deficiencies in behaviors that depend on normal hippocampal and cortical function and predict deficits in behaviors that will be present throughout life. The results from these studies suggest that gestational exposure results in plasticity deficits mediated through down-regulation of developmental glutamate receptor subunit expression and function at a time when excitatory synapses are being formed for the first time in the developing central nervous system. Supported by NS041071

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EFFECTS OF MATERNAL DIET ON OFFSPRING'S VULNERABILITY TO ENVIRONMENTAL EXPOSURE. Didima de Groot, Celine de Esch, Simone Eussen, Annick Hundscheid, Ivana Bobeldijk, John Groten, Henk Hendriks, Wilrike Pasman, Florence Salmon, Heleen Wortelboer, André Wolterbeek. *TNO Quality of Life, Zeist, The Netherlands*

The nutritional status of the mother during gestation and early lactation may affect the development of the offspring, resulting in lasting alterations during adolescence and ageing. In this reasearch we studied the impact of maternal diet on vulnerability of the offspring to environmental exposure.

Three groups of female Wistar rats (n=28-32) were kept on a control diet (C), a high (H) or a low (L) calorie diet during 6 weeks premating, mating, gestation and lactation. Immediately after birth (postnatal day (PN) 1), F1-pups were cross-fostered to dams of the same group or to dams of the other two dietary groups resulting in 9 different groups and fed the various diets up to PN 70. To investigate environmental influences, the vulnerability for chemically induced neurotoxicity was studied. Hereto, the F1-pups were dosed subcutaneously with MAM (PN day 2-5) or MeHg (PN 2-21).

Food consumption, energy intake and body weight of the dams differed significantly between the groups. Differences in birth weight were not found; the lower number of pups per litter and slightly lower birth weights in the low calorie group did not reach significance. Early pup growth was affected showing 'catch-up' growth of the low calorie pups fostered to dams on high calorie diet and, vice-versa, decreased growth of the high calorie diet pups fostered to dams on a low calorie diet. Increased vulnerability for exposure to MAM or MeHg was observed in both high and low calorie pups when fostered to low calorie dams as demonstrated in several neurobehavioural tests with delayed/disturbed neurodevelopment. MAM or MeHg did not induce neurotoxic effects in pups, raised on standard diet before and after birth.

It was concluded that maternal diet manipulation may have (transitory) effects on the offspring which, in turn, may be responsible for increased vulnerability of the offspring for (subtle) environmental influences causing life-long changes in metabolism, as suggested here by changes in body weight. Keywords: Maternal Diet, Cross-Fostering, Postnatal Exposure.

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A PROXY MEASURE OF PRENATAL TESTOSTERONE EXPOSURE IS RELATED TO GRAY MATTER VOLUME IN HUMAN NEONATES Rebecca C. Knickmeyer[1], Y. Sampath K. Vetsa[2], Bradley Moore[2], Guido Gerig[1,2] and John Gilmore[1] [1]*Dept. of Psychiatry, University of North Carolina, Chapel Hill, NC 27599,* [2]*Dept. of Computer Science, University of North Carolina, Chapel Hill, NC 27599.*

There is growing concern that endocrine-active compounds (EACs) in the environment may exert toxicological effects on the developing human brain. Experiments in animals have routinely demonstrated that manipulation of testosterone levels during early life and exposure to EACs that alter the production, action, and conversion of testosterone, have profound effects on brain structure and subsequent behavior. In contrast, information on whether testosterone is related to individual differences in brain development in human beings is extremely limited, especially at early ages. We report the first study to link variation in an index of prenatal testosterone exposure (2D:4D ratio, the ratio of the 2nd to the 4rth digit) to structural brain development assessed via high-resolution MRI at 2 weeks after birth. 31 female and 33 male neonates were scanned unsedated on a Siemens head-only 3T scanner (Allegra, Siemens Medical System

Inc., Erlangen, Germany). Tissue segmentation was accomplished using a novel automated approach developed at UNC for neonatal MRIs. 2D:4D was calculated from photocopies of the infants' hands. We focused on left 2D:4D as this showed the greatest sex difference in our sample. Left 2D:4D was significantly correlated with gray matter volume in males (k = -0.347, p < 0.05); males with higher 2D:4D (i.e. lower testosterone exposure) had smaller gray matter volumes. There was also a trend relating left 2D:4D to total intracranial volume (k= -0.267, p = 0.1). No relationship was seen between 2D:4D and volume of unmyelinated white matter. No relationships were seen within females. These results may have important implications for predicting the effects of exposure to EACs on the developing human brain. Keywords: Testosterone, Brain Development, MRI.

Platform Session

SESSION 7-A-2: ROLE OF ENVIRONMENTAL CONTAMINANTS IN THE ETIOLOGY OF ADHD-LIKE BEHAVIORS

Session Chair: Richard Seegal, PhD
Co-Chair: Paul W. Stewart, PhD

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AN OVERVIEW: DEVELOPMENTAL NEUROENDOCRINE EFFECTS OF PCBS AND PBDES: PARALLELS WITH ADHD. RF Seegal. *Wadsworth Center, New York State Department of Health and Department of Environmental Health Sciences, University at Albany, Albany, NY, USA.*

Attention deficit disorder with hyperactivity (ADHD) is one of the most commonly diagnosed developmental disorders. Although genetic and endocrine factors are implicated, environmental contaminants may also influence the incidence of this disorder. Thus, Jacobson *et al.* and Stewart *et al.* have reported deficits in attentional domains, including impulsive responding, in children exposed during development to PCBs and other fish-borne contaminants. Here I briefly review changes in neurochemical and endocrine function in rodents following exposure to polychlorinated biphenyls (PCBs) and, to a lesser extent, polybrominated diphenyl ethers (PBDEs). These changes are similar to those seen in children diagnosed with ADHD. First, PCBs reduce central dopamine (DA) and norepinephrine (NE) concentrations and inhibit the dopamine transporter (DAT) leading, over the long term, to significant decreases in extra-neuronal DA. Most importantly, clinical studies describe increases in basal ganglia DAT densities in children with ADHD that are also likely to lead to decreases in synaptic DA. Second, PCBs significantly reduce circulating thyroid hormone concentrations in developmentally exposed rats—findings reminiscent of those seen in children diagnosed with ADHD. Examination of these parallels may not only strengthen the hypothesis that these contaminants contribute to the etiology of ADHD, but may also provide a mechanistic basis for understanding the recent epidemiological observations of ADHD-like behaviors in children developmentally exposed to PCBs and other fish-borne contaminants. Supported in part by NIEHS grant 1P01ES11263 and USEPA grant R829390.

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PERINATAL PCB EXPOSURE, DEFICITS IN INHIBITORY CONTROL AND HYPOFUNCTION OF PREFRONTAL DOPAMINE: PARALLELS WITH ADHD. Susan L Schantz. *University of Illinois at Urbana-Champaign, Urbana, IL, USA.*

Polychlorinated biphenyls (PCBs) are industrial chemicals that are no longer in use, but are still prevalent in the environment. Previous research in animal models and humans has revealed deficits in response inhibition following PCB exposure. Perinatally PCB-exposed animals show increased perseverative responding on both reversal learning and delayed spatial alternation, learning tasks that require inhibitory control for their successful execution. PCB-exposed rats and monkeys also perform more poorly on schedule-controlled operant tasks where low rates of responding are optimal, including fixed interval and differential reinforcement of low rate (DRL) schedules, although not all investigators have observed deficits on these tasks. We have previously reported increased perseverative responding on both reversal learning and delayed spatial alternation tasks in PCB-exposed rats. More recently we found that rats exposed to an environmentally relevant PCB mixture during the perinatal period made more responses during the extinction phase of a DRL task and more errors of commission on an auditory discrimination task. The deficits in response inhibition seen following early PCB exposure closely parallel those observed in children with attention deficit hyperactivity disorder (ADHD) and in animal models of ADHD. The effects of PCBs on attentional processes—which are also deficit in ADHD—are not as clear. Our future studies will focus on further elucidating the effects of PCBs on attention and inhibitory control and investigating the role of altered prefrontal dopamine function in mediating these effects. Because of the similarities between the cognitive and neurochemical deficits resulting from early PCB exposure and those observed in ADHD, these studies have the potential to further our understanding of this common childhood disorder. Supported by ES11263 from NIEHS and R82939001 from USEPA.

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IS IMPULSIVE BEHAVIOR AND IMPAIRED RESPONSE CONTROL A FINAL COMMON PATH FOR PCB, MEHG AND PB NEUROTOXICITY IN CHILDREN? PW Stewart, J Reihman, E Lonky, B Gump, T Darvill & J Pagano. *Psychology Department, State University of New York at Oswego. Oswego, NY. USA.*

Several experts in neurobehavioral toxicology have long argued for the assessment of behavioral domains in children which are sensitive to toxicant exposures in animals. Unfortunately, this advice has largely been ignored. Tests of "global cognition" dominate the assessment of exposed children. These tests are not only rely heavily on language, they also amalgamate and confound several disparate abilities into a single score – creating what may be pragmatic for the clinician but entirely problematic for the scientist. Recently, we have examined neurobehavioral toxicity in children exposed to a variety of contaminants (PCBs, MeHg and Pb) using domain-specific tasks which are conceptually related, or

identical to, tasks which are sensitive to these exposures in animals. Using these tools, we have repeatedly demonstrated impulsivity and response inhibition deficits associated with PCB exposure These deficits have been demonstrated using a battery of signal detection tests designed to target response inhibition. More recently, we have employed an operant task (DRL) which has been sensitive to PCB and Pb exposure in rats and primates. Data generated from this task has revealed a surprisingly large number of deleterious associations with ostensibly different contaminants: PCBs, MeHg and Pb. Each of these contaminants has predicted impaired performance on the DRL task, and each of the associations are each statistically independent of the other. Children exposed to any of these contaminants responding impulsively and inefficiently and earn fewer reinforcements. These findings have been robust in a multivariate environment where more than 50 potentially confounding variables have been assessed. These data have specific implications for the nature of the tests used in assessing children exposed to environmental contaminants, and more broadly, how we think about and conceptualize "behavioral" toxicity.

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DEVELOPMENTAL PESTICIDE EXPOSURE REPRODUCES FEATURES OF ATTENTION-DEFICIT HYPERACTIVITY DISORDER. Jason R. Richardson[1], Thomas S. Guillot[2], W. Michael Caudle[2], and Gary. W. Miller[2]. [1]*Department of Environmental and Occupational Medicine, University of Medicine and Dentistry-New Jersey/Robert Wood Johnson Medical School and Environmental and Occupational Health Sciences Institute* [2]*Center for Neurodegenerative Disease, Emory University.*

Attention deficit hyperactivity disorder (ADHD) is estimated to affect 3-12% of school-age individuals in the United States alone. Although the pathophysiology of ADHD is not completely understood, disruption of the dopamine system appears to play a central role in the pathologic manifestation of disease. Specifically, polymorphisms and elevated levels of the dopamine transporter (DAT) have been found in both adolescents and adults with ADHD. Thus, alteration of DAT levels or function appears to contribute to the pathogenesis Accordingly, exposure to environmental agents that alter the proper development of the dopaminergic system and enhance DAT levels may contribute to development of behaviors associated with ADHD. Here, we demonstrate that developmental exposure to deltamethrin, a widely used pyrethroid insecticide, causes long-term up-regulation of the DAT (21-70%) and hyperactivity (98-185% increase in locomotor activity) in adolescent mice at 6 weeks of age. These effects were sex-specific, with males being more affected than females. The hyperactivity observed appears to be related to supersensitive D1-dopamine receptors, and could be reversed by dopamine receptor antagonists and low-dose methylphenidate administration. Additionally, the offspring of deltamethrin treated animals exhibited deficits in working memory and attention assessed in a Y-maze. Gene-profiling studies in the midbrain of these animals revealed a dose-related increase in the transcription factors Nurr1 (22-99%) and Pitx3 (49-114%), which are required for proper development and maintenance of the dopamine system and the dopamine transporter. The parallels between mice developmentally exposed to deltamethrin and individuals with ADHD suggest that pesticide exposure during development may represent a risk factor for ADHD.

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SMOKING DURING PREGNANCY: EFFECTS ON AROUSAL AND ATTENTIONAL BRAIN SYSTEMS. E. Garcia-Rill[1], R. Buchanan[2], [1]*CTN, University Arkansas for Medical Sciences,* [2]*Arkansas Bioscience Institute, Arkansas State University*

Prenatal exposure to cigarette smoke is known to produce lasting arousal, attentional and cognitive deficits in humans. The pedunculopontine nucleus (PPN), as the cholinergic arm of the reticular activating system (RAS), is known to modulate arousal, waking and rapid eye movement (REM) sleep. The PPN projects to the intralaminar thalamus, where it modulates thalamocortical attentional systems. We studied the properties of PPN neurons during the developmental decrease in REM sleep between 10 and 30 days postnatally in the rat. Pregnant dams were exposed to cigarette smoke for 15 min, 3 times per day, from day E14 until birth, and the pups allowed to mature. Intracellularly recorded PPN neurons in 12-21 day rat brainstem slices were tested for intrinsic membrane properties, including the hyperpolarization-activated cation current Ih, which is known to drive oscillatory activity. We found that PPN cells from the offspring of treated animals had higher Ih amplitude at both 12-16 days and 17-21 days compared to those in control offspring. In addition, offspring of treated animals had higher resting membrane potential and lower action potential threshold. These data suggest that prenatal exposure to cigarette smoke induced marked changes in cells in the cholinergic arm of the RAS, rendering them more excitable. Such data could partially explain the differences seen in individuals whose parents smoked during pregnancy, especially in terms of their hypervigilance and increased propensity for attentional deficits and cognitive/behavioral disorders. Supported by USPHS grant RR020146.

Symposium

SESSION 7-B: NEUROPROTECTION BY ALZHEIMER'S DRUGS

Session Chair: Toshio Narahashi, PhD
Co-Chair: Edson X. Albuquerque, PhD

Theme: *Alzheimer's disease is one of the most serious neurological disorders afflicting aged individuals. While many studies have been performed to determine the causes of the disease, no definitive explanations have yet been offered, and no prevention or cure of the disease has yet been developed. Thus, only symptomatic drug treatments to improve the patients' conditions are available at the present moment. In the brain of an Alzheimer's disease patient, both the cholinergic system and the glutamatergic system are down-regulated, and most drugs currently target these neuroreceptor systems. The actions of these drugs are basically "neuroprotection" via a variety of mechanisms. The present symposium will address this issue.*

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SYNUCLEIN-LINKED NEUROIMMUNITY AND THE PATHOGENESIS OF PARKINSON'S DISEASE. Howard E. Gendelman. *University of Nebraska, Omaha, Nebraska, USA*

The pathology of Parkinson's disease (PD) includes loss of dopaminergic neurons in the substantia nigra, nitrated α-synuclein (□syn) enriched inclusions or Lewy bodies and neuroinflammation. We reasoned that PD-associated oxidative protein modifications create novel antigenic epitopes capable of both microglial and peripheral adaptive T cell responses exacerbating nigrostriatal degeneration. Nitrated □-syn was readily detected in cervical lymphoid tissue in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) intoxicated mice. Using a combination of genomic (gene arrays) and proteomic (SELDI-TOF, liquid chromatography-tandem mass spectrometry, Ettan DIGE, and protein array) methods, a fingerprint of □-syn activated microglia was also generated. Nuclear factor-□B transcriptional activation and its related signaling cascades that affect cell metabolism and immune response signaled this □-syn microglial response. Microglial ROS production was also induced, in a dose-dependent manner, by activation with aggregated nitrated □-syn. This was inhibited by voltage-gated potassium current blockade, and to a more limited degree, by chloride current blockade. Transfer of T cells from mice immunized with oxidized α-syn led to prolonged neuroinflammation and significant increases in dopaminergic cell loss. These data support the notion that nitrotyrosine modifications in α-syn induce both an innate neuroinflammatory response as well as serving to break immunological tolerance to self. Both processes serve to exacerbate the pathobiology of PD.

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GALANTAMINE-MEMANTINE CO-APPLICATION: BENEFICIAL EFFECT. Toshio Narahashi, PhD. *Northwestern University Medical School, Chicago, IL, USA*

Alzheimer's disease is associated with down-regulation of the cholinergic and NMDA systems. Whereas several drugs have been used for the symptomatic treatment of Alzheimer's disease patients, their efficacy is limited. Thus, combined use of two drugs with different mechanisms of action might improve the effectiveness. Here we present the *in vitro* basis for the beneficial action of combining galantamine, a stimulator of the ACh and NMDA systems, with memantine, a blocker of the NMDA system.

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A NOVEL APPROACH FOR THE TREATMENT OF ORGANOPHOSPHOROUS COMPOUND POISONING: IMPLICATIONS FOR ALZHEIMER'S DISEASE. Edson X. Albuquerque, PhD. *University of Maryland, Baltimore, MD, USA*

The need to identify a safe and effective means to protect against the lethal effects of organophosphorous (OP) compounds, including OP pesticides and nerve gases such as soman and sarin, has become even more pressing in view of terrorist threats. In our recent studies using guinea pigs, we have been able to identify a combination of agents that confers a degree of protection that far surpasses any currently available treatment regimens. Protection can be achieved by administration prior to OP exposure, a feature that could be very helpful in the case of first responders to an incident involving OP assault. At the most effective doses for avoiding lethality, the combination of agents has been shown to produce minimal side effects. This represents a significant advance in measures available to counteract OP toxicity, and a study of the mechanisms involved has relevance to wider issues, including the treatment of Alzheimer's disease. This antidotal therapy is protected under an international patent application PCT/US05/33789 filed on 9/23/05. Support: US Army Med. Res. Devel. Comm. Contract DAMD- 17-95-C-5063 and Battelle Scientific Services contract TCN 03132.

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NICOTINIC TREATMENT FOR BOTH SYMPTOMATIC RELIEF AND ATTENUATION OF COGNITIVE DECLINE WITH AGING AND ALZHEIMER'S DEMENTIA. Edward D. Levin, PhD. *Duke University Medical Center, Durham, NC, USA*

Nicotinic receptor systems have been found to be critically important for cognitive function including attention, learning and memory. Nicotinic antagonists generally impair cognitive performance and nicotinic agonists including nicotine improve it. Nicotine skin patches have been shown to significantly improve cognitive function impaired by aging as well as Alzheimer's disease. In addition, there is preclinical evidence that nicotinic treatment can attenuate neurodegeneration and may hold promise for reducing the decent into Alzheimer's dementia.

Poster Session

SESSION 8: GENERAL POSTER SESSION

Posters will be on display from Sunday Afternoon through Wednesday Noon.

SEE PAGES 30 - 45 for abstracts presented from Poster. Poster abstracts are numbered from P-83 to P125.

The poster session is a highlight of this conference series and provides an ideal opportunity for one-on-one personal exchange of research information and ideas in an informal setting with a unique consortium of participants expert in various aspects of the theme and neurotoxicology in general. The General Poster Session has proven to be a wonderful venue for informal, in-depth discussion, collaboration building, and mentoring of young scientists. It is an important networking opportunity for students. Judging and selection of Pre – and Post-Doctoral Student Awardees will be made during the session.

Symposium

SESSION 9-A. HEALTH EFFECTS OF MANGANESE EXPOSURE: HUMANS & ANIMAL MODELS

Session Chair: Michael Aschner, PhD
Co-Chair: Anumantha Kanthasamy, PhD

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.*

Timely Topics to be Addressed:

- Consideration of the relevant heath issues associated with over exposure to manganese.
- Characterization of exposures
- Development of appropriate biomarkers of exposure.
- Quantifying the relationships between exposure and ill health, including pharmacokinetics.
- Understanding the mechanisms of transport, damage and repair
- Understanding and utilizing invertebrate models such as the *c. elegans* to probe for mechanisms of Mn neurotoxicity

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POTENTIAL NEUROTOXIC RESPONSES IN RATS AFTER PULMONARY ADMINISTRATION OF WELDING FUME WITH VARYING CONCENTRATIONS OF MANGANESE. JM Antonini. *Health Effects Laboratory, NIOSH, Morgantown, WV, USA.*

Questions persist regarding a possible causal association between neurological effects in welders and the presence of manganese (Mn) in welding fume. Here, our objective was to examine the potential neurotoxic effect of Mn in rats after pulmonary administration of different welding fumes. Three welding fumes were collected: gas metal arc-mild steel fume (GMA-MS); flux-cored arc hard-surfacing fume (FCA-HS); and manual metal arc hard-surfacing fume (MMA-HS). The amount of Mn in the FCA-HS (2.0 mg Mn/gm total metal) and MMA-HS (1.8 mg Mn/gm total metal) fumes was ~6x higher than in the GMA-MS (0.32 mg Mn/gm total metal) fume. Male Sprague-Dawley rats were instilled intratracheally with 0.5 mg/rat of one of the welding particle samples once weekly for seven weeks. Control animals received saline using the same treatment regimen. Four days after the last treatment, the animals were sacrificed, and lungs and discrete brain regions were recovered for metal and toxicity analyses. Significant increases in Mn levels were observed in the lungs of the animals treated with FCA-HS and MMA-HS fumes compared to the GMA-MS group. Slight elevations in Mn levels were observed in the striatum and cortex of brains from animals treated with each of the welding fumes compared to control levels. Measurement of glial fibrillary acidic protein (GFAP) and neurotransmitter levels as well as Fluoro-Jade B (FJB) staining provided an assessment of neurotoxicity. No FJB staining was observed but slight, although not significant, increases in striatal GFAP were found in the welding fume groups compared to the controls. Also, an increase in striatal dopamine and its metabolite DOPAC were observed in some of the welding fume groups. These preliminary findings indicate that Mn that has deposited in the lungs of animals treated with welding fume may potentially reach specific brain regions. Questions still exist as to whether inhaled welding fumes induce neurotoxicity. Further study at more chronic time points is needed.

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WHERE DOES Mn2+ INHIBIT OXIDATIVE PHOSPHORYLATION? T. E. Gunter*, M. Aschner#, J. Salter*, K. K. Gunter*; *Dept. of Biochem. & Biophys.; Univ. of Rochester Med. School; Rochester, NY 14642*; and Dept. of Pediatrics; Vanderbilt Med. School; Nashville, TN, 37232#.*

Oxidative phosphorylation produces about 92% of the ATP used by animal cells. Intramitochondrial [Ca^{2+}], an important factor controling ATP production, activates a series of sites in the metabolic pathways which would otherwise limit the ATP production rate. Ca^{2+} activates NADH production at pyruvate dehydrogenase, isocitrate dehydrogenase, and □-ketoglutarate dehydrogenase, and ATP production at the F_1F_0 ATP synthase and can increase the rate of ATP production up to several fold (Balaban, R.S. J. Mol. Cell. Cardiology 34:11259, 2002). Loss of this additional Ca^{2+}-induced ATP production could be vital to neurons in the basal ganglia which require large amounts of ATP. Mn^{2+} is known to bind to Ca^{2+} binding sites and intracellular Mn^{2+} to be sequestered by mitochondria, where it could displace Ca^{2+} from the sites activating ATP production. Both we and others have found that Mn^{2+} inhibits oxidative phosphorylation (Gavin, C. E. et al. Toxicol. Appl. Pharmacol. 115:1,1992; Zwingmann, C. et al. J. Cereb. Blood Flow Metab. 23: 756, 2003) Part of this inhibition is likely caused by interference by Mn^{2+} with Ca^{2+} activation at the sites listed above. We report the results of experiments on NADH production by the dehydrogenases and on mitochondrial oxidation rate designed to determine the sites at which Mn^{2+} inhibits oxidative phosphorylation and whether Mn^{2+} does interfere with Ca^{2+} activation of NADH production. Keywords: Mn2+ Toxicity, Mitochondria, ATP Production.

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MANGANESE UPREGULATES CELLULAR PRION PROTEINS AND INHIBITS THE RATE OF PROTEINASE-K DEPENDENT PROTEOLYSIS IN CELL CULTURE MODELS OF PRION DISEASES. Anumantha Kanthasamy, Christopher Choi, Vellareddy Anantharam, Arthi Kanthasamy, *Department of Biomedical Sciences, Iowa State University, Ames, IA 50011-1250*

The accumulation of the proteinase-K resistant form of prion protein (PrPsc) is a key pathological feature of prion diseases. However, the cellular mechanisms underlying conversion of normal cellular prion (PrPc) to PrPsc in prion disease is not well understood. Binding of the divalent cation copper to the octapeptide repeat sequences of PrPc has been shown to be critical to the stability of the protein. In the present study, we tested a novel hypothesis that transition metals, including manganese (Mn^{2+}), may alter the stability of PrPc, resulting in accumulation of prion protein with reduced susceptibility to proteolysis. First, we examined the effect of manganese on PrP expression and degradation in neuronal cells expressing mouse prion proteins with an altered epitope (3F4). Mn (100 μM) treatment over 0-24 hr increased PrP levels in both cytosolic and membrane-rich fractions in a time-dependent manner. Interestingly, Mn treatment neither increased PrP mRNA transcripts, as measured by qRT-PCR, nor altered the mRNA stability, indicating that the effect of Mn may be at the level of post-translation and/or degradation of PrP. In order to determine whether the accumulation of PrP is due to impairment of the proteasomal degradation pathway by Mn, proteasomal activity and ubiquitination were measured, but the results showed no significant alterations of the proteasomal degradation pathway. The pulse-chase analysis showed that the PrPc turnover rate is significantly increased with manganese treatment. Notably, limited digestion with proteinase-K also revealed that manganese treatment decreased the digestion rate of PrP. Collectively, these data clearly indicate that the divalent metal manganese can alter the normal processing of PrP, resulting in the accumulation of high concentrations of prion proteins with reduced susceptibility to proteolysis [supported by MHRP grant W81XWH-05-1-0239]. Keywords: Manganese, Neurotoxicity, Prion Diseases.

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CHANGES IN DIETARY IRON LEVELS AFFECT BRAIN MANGANESE ACCUMULATION AND DISTRIBUTION. VA Fitsanakis[1], N Zhang[2], JG Anderson[3], KM Erikson[3], JC Gore[2,5], MJ Avison[2] and M Aschner[1,4,5]. *[1]Dept Pediatrics, Vanderbilt University School of Med, Nashville, TN. [2]Vanderbilt University Institute of Imaging Science, Vanderbilt University Med Ctr, Nashville, TN.[3]Dept Nutrition, University of North Carolina—Greensboro, Greensboro North Carolina. [4]Dept Pharm and [5]the Kennedy Center, Vanderbilt University Med Ctr, Nashville, TN.*

Occupational exposure to manganese (Mn) has been associated with the onset of psychological and motor symptoms in some individuals, leading to a phenotype called manganism. Brain Mn accumulation has also been documented in patients with liver failure, those receiving parenteral nutrition or individuals with iron (Fe) deficiency. As it is known that Mn and Fe are transported by the same proteins, we wanted to determine whether changes in dietary Fe levels alter the brain Mn deposition patters. Previous data from magnetic resonance imaging (MRI) and atomic absorption (AA) spectroscopic techniques demonstrated early regionally specific and latter more global bran Mn accumulation in treated populations compared to controls. In the current study, two groups of adult male Sprague-Dawley rats were fed either Fe-deficient (3 mg Fe/kg) or Fe-supplemented (30 mg Fe/kg) chow for 14 weeks. Both groups were also given weekly intravenous injections of 3 mg Mn/kg. Animals were weighed weekly, a measure of general health. At the conclusion of the study, brains were removed and dissected into various regions (cerebellum, brain stem, midbrain, hippocampus, striatum or cortex) and the amount of Mn and Fe determined. Data was compared to control and Mn-treated animals from a previous study. Fe-supplemented animals weighed significantly less than other groups throughout the course of the study (one-way repeated measure ANOVA, $p < 0.05$). Atomic absorption spectroscopy indicated no difference in brain Mn or Fe levels between the Fe-deficient or Fe-supplemented groups. However, both groups showed an increase in brain Mn levels in the cerebellum, brain stem, striatum and cortex compared to control and Mn-treated cohorts ($p < 0.05$). Interestingly, there was a trend to decreased brain Fe levels in both Fe-modulated groups. This reached statistical significance in the Fe-deficient populations in the cerebellum, brain stem, midbrain and cortex ($p < 0.05$) compared to control. These data, which will also be correlated with MRI results, suggest that any change in dietary Fe levels exacerbates brain Mn accumulation, and alters normal brain Fe distribution in multiple discrete brain regions. This work was supported by Dept of Defense, Manganese Research Health W81XWH-05-1-0239 project 04149002 (MA and VF), and NIEHS ES 10563 (MA).

Platform Session:

SESSION 9-B: DEVELOPMENTAL NEUROTOXICITY - II

Session Chair: Merle G. Paule, PhD
Co-Chair: Mary Ann Wilson, PhD

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A NOVEL RAT BRAIN ATLAS FOR NEUROTOXICITY TESTING Didima de Groot[1], Anouk Gloudemans[1], Marja Moerkens[1], Sarita Hartgring[1], Jimmy Israel[1], Marga Bos-Kuijpers[1], James O'Callaghan[2], Hans-Jorgen Gundersen[3], Wolfgang Kaufmann[4], Jan Lammers[1], Ine Waalkens[1], Bente Pakkenberg[5] *[1]TNO Quality of Life, Zeist, The Netherlands; [2]NIOSH, Morgantown, USA.; [3]University of Aarhus, Aarhus, Denmark; [4]BASF, Ludwigshafen, Germany; [5]Research Laboratory for Stereology and Neuroscience, Copenhagen, Denmark*

A neuro-anatomical rat brain atlas was designed for potential use in (regulatory) developmental neurotoxicity testing. In this atlas, ten major regions, together composing the total brain, are defined and delineated using natural and artificial borders between regions: i.e. *neocortex*, *limbic cortex*, *olfactory bulb*, *hippocampal formation*, *callosal body*, *caudate putamen*, *amygdala* and *thalamus/hypothalamus*.

To validate the atlas, the volume of the regions was estimated by two independent investigators using stereology (Cavalieri principle). For validation a group of ten 22 day old male Wistar rats was used. Once the outcome of the validation study proved to be satisfactory in terms of inter-investigator variation and reproducibility, the usefulness of the atlas as a screening tool for developmental neurotoxicity testing was evaluated, using the effects of prenatal exposure to methylazoxy methanol

(MAM) as a model [1, 2]. MAM (7.5 mg/kg bw/day), a compound inhibiting cell division, was dosed to female rats from gestation days 13 to 15 when mainly the forebrain and hippocampus are in proliferation whereas the cerebellum is not as this region develops mainly after birth. Control animals received saline. Groups for analyses comprised offspring of both sexes aged 22 and 62 days, both control and MAM exposed F1-animals (n=10 litters/group; 1 rat/sex/litter; 8 groups).

The brain atlas appears to be an adequate and efficient tool to study the normal and abnormal development of the rat brain; the regions defined according to this atlas can be demarcated easily and reproducibly. In line with the exposure schedule used, MAM showed a remarkable growth suppressing effect on the different brain regions except for cerebellum and medulla oblongata. This effect is more pronounced in the male than in the female rat brain. This is not surprising as also the normal development of brain morphology differed between the sexes, implying a different time window of development and, hence, a different window for maximal vulnerability for neurodevelopmental toxicity. *This work is supported financially by the American Chemistry Council.* Keywords: Rat Brain Atlas, Neurotoxicity, Prenatal Exposure.

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LOW DOSE EARLY POSTNATAL DIAZINON EXPOSURE CAUSES IMPAIRED WORKING MEMORY ON THE RADIAL-ARM MAZE IN RATS DURING ADULTHOOD. Edward D. Levin, Cindy Roegge, Olga Timofeeva, Frederic J. Seidler and Theodore A. Slotkin. *Departments of Psychiatry and Pharmacology, Duke University Medical Center, Durham, NC, USA.*

Organophosphate pesticides inhibit acetylcholinesterase but can have adverse effects on brain development through multiple mechanisms. Previously, we found that of fetal or neonatal exposure of rats to chlorpyrifos at doses insufficient to inhibit cholinesterase nevertheless altered cognition, emotional response, and spontaneous locomotor behavior in adulthood. We are now extending these studies to other organophosphate pesticides. Diazinon was assessed in the current study. Diazinon was administered at doses spanning the threshold for cholinesterase inhibition to Sprague-Dawley rat pups (0, 0.5 and 2 mg/kg/d of diazinon on days 1-4 after birth, N=11-12 of each sex/ dose). These animals were trained on the 16-arm radial maze starting in young adulthood to assess spatial learning along with working and reference memory. Twelve of the sixteen arms were baited with food reinforcements at the beginning of each session and not thereafter. The same four arms were left unbaited for all the training sessions for any particular rat. Each session lasted until all of the baited arms had been chosen with a maximum of ten minutes. There were 18 training session. Re-entries into previously baited arms were counted as working memory errors, while entries into arm, which were never baited were counted as reference memory errors. Response latency was determined by dividing the total session length by the number or arm entries made resulting in the seconds per entry measure. Diazinon treatment caused a significant main effect ($p<0.05$) with working memory errors. Paired comparisons showed that the 0.5 mg/kg diazinon dose group had significantly ($p<0.025$) more working memory errors (8.6±0.5) per session than either the controls (7.3±0.4) or the 2 mg/kg diazinon group (7.3±0.5). All dose groups learned the task. The greatest deficit was seen during the middle portion of training, although there was not a significant diazinon x session block interaction. This memory impairment did not seem to be secondary to generalized sensory, motor or motivational effects. No diazinon effects were seen with reference memory or response latency. We have previously seen with chlorpyrifos that developmental exposure can cause a selective memory impairment at low but not higher doses. This suggests that mechanisms other than acetylcholinesterase inhibition occurring with the low dose are causing the working memory impairment and that the acetylcholinesterase inhibition which comes to bear with the higher dose may be ameliorating the effect. Our results show that there are persistent neurobehavioral effects of early postnatal exposure to doses of diazinon that lie below the threshold for signs of systemic toxicity or cholinesterase inhibition. (Supported by the Duke University Superfund Basic Research Center ES010356). Keywords: Diazanon, Memory, Organophosphate.

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NEONATAL LEAD EXPOSURE IMPAIRS PLASTICITY IN DEVELOPING RAT SOMATOSENSORY CORTEX. MA Wilson, ME Blue, RC Patra, EM Gordon-Lipkin, and MV Johnston. *Kennedy Krieger Research Institute and Johns Hopkins University School of Medicine, Baltimore, MD, USA*

Exposure to lead during childhood impairs cognition and behavior; these effects are greatest in children exposed between 1 and 3 years of age, a period of activity-dependent development and refinement of synaptic connections in cerebral cortex. In animal models, lead alters neurotransmission and postsynaptic signaling. These data suggest that the detrimental effects of childhood lead exposure may be due to disruption of activity-dependent cortical plasticity. We used a rodent model of activity-dependent plasticity in somatosensory cortex to evaluate this hypothesis. Rat somatosensory cortex contains visible clusters of layer IV neurons, called barrels, that receive sensory input from individual whiskers. Barrels are arranged in a topographic array corresponding to the long vibrissae on the snout; this part of S1 cortex is referred to as the barrel field. This topographic map can be modified by whisker follicle ablation within a few days of birth. Rats were exposed to 0 or 0.15% lead acetate via the dam's water beginning on the day of birth (P1). Whisker follicles C1 to C4 were ablated on postnatal day 2 (P2) or P4, and barrel field morphology was examined on P10. In controls, follicle ablation produced a reduction in size and fusion of the denervated barrels and expansion of adjacent barrels. Lead exposure blunted this plastic response, increasing the area and number of barrel-like clusters remaining in the denervated row ($p<0.05$). These data are consistent with a model in which lead increases spontaneous neuronal activity in the denervated row, interfering with activity-dependent processes that shape cortical circuitry. Our results suggest that the detrimental

effects of developmental lead exposure may be due to disruption of activity-dependent plasticity in cerebral cortex. Keywords: Lead, Developmental Neurotoxicity, Plasticity.

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NEUROBEHAVIORAL ASSESSMENT USING A FUNCTIONAL OBSERVATIONAL BATTERY AND MOTOR ACTIVITY IN RATS PERINATALLY EXPOSED TO DE-71. VC Moser, CG Coburn, JD Farmer, KA Jarema, RC MacPhail, KL McDaniel, PM Phillips, PRS Kodavanti. *Neurotoxicology Division, NHEERL/ORD, US EPA, Research Triangle Park, NC.*

Polybrominated diphenyl ethers (PBDEs) have been widely used as flame retardants in a variety of commercial products. Their persistence in the environment and detection in populations throughout the world has raised concern about their toxic effects. Developmental neurotoxic effects have been suspected due to their structural similarities to polychlorinated biphenyls (PCBs). In this study, we evaluated behavior in offspring exposed to a widely used pentabrominated commercial mixture, DE-71. Pregnant Long-Evans rats were exposed to 0, 1.7, 10.2, or 30.6 mg/kg/day in corn oil by oral gavage from gestational day 6 to weaning. On postnatal days (PND) 24 and 60, male offspring (n=8-13/dose) were tested using a functional observational battery (FOB). Horizontal and vertical motor activity were measured in figure-eight chambers immediately after FOB testing. Offspring of both sexes (n=3-11/dose/gender) were also tested in Motron® chambers on PNDs 100 and 114. For the FOB, there were no effects on reflexes, excitability, or measures of physiological, autonomic, sensorimotor, or neuromuscular function. The only statistically significant finding was a dose-by-age interaction in the number of rears in the open field, wherein the control group showed similar levels at both time points but the treated rats (low and high dose only) showed higher rearing on PND60. Motor activity measured in the figure-eight chambers was not affected, nor was habituation. Neither horizontal nor vertical activity measured in the Motron® chambers showed significant effects of dose or time (age) of testing. Thus, perinatal DE-71 exposure may cause subtle changes in rearing activity that are detected in an open field. (*This abstract does not necessarily reflect USEPA policy*). Keywords: PBDE, Motor Activity, Developmental.

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NMDA-TYPE GLUTAMATE RECEPTORS AND ANESTHETIC-INDUCED NEURONAL OXIDATIVE STRESS DURING DEVELOPMENT Cheng Wang, *Division of Neurotoxicology, National Center for Toxicological Research/FDA*

Advances in pediatric and obstetric surgery have resulted in an increase in the duration and complexity of anesthetic procedures. The most frequently used general anesthetic agents have either NMDA receptor blocking or GABA receptor enhancing properties. It has been reported that anesthetic drugs cause widespread and dose-dependent apoptotic neurodegeneration in the developing rat brain. The window of vulnerability for this effect is restricted to the period of rapid synaptogenesis which occurs after birth in the rodent. Because the brain growth spurt period in humans and nonhuman primates extends over a much longer period than in the rodent, it is difficult to match between species the exact stage of development during which a particular developmental event occurs. However, the similarity of the physiology, pharmacology, metabolism and reproductive systems of the nonhuman primate to that of the human, especially during pregnancy, make the monkey an exceptional animal model in assessing potential neurotoxic effects of anesthetic agents.

To understand the biochemical and molecular mechanisms that underlie anesthetic-induced neurotoxicity, particularly at the most sensitive developmental stages, *in vitro* monkey preparations that parallel our *in vivo* developing monkey and rat models have been applied. Our *in vitro* data suggest that ketamine administration results in a dose related increase in neurotoxicity in the developing monkey. Ketamine-induced neuronal cell death in the monkey is probably both apoptotic and necrotic in nature. Ketamine-induced cell death may involve the up-regulation of NMDA receptor NR1 mRNA and protein during development: co-administration of NR1 antisense oligonucleotide specifically prevents synthesis of NMDA receptor NR1 protein and subsequently blocks the neuronal loss induced by ketamine.

In vivo, pregnant rhesus monkeys (gestational day 122), PND 5 and PND-35 monkey infants were infused intravenously with ketamine to maintain a steady anesthetic plane for 3 or 24 hrs followed by a 6-hr withdrawal period. We have demonstrated that the early developmental stages (GD 122 and PND 5) appear to be more sensitive to anesthetic neurotoxicity than later developmental stages (e.g., PND 35) in the monkey. Shorter duration anesthetic exposures (ketamine infusion for 3 hours versus 24 hours) do not produce neuronal cell death in this model.

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CROSS-CULTURAL COMPARISON OF A NEUROBEHAVIORAL TEST BATTERY FOR CHILDREN. DS Rohlman[1], E Villanueva-Uy[2], E Ramos[3], P Mateo[3], D Bielawski[4], L Chiodo[4], VD Black[4], L McCauley[5], EM Ostrea[4], Jr. *[1]Center for Research on Occupational and Environmental Toxicology, Oregon Health & Science University, Portland, OR, USA; [2]University of the Philippines National Institutes of Health, Manila, Philippines; [3]Bulacan Provincial Hospital, Bulacan, Philippines; [4]Department of Pediatrics, Wayne State University School of Medicine, Detroit, Michigan, USA; [5]University of Pennsylvania, Philadelphia, PA, USA*

Neurobehavioral tests are used to assess health effects in exposed working adult populations. The heightened concern over the potential impact of environmental exposures on neurological functioning in children has led to the development of neurobehavioral test batteries for use with children. There is a need for reliable, easy-to-administer batteries to assess neurotoxic exposure in children. One such test battery used with Spanish- and English-speaking children ages 4 and older, combines computerized tests from the Behavioral Assessment and Research System (BARS) with non-computerized tests. The present study extended that battery to a Filipino population. Test instructions were translated into the vernacular, Tagalog or Tagalog-English ("Taglish") and some instructions and materials were modified to be

appropriate for the target populations. The battery was administered to 4 to 6 year old Filipino children (N=50). The performance of the Filipino children was compared to data previously collected from Hispanic and Caucasian children tested in the US. The majority of children had no difficulty completing the tests in the battery with the exception of the Symbol-Digit test and Digit Span Reverse. Younger children had more difficulty completing these tests than older children. The groups had similar performance on the neurobehavioral tests and the older children performed better then the younger children on all of the tests. The findings from this study demonstrate the utility of using this test battery to assess cognitive and motor performance in Filipino children. Tests in the battery assess a range of functions and the measures are sensitive to differences in ages. Keywords: Neurobehavioral, Children, Cross-cultural.

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NEUROPSYCHOLOGICAL EFFECTS OF DENTAL AMALGAM IN CHILDREN: A RANDOMIZED TRIAL. David C. Bellinger, PhD, MSc. *Harvard Medical School, Boston, Massachussetts*

There has been great concern (and controversy) about the possible health effects associated with the use of mercury-containing amalgam to restore dental caries. While observational studies of dental professionals have suggested adverse effects, few data are available with regard to the effects on children, a potentially sensitive subgroup. The study to be reported is one of the first two randomized trials to evaluate such effects. *Context:* Concern persists that inhalation of mercury vapor released by amalgam dental restorations causes adverse health effects. No randomized trials have been published comparing amalgam and alternative restorative materials. *Objective:* To compare the neuropsychological and renal function of children whose dental caries were restored using amalgam or mercury-free materials. *Design:* The New England Children's Amalgam Trial, a two-arm randomized safety trial. *Setting:* Five community-health dental clinics in Boston and one in Farmington, Maine, between September 1997 and March 2005. *Participants:* Children 6 to 10 years old at baseline, with no prior amalgam restorations and two or more posterior teeth with caries. *Intervention:* Restoration of baseline and incident caries during a 5-year follow-up period using either amalgam or composite materials according to randomized assignment. *Main Outcome Measures:* The primary neuropsychological outcome was 5-year change in Full-Scale IQ scores. Secondary outcomes included tests of memory and visual motor ability. Renal glomerular function was measured by creatinine-adjusted albumin in urine. *Results:* Children had a mean of 15 tooth surfaces restored during the 5-year period (range 2-55). Assignment to the amalgam arm was associated with a significantly higher mean urinary mercury level (0.9 vs. 0.6 ?g/gC at year 5, P<0.001). Adjusting for randomization stratum and other covariates, no significant differences were found between children in the amalgam and composite arms in 5-year change in Full-Scale IQ score (3.1 vs. 2.1, P =0.21), 4-year change in General Memory Index (8.1 vs. 7.2, P =0.34), 4-year change in Visual Motor Composite (3.8 vs. 3.7, P =0.93), or year 5 urinary albumin (median 7.5 vs. 7.4, P =0.61). *Conclusion:* No adverse neuropsychological or renal effects were observed over the 5 year period in children whose caries were restored using dental amalgam. Health effects of amalgam restorations in children need not be the basis of treatment decisions when choosing restorative dental materials.

Platform Session

SESSION 10-A: HEALTH EFFECTS OF MANGANESE EXPOSURE: HUMANS AND MODELS - II

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ENHANCED PROINFLAMMATORY CYTOKINE PRODUCTION BY STRIATAL SLICES EXPOSED TO MANGANESE AND LPS *IN VITRO*. NM Filipov, AB Norwood and SC Sistrunk. *Center for Environmental Health Sciences, Department of Basic Sciences, College of Vet. Medicine, Mississippi State University, Mississippi State, MS, USA.*

Proinflammatory cytokines and other inflammatory mediators, when produced in substantial amounts, may be involved in neurodegenerative diseases such as Parkinsons Disease (PD). Microglia, the main producers of proinflammatory cytokines in the brain, are not uniformly distributed and are concentrated in the basal ganglia. The basal ganglia are damaged in PD and are also a target for excessive manganese (Mn) exposure. Mn neurotoxicity resembles many of the features of PD and we have already demonstrated that Mn can enhance the production of lipopolysaccharide (LPS)-induced proinflammatory cytokines such as IL-1□, IL-6, and TNF-□by microglial cells *in vitro*. Hence, the objective of this study was to determine whether Mn, either by itself or in combination with LPS, will have differential effects on proinflammatory cytokine production by slices obtained from the cortex, striatum, and hippocampus of adult male C57BL/6 mice. Acutely isolated slices were exposed for 24 h to Mn and/or LPS and levels of several different proinflammatory cytokines in the culture medium were analyzed by flow cytometry. Basal TNF-□and IL-6 levels were the lowest in the cortical slices. Both Mn and LPS enhanced the production these two cytokines in all slices, but the magnitude of the increases was the greatest in the striatal slices and the lowest in the cortical slices. Importantly, striatal slices exposed to both Mn and LPS, produced substantially more TNF-□and IL-6 than slices exposed only to Mn or LPS. Thus, Mn-caused enhanced production of proinflammatory cytokines may be contributing to the mechanism(s) of Mn neurotoxicity and it appears to be area-specific which is possibly due to the differential distribution of microglia. Supported by NIEHS ES11654.

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MANGANESE EXPOSURE AND EFFECTS OVER THE LIFESPAN : AGE AND GENDER CONSIDERATIONS. Donna Mergler, *CINBIOSE, University of Quebec at Montreal, Quebec, Canada*

Our research group has examined manganese exposure and effects in several different groups, spanning lifetime and gender. In cohorts of pregnant women, children, workers exposed to Mn, communities of men and women whose age ranged from 20-70 years, we have assessed bioindicators of Mn in relation to exposure

sources and neurotoxic effects. This presentation will examine the relation between exposure and effects at each of these stages. We will synthesize the data within a lifespan framework, with emphasis on biomarker differences with respect to age and gender differences and the implications for health and well-being.

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NEUROIMAGING AND NEURODEVELOPMENTAL CORRELATES OF INTRAVENOUS MANGANESE EXPOSURE IN PARENTERALLY-FED INFANTS: A CLINICAL TRIAL IN THE NEONATAL INTENSIVE CARE UNIT (NICU). Judy L Aschner, Heather Furlong, Donna Daily and Michael Aschner. *Department of Pediatrics, Vanderbilt University Medical Center, Nashville, TN, USA.*

Manganese (Mn), an essential metal needed for normal growth and development, can be neurotoxic upon excessive environmental or dietary exposure. Sick infants requiring parenteral nutrition (PN) may be at increased risk of Mn neurotoxicity because neonatal PN solutions contain high concentrations of Mn and PN bypasses the normal intestinal absorptive control and biliary excretory mechanisms for Mn. Furthermore, iron (Fe) deficiency, a common problem among sick neonates, increases Mn brain uptake because Mn and Fe compete for the same carrier transport systems in the central nervous system. We describe an on-going clinical study in the NICU at Vanderbilt Children's Hospital with the **objectives** of identifying neonatal populations at increased risk of excessive brain Mn deposition and altered cognitive and motor development based on their dietary parenteral Mn exposure. Mn neurotoxicity is assessed by longitudinal evaluations of cognitive, neurodevelopmental, and psychophysiological (event-related potential) measures and will be correlated with blood Mn levels measured by ICP-MS and brain deposition of Mn using the technique of magnetic resonance (MR) relaxometry. Our study is designed to test the **hypotheses** that compared with unexposed age-matched controls infants receiving prolonged Mn-supplemented PN will have increased deposition of Mn in their brains and lower scores on neurodevelopmental, cognitive and psychophysiological assessments. The impact of dietary Mn, and especially parenterally delivered dietary Mn, gestational age, Fe status, and hepatic dysfunction on the ability of the neonatal brain to regulate Mn deposition has not been scientifically addressed. This clinical investigation may shed light on the development and progression of neurological dysfunction in infants on prolonged PN and provide a basis for sound and evidence-based recommendations for appropriate Mn supplementation and monitoring of infants receiving PN. Keywords: Mn Neurotoxicity, Parenteral Nutrition, Neonatal Clinical Trial.

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HIGH SIGNAL INTENSITIES ON T1-WEIGHTED MRI IN THE SPECTRUM OF MANGANESE SYMPTOMATOLOGY. J Park[1)] and Y Kim[2)]. [1)]Occupational Safety and Health Research Institute, Korea Occupational Safety and Health Agency, Incheon, South Korea. [2)]Department of Occupational and Environmental Medicine) Ulsan University Hospital, University of Ulsan College of Medicine, Ulsan, South Korea.

Bilateral symmetrical increase in signal intensities, confined to the globus pallidus and midbrain observed on T1-weighted magnetic resonance images (MRI), but with no alteration on the T2 weighted image were observed in the experimental manganese (Mn) poisoning of the non-human primate and a patient with Mn intoxication. Mn concentration highly correlated with pallidal index (PI) in Mn-exposed workers and liver cirrhotics. Moreover, PI significantly affected correct score of pursuit aiming tests and finger tapping of the dominant hand after control of age and education among neurobehavioral performances. Thus, T1 signal on MRI may reflect target organ dose of occupational Mn exposure in the spectrum of Mn symptomatology. The increased signals in MRI were highly prevalent (41.6%) in asymptomatic Mn-exposed workers. These changes in MRI disappeared following the withdrawal from the source of Mn accumulation. Thus increased signal intensities do not necessarily reflect manganism. In conclusion, MRI T1-signal reflects target organ dose in the spectrum of Mn symptomatology. At which increase of signal intensity, the progression to overt manganism through preclinical neurobehavioral impairments from Mn exposure occurs, however, remains to be solved.

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UTILIZATION OF BEHAVIORAL TESTING, ATOMIC ABSORPTION SPECTROSCOPY (AAS) AND ISOPROSTANE ANALYSIS TO DETERMINE THE EFFECTS OF BRIAN MANGANESE (Mn) ACCUMULATION IN MALE C57BL/6J MICE. LA Miyatake[1], V Fitsanakis[1], D Milatovic[1], J Anderson[2], KM Erikson[2], M McDonald[3], and M Aschner[1, 4, 5]. [1]*Department of Pediatrics, Vanderbilt University School of Medicine, Nashville, TN.* [2]*Department of Nutrition, University of North Carolina – Greensboro, Greensboro North Carolina.* [3]*Department of Pharmacology, Vanderbilt University, Nashville, TN.* [4]*Department of Pharmacology and the* [5]*Kennedy Center, Vanderbilt University Medical Center, Nashville, TN*

Manganese (Mn) is an important and essential metal required by both animals and humans. Both Mn in excess and deficiency can lead to serious health problems. Manganism is a disease associated with elevated levels of Mn in the brain. It progresses from a psychological to a physically debilitating disease. In this study, male C57BL/6J mice received food with either normal (10mg Mn/kg) or high (100mg Mn/kg) Mn levels for a total of 22 weeks. Animals were weighed weekly as a measure of general health. During the last eight weeks of the study, the mice underwent a battery of behavioral experiments, including tests for anxiety, learning and memory, motor and sensorimotor abilities. At the end of the study, blood was collected and the brains were removed. One hemisphere was used to determine levels of oxidative stress as measured by isoprostanes and neuroprostanes levels. The second hemisphere was dissected into cerebellum, brain stem, midbrain, hippocampus, striatum or cortex to determine Mn accumulation via atomic absorption spectroscopy. AAS results indicate a statistically significant increase in striatal Mn ($p = 0.014$), but not Fe, levels in the absence of increased isoprostane or neuroprostane levels in each hemisphere. Interestingly, the elevated plus maze

test suggests that treated animals may be more anxious than control animals. This data is useful in characterizing the relationship between brain Mn accumulation and potential behavioral correlates.

Symposium

SESSION 10-B. THE SEYCHELLES CHILD DEVELOPMENT STUDY OF METHYLMERCURY EXPOSURE FROM FISH CONSUMPTION: NEW RESULTS AND CONCLUSIONS

Session Chair: Christopher Cox, PhD

Theme: *The Seychelles Child Development Study (SCDS) was designed to examine the relationship between exposure to prenatal MeHg from maternal fish consumption and the children's neurodevelopment. Three cohorts have been evaluated over nearly 20 years and the study is continuing to explore the relationships between MeHg exposure, fish consumption, nutrients and children's development.*

This session is sponsored by National Institute of Environmental Health Sciences/NIH

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OVERVIEW OF CHILD DEVELOPMENT STUDIES IN SEYCHELLES, PAST, PRESENT AND PLANNED. Conrad F Shamlaye, MD, MPH, M Econ. *Ministry of Health, Republic of Seychelles*

The Seychelles Child Development Study (SCDS) was designed to examine the relationship between exposure to prenatal MeHg from maternal fish consumption and the children's neurodevelopment. Three cohorts have been evaluated over nearly 20 years and the study is continuing to explore the relationships between MeHg exposure, fish consumption, nutrients and children's development. An overview of the study conducted to date, the studies currently ongoing and future plans will be presented. In addition, some of the underlying epidemiological principles that we have adhered to will be presented along with the reasons we feel they are important.

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BIOMARKERS FOR METHYL MERCURY. Thomas W Clarkson, PhD, Elsa Cernichiari, MS, Grazyna Zareba, PhD and Gary J. Myers, MD. *University of Rochester Medical Center, Rochester, NY*

Several biological media have been used as indicators of the fetal body burden of methyl mercury and the levels in the primary target tissue, the developing brain. These media include maternal hair and blood, cord blood and placental tissue. The relative merits of each media will be considered both with regard to current knowledge of the physiology of mercury disposition in the body and also the practicality of field application with respect to sample collection, transport, storage and processing.

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POTENTIAL BENEFITS OF MATERNAL FISH CONSUMPTION TO FETAL AND CHILD DEVELOPMENT. .J.J. Strain, PhD. *Ulster University, Northern Ireland, UK*

Fish consumption is associated with both the exposure to MeHg and the intake of nutrients essential in brain development. The interaction of nutrients and toxic agents is complex and important in understanding the toxicity. The SCDS has been investigating this relationship in a new cohort over the past five years. This presentation will focus on the nutrients that were selected for evaluation during this study and describe the reasons for their selection. The nutrients include the long chain polyunsaturated fatty acids (LCPUFA), docosahexanoic and arachadonic acid, iodine, iron, and choline. Both of the LCPUFA appear to affect neurodevelopnent in the latter stages of fetal growth but only DHA is strongly associated with fish consumption. Iodine, iron and choline are found in fish and other foods and are less strongly associated with fish consumption. Evidence from human studies indicate that the effects of iodine on neurodevelopment are mainly prenatal and those of iron largely postnatal. In rodents, choline has effects on neurodevelopment at both prenatal and postnatal stages. The status of two other trace elements, copper and zinc - known to be important in neurodevelopment and accumulate in seafoods - are difficult to measure in humans. A further trace element found in relatively high amounts in fish is selenium, which appears to attenuate the toxic effects of MeHg but has no known direct effect on neurodevelopment.

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FINDINGS FROM THE SEYCHELLES CHILD DEVELOPMENT AND NUTRITION STUDY. Philip W. Davidson, PhD, Gary J. Myers, MD, JJ Strain, PhD, Sally W. Thurston, Thomas W. Clarkson, PhD ~ *University of Rochester Medical Center, Rochester, NY*

We tested the hypothesis that brain selective nutrients resulting from fish consumption during pregnancy foster neurodevelopment to a point that equals or exceeds any impairment attributable to methylmercury. We measured prenatal methylmercury exposure and maternal nutrition in 229 mother-infant pairs and evaluated the children's development at four ages through 30 months. Our multiple regression analyses allowed an examination of the effects on child development of both prenatal MeHg exposure and each of the nutrients after adjustment for standard covariates. The results showed that the benefit of fish consumption during pregnancy may outweigh any hypothetical adverse effects of MeHg on neurocognitive outcomes in offspring. These results suggest that assessing the overall diet is critical when determining the risks and benefits of fish consumption.

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POSTNATAL EXPOSURE ISSUES. Gary J. Myers, MD, Sally W. Thurston, PhD, Alexander T Pearson, MA, and Thomas W. Clarkson, PhD. *University of Rochester Medical Center, Rochester, NY*

Fish consumption and exposure to MeHg begins as early as 1 year of age in Seychelles. The brain continues rapid development following birth and is still sensitive to

MeHg exposure. However, the level of exposure needed to adversely affect the postnatal brain is not known. One difficulty in investigating postnatal exposure has been determining a metric to adequately measure it. In the Seychelles Child Development Study, we determined postnatal childhood exposure from hair as convenience samples at 6, 19, 66, and 107 months. We will present two different metrics and their relationship to the children's IQ measured at their 9 year evaluation using covariate-adjusted linear regression. The first metric categorized exposures as high versus low since peak exposure can be critical. We used the mean exposure of 6 ppm measured at both 19 and 66 months of age for the cohort as the cutoff. The second metric examined cumulative exposure or the area under the curve since Hg entering the brain persists and may be damaging. We used the exposure between 6 and 66 months of age to determine this. We examined these metrics in relationship to the children's IQ at the 9 year evaluation.

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METHYLMERCURY AND BLOOD PRESSURE. Sally Thurston, PhD, Gary J. Myers, MD, Pascal Bovet, MD. *University of Rochester Medical Center, Rochester, NY*

Hypertension is a major global health problem. Prenatal exposure to organic methylmercury (MeHg) from fish consumption has been proposed as one cause. A study from the Faroe Islands reported a direct association between BP at age 7 years and prenatal MeHg exposure. As cord blood mercury levels increased both diastolic and systolic BP increased. We examined this relationship in the Seychelles Islands to determine if this effect was consistent across studies. We studied the Seychelles Child Development Study (SCDS) main cohort that includes 789 children with known prenatal MeHg exposure. The children's BP was measured at ages 12 and 15 years. We analyzed the association between prenatal MeHg exposure and BP using linear regression and additive models. Blood pressure and key covariates were available on 644 subjects at 12 years, 559 subjects at 15 years, and 524 children at both ages. The findings and interpretation of these analyses will be discussed.

Panel Discussion

Topic:

"The Seychelles Child Development Study and Epidemiology of Methylmercury Exposure from Fish Consumption"

Panelist Discussants: Session Speakers plus:

Representatives from governmental agencies:

Michael Bolger, PhD ~ FDA
Christopher DeRosa, PhD ~ ATSDR
Kevin Teichman, PhD ~ EPA
Annette Kirshner, PhD ~ NIEHS

Symposium

SESSION 11-A: THE FETAL BRAIN ON ALCOHOL

Session Chair: Cynthia (Cindy) J.M. Kane

Theme: *Prenatal ethanol exposure is the leading known cause of mental retardation in the Western World. In the U.S. the incidence of Fetal Alcohol Spectrum Disorders (FASD) is estimated at 1-10 per 1000 births and costs $5.4 billion annually. We will discuss the most recent advances in understanding of ethanol pathogenesis in the developing brain including: inhibition of neural stem cell proliferation, the role of NMDA receptor in cortical damage, and differential genetic vulnerability to ethanol teratogenesis. In addition, emerging knowledge of the role of glia, astrocytes and microglia, as direct targets of ethanol toxicity will provide an intriguing conclusion to the discussion.*

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LATENT EFFECTS OF FETAL ALCOHOL EXPOSURE ON NEURAL STEMCELLS: BRAINSTEM NEURONS. MW Miller. *Department of Neuroscience and Physiology. SUNY- Upstate Medical University, Syracuse NY 13210 USA.*

One contributor to fetal alcohol syndrome (FAS) associated microencephalyis a targeted effect of ethanol on proliferating neural stem cells (NSCs). NSCsappear with the formation of the neuroectoderm at gastrulation and continue todivide for weeks or months until the time of cell definition, i.e., when neuronalprecursors pass through their final mitotic division. Timing of ethanol exposure iscritical for the outcome. Ethanol can alter cell proliferation during the period ofneuronogenesis for a particular structure. The present study tests the hypothesis thatexposure to ethanol during gastrulation can affect proliferation at later stages in braindevelopment. This notion is supported by evidence that craniofacial dysmorphiastypical of FAS are caused by exposures at gastrulation and that skull developmentis driven by brain growth. Rodents and non-humans primates were exposed toethanol at the time of gastrulation or during neuronogenesis, and the numbers ofneurons in cranial nerve nuclei of the mature offspring were determinedstereologically. The trigeminal nuclei were affected by exposure during gastrulationor the period of neuronal generation, whereas other nuclei were affected, but onlywhen the exposure occurred during neuronogenesis. Thus, ethanol exposure atgastrulation can affect NSC proliferation, changes that remain latent until neuronalprecursors pass through their final mitotic division. Keywords: Fetal Alcohol, Neurogenesis, Stem Cells.

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THE RELATIONSHIP OF CORTICAL DEVELOPMENT TO PRENATAL ALCOHOL EXPOSURE AND NMDA-NR1 RECEPTORS: NEW MECHANISMS FOR TOXIC EFFECTS OF ETHANOL. Andrea J. Elberger. *Department of Anatomy and Neurobiology, The University of Tennessee Health Science Center, Memphis, TN, USA*

Prenatal alcohol exposure is a major public heath issue worldwide. Specific mechanisms of ethanol toxicity have been studied in mice with deletion of the NMDA-NR1 (NR1) receptor gene. Cortical development was altered in mice with and without prenatal ethanol (EtOH) exposure. Littermates with total deletion (NR1-/-), 50% deletion (NR1+/-), and no deletion (NR1+/+) show that a range of prenatal alcohol exposure effects are blocked in NR1-/- mice and attenuated in NR1+/- mice. NR1+/+ mice and outbred rats show similar EtOH effects as rats in neocortex including altered developing corpus callosum projection neurons. Possible mechanisms by which EtOH toxicity is modified by NR1 receptors have been further studied through the relationship of NR1 with the endocannabinoid (eCB) pathway and its receptors CB1 and CB2. Morphological studies show that CB1, CB2 and NR1 receptors regionally colocalize in brain in NR1+/+ and NR1+/-, although the distribution of all three receptors is reduced in NR1+/-. Following a single dose of EtOH, NR1+/- mice fail to show the increased brain and blood levels of eCBs that NR1+/+ show. In NR1+/+ the blood levels of lipid precursors for eCBs are gradually reduced by increasing EtOH levels. Overall, these results support the possibility that the change in eCB levels is a physiologically significant consequence of EtOH exposure and is potentially responsible for toxicity at all ages. Supported by NIH grants AA12163, AA13516.

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THE INFLUENCE OF GENETICS IN ETHANOL'S TERATOGENIC ACTIONS. K.M. Hamre and X.P. Du. *Dept. of Anat. & Neurobiol., Univ. of Tennessee Health Science Center, Memphis, TN, USA.*

In both human populations and in animal studies, the genetics of the organism has been shown to affect the severity of ethanol's teratogenic actions. However, the cellular and molecular mechanism(s) behind this differential sensitivity remain unknown. We are using several mouse strains or lines to examine the effects of genetics. Initially, the sensitivity of the various strains was ascertained by examining the effects of ethanol on cell death within the developing CNS. Cell death was determined using a number of markers including TUNEL staining to label dying cells as well as the presence or absence of DNA laddering in tissue samples. Additionally, multiple doses of ethanol were examined to determine the dose at which significant cell death is first detected. From this, both differences in the level of cell death at equivalent doses as well as the dose at which significant levels of cell death are observed to differ among strains. Two strains, C57BL/6J (B6) and ICR were found to be highly sensitive showing the highest level of cell death as well as being sensitive at the lowest dose. Two other strains, DBA/2J (D2) and short-sleep, were shown to be insensitive to ethanol's teratogenic actions while a fifth line, Long-sleep, was found to be intermediate. A second experiment used microarray analyses with real-time qPCR validation to examine changes in gene expression following ethanol exposure. In these analyses, one insensitive strain and one sensitive strain were examined, B6 and D2 respectively. Ethanol-exposed embryos were compared both to maltose-dextrin controls of the same strain, as well as to both groups of the other strain. In the sensitive B6 embryos, a large number of changes in gene expression were detected following ethanol exposure while in the insensitive D2 embryos only a small number of genes changed expression after ethanol exposure. However, the most differences in gene expression were observed between the two strains irrespective of treatment condition. This suggests that it is the baseline differential expression that is responsible for the significantly different responses of the two strains to ethanol exposure. Thus, the differential susceptibility to ethanol's teratogenic actions in embryos of differing genetic backgrounds was mediated, at least in part, by differential responses at the cellular and molecular levels. Support: UTHSC Genomics & Bioinform. COE & Neurosci. Institute. Keywords: Fetal Alcohol, Genetics, Cell Death.

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THE CHOLINERGIC SYSTEM IN ASTROCYTES IS A TARGET FOR ETHANOL. M Guizzetti, G Giordano, L G Costa. *Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA, USA*

Astrocytes modulate many neuronal functions including dendrite and axon outgrowth, synaptogenesis and neuronal fate. Astrocytes express several subtypes of muscarinic receptors which have been hypothesized to have a morphogenic role during brain development. We have shown that activation of M3 muscarinic receptors in astrocytes induces cell proliferation that it is strongly inhibited by ethanol through the inhibition of the carbachol-activated PLD $\rightarrow$ PA $\rightarrow$ PKC $\rightarrow$ p70S6K $\rightarrow$ NF-κB pathway, suggesting that muscarinic receptor signaling may be a target of ethanol in astrocytes. We recently found that muscarinic stimulation of astrocytes also induces neurite outgrowth in hippocampal neurons co-cultured with astrocytes. Hippocampal neurons grown on top of a monolayer of astrocytes previously treated with carbachol increased their neurite length measured by a spectrophotometric method; this effect was mediated by muscarinic receptors. Furthermore, co-incubation of ethanol (25-100 mM) and carbachol resulted in a concentration-dependent decrease in the effect of carbachol. The effect of ethanol was confirmed by morphometric analysis of β-tubulin-stained neurons observed at fluorescence microscope. Carbachol-treated astrocytes dramatically increased the length of the axon and of the minor neurites and the total number of neurites per cell. Neurons grown in the absence of astrocytes, but in the presence of conditioned medium from carbachol-treated astrocytes, displayed a longer axon, suggesting that the effect of carbachol-stimulated astrocytes on neuronal differentiation is mediated in part by soluble factors secreted by astrocytes and in part by membrane factors or extracellular matrix proteins. In conclusion, our findings indicate that muscarinic receptor activation in astrocytes plays important roles during brain development, and that inhibition of astrocytic muscarinic receptor signaling by ethanol is an important mechanism involved in its developmental neurotoxicity. (Supported in part by AA08154 and P30ES07033). Keywords: Astrocytes, Ethanol, Muscarinic Receptors.

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DEVELOPMENTAL INTERACTIONS BETWEEN MICROGLIA AND NEURONS ARE DISRUPTED BY ETHANOL. Cynthia J.M. Kane. *Department of Neurobiology and Developmental Sciences, University of Arkansas for Medical Sciences, Little Rock AR 72205*

Not surprisingly, neurons have been the focus for investigation of ethanol neurotoxicity for several decades. However, there is more to ethanol pathogenesis during brain development than just neurons. We have discovered that ethanol has direct detrimental effects on microglia, the monocyte-derived cells that represent the innate immune system in the brain. Ethanol exposure during the neonatal rat equivalent of the third trimester of human gestation produces >35% loss of microglia in the cerebellar vermis. Microglial loss appears to be due to both inhibition of proliferation and induction of apoptosis. The microglia that survive ethanol exposure exhibit marked pathology with broad, short cytoplasmic extensions and enlarged soma. This phenotypic change does not correlate with the characteristic, neurotoxic activation of microglia; activation markers and neuroinflammation are absent. In contrast, our data suggest that inhibition of microglial development and function increases neuronal vulnerability to ethanol by disrupting protective interactions between microglia and neurons. Analysis of the molecular mechanisms of ethanol action in microglia has revealed disturbance of specific signaling pathways. Further study reveals that pharmacologic manipulation of these molecular targets holds potential for reducing ethanol toxicity. Using the *in vivo* model, we have found that a pharmacologic agonist of the peroxisome proliferator activated receptor gamma can partially protect both neurons and microglia from ethanol toxicity *in vivo*. Thus, recent identification of microglia as targets of ethanol pathogenesis in the developing brain provides insight into ethanol disruption of neuronal-microglial interactions that may augment intervention in the neurotoxicity of Fetal Alcohol Spectrum Disorders. Supported by NIH grants AA014645 and AA014888.

Platform Session

SESSION 11-B: MERCURY TOXICITY, DITHIOCARBAMATES AND OXIDATIVE STRESS

Session Chair: W. M. Valentine, PhD
Co-Chair: Nick VC Ralston, PhD

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DITHIOCARBAMATE-MEDIATED OXIDATIVE STRESS IN PERIPHERAL NERVE. OM Viquez, HL Valentine, DM Milatovic and WM Valentine. *Departments of Pathology and Pediatrics, Vanderbilt University Medical Center, Nashville, TN, USA*

Previous studies have demonstrated the ability of certain dithiocarbamates to produce myelin injury and copper accumulation in peripheral nerve with correlative associations between the level of copper and severity of lesions observed. This suggests that disruption of copper homeostasis may contribute to dithiocarbamate-mediated peripheral neuropathy possibly through the accumulation of either free copper or a redox active copper complex that promotes oxidative stress. In the present study, an established model for dithiocarbamate induced myelinopathy using parenteral administration of N,N-diethyldithiocarbamate (DEDC) in rats was used to examine the potential of dithiocarbamates to enhance oxidative stress in peripheral nerve. Following administration of DEDC, peripheral nerves were collected and F_2 isoprostanes and protein carbonyl levels determined by GC/MS and fluoresceinamine immunoassay, respectfully. Protein expression levels were also examined using 2D DIGE and immunohistochemistry to further characterize the cellular response in nerve produced by DEDC. Significant increases in lipid peroxidation, protein carbonyl content and expression of three isoforms of glutathione transferase were observed in the nerves obtained from DEDC exposed rats relative to controls. Additionally, immunostaining for glutathione transferase pi localized the elevated expression of this enzyme to the cytoplasm of Schwann cells within the DEDC exposed nerves. These results support the ability of DEDC to enhance oxidative stress within peripheral nerve. Additional studies are required to determine whether the oxidative stress is a specific copper-mediated contributing event in dithiocarbamate myelinopathy or results from nonspecific processes associated with demyelinating injury in general.

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SELENIUM'S PROTECTIVE EFFECT AGAINST MERCURY TOXICITY NVC Ralston and LJ Raymond. *Energy & Environmental Research Center, University of North Dakota, Grand Forks, North Dakota, USA*

Selenium (Se) is a nutritionally essential element required for synthesis of selenocysteine, the active component of a number of homeostatically conserved proteins in brain and neuroendocrine tissues. Selenium is known to protect against mercury (Hg) toxicity, apparently because supplemental Se can offset losses to intracellular (HgSe) formation and thus overcome the inhibitory effects excessive methylmercury (MeHg) normally has on Se-dependent enzyme activities. Maternal MeHg exposure from ocean fish consumption has not been associated with harm in studies of child development in the Seychelle Islands, but studies in the Faroe Islands indicate maternal MeHg exposure from eating pilot whale meat is harmful. The differences in observed results of these studies may be due to distinctions in the molar ratios of Hg and Se in the diet. Ocean fish tend to be rich in Se, typically having 5 to 20 moles of Se for every mole of Hg. Meanwhile, meats of predatory whales such as the pilot whales eaten in the Faroe Islands are virtually unique in having ~4 moles of Hg for every mole of Se. Results of our present studies support the hypothesis that disproportionately high molar ratios of Hg:Se are hazardous, while the same amounts of Hg are not harmful when Se is abundant. Because of the rich amounts of Se they contain, ocean fish appear more likely to prevent Hg toxicity than to cause it. Keywords: Mercury, Toxicity, Selenium.

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SELENIUM PREVENTS AND REVERSES METHYLMERCURY TOXICITY. LJ Raymond and NVC Ralston. *Energy & Environmental Research Center, University of North Dakota, Grand Forks, North Dakota, USA*

Reports on episodes from poisoning incidents confirm that the developing brain is susceptible to methylmercury (MeHg) toxicity. This has provoked speculation that neurodevelopmental harm to children may occur as a result of maternal exposure to MeHg from seafood consumption. However, it is important to recognize that selenium (Se), an important nutrient present in many foods including fish, has a potent "protective effect" against Hg toxicity. Due to its exceptionally high binding affinity for Hg it has been thought that Se's protective effect against MeHg toxicity was the result of Se sequestration of Hg. Instead, it appears that Hg toxicity is the result of Hg's ability to sequester intracellular inorganic Se and inhibit the formation of selenoenzymes that are vital to the health of the central nervous system. Therefore, MeHg toxicity will not occur when supplemental dietary Se extends this threshold. We conducted a series of studies to investigate the influence of Se status on sensitivity to MeHg toxicity. Rats were fed Se deficient, adequate, or rich diets supplemented with either no, low, or high levels of MeHg. Neurofunctional and biochemical endpoints of MeHg toxicity were monitored. Signs of toxicity were only evident in animals fed Se deficient diets with high concentrations of MeHg. Additionally, reversal of Hg toxicity symptoms was evident in rats that were switched to Se rich diets. Keywords: Mercury, Toxicity, Selenium.

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EVIDENCE OF IMPAIRED MERCURY EFFLUX IN AUTISM. Dan Rossignol *Department of Family Medicine, University of Virginia, Charlottesville, VA.*

Autism is a neurodevelopmental disorder currently affecting as many as 1 out of 166 children in the United States. Over the last 15 years, the prevalence of autism has increased more than 10-fold, which strongly suggests an environmental trigger. The search for such a trigger has led to the observation that autistic individuals might not properly excrete mercury due to certain underlying genetic susceptibilities. The amount of mercury burden in the environment has increased three-fold since 1900 and 1 in 6 children born today is predicted to have blood levels of mercury high enough to impair neurological development. The prevalence of autism has been correlated with increasing environmental mercury exposure. The symptoms of mercury poisoning can mimic autism and a genetic susceptibility to mercury toxicity causes an autism-like illness in animals exposed to ethylmercury. Several recent studies reveal that a higher mercury exposure and burden exist in autistic children when compared to controls. Autistic children excrete less mercury in hair than typical children and the severity of autism is inversely correlated with the amount excreted. Children with autism also excrete more mercury in the urine upon challenge with a chelator when compared to control children and have more precursors of heme present in the urine, which might be indicative of mercury toxicity. Previous mercury poisoning events, including acrodynia, suggest an underlying genetic susceptibility to mercury toxicity which might occur in approximately 1% of the general population and could explain the wide variation in the half-life of mercury in human blood, which ranges from 37 days to as high as 250 days. Genetically determined abnormalities, including low glutathione and cysteine levels, impaired sulphation, decreased metallothionein function, and polymorphisms in metal transporter genes, suggest a decreased ability to efflux mercury and an increased susceptibility to mercury toxicity in autistic individuals. Autism afflicts 4 times as many males as females. Estrogen is protective while testosterone increases the toxicity of mercury and elevated prenatal testosterone levels might predispose an individual to develop autism. Males also have lower glutathione levels and normally excrete less mercury than females, and a mercury poisoning event demonstrated an increase in male fetal deaths compared to females. Finally, reports indicate that chelation of lead and mercury might ameliorate symptoms in some autistic children. Keywords: Autism, Mercury, Glutathione.

Plenary Session

SESSION 12. NEUROTOXICITY IN DEVELOPMENT AND AGING: TRANSLATIONAL RESEARCH

Session Chair: Evelyn Tiffany-Castiglioni, PhD
Co-Chair: Richard M. LoPachin, PhD

Theme. *This session will address how research on mechanisms can translate into improved public health. The spectrum of biological complexity will be examined in a broad overview, followed by specific presentations on various reductionist systems and how these may have potential for screening, risk assessment and development of prophylactic or therapeutic strategies. The large gap between basic bench science, informed by epidemiologic studies that identify toxicologic hazards, and clinically useful outcomes will be addressed in a roundtable discussion by panelists whose expertise ranges from genetics mechanism, to cells, tissues, organ systems, organisms, and populations.*

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TRANSLATIONAL MEDICINE AND THE NIEHS STRATEGIC PLAN. Annette Kirshner, PhD. *NIEHS/NIH, Research Triangle Park, North Carolina.*

Recent technological advances and a growing appreciation that environmental factors contribute to most complex diseases provide unprecedented opportunities for developing new research paradigms that bring together interdisciplinary teams of scientists to move basic environmental health sciences research into clinical and public health practice. In support of this, the NIEHS has been fostering and will continue to foster scientific collaborations between clinical and basic investigators to accelerate the application of basic research results into the clinical setting to improve human health in those areas where environmental factors are known or expected to influence the development or progression of human disease. This support is evident from the NIEHS Strategic Plan in which the first three of its seven goals relate to and encourages research that emphasizes the study of environmental exposures to inform clinical research.
Traditionally, environmental impacts on disease have been studied from either the perspective of the exposure or the perspective of the disease. NIEHS' goal now is to address environmental disease from a more integrated perspective

enabling us to gain a greater understanding of human disease by strengthening the evidence that a given exposure is toxic, determining how specific environmental exposures affect disease etiology and progression, and using environmental exposures to identify molecular targets to determine susceptibility and intervention. This presentation will address those goals of the NIEHS Strategic Plan that relate to this integrative approach to research.

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ARE AGE-RELATED NEURODEGENERATIVE DISEASES MEDIATED BY THIOL ADDUCT FORMATION IN NERVE TERMINALS? Richard M. LoPachin, PhD. *Albert Einstein College of Medicine, Bronx, New York.*

This talk will present a toxicologic approach to understanding possible links between human exposures to Type-2 conjugated alkenes such as acrylamide (ACR) and neurologic disease. Humans are exposed on a daily basis to conjugated alkenes in air and food. The interaction of an alkene electrophile with a sulfhydryl group can impair the function of proteins in the nerve terminal, which could disrupt brain activity. Our research suggests that this early nerve terminal injury is caused by the endogenous production of conjugated alkenes. Based on the possible neurotoxic consequences of environmental exposure which will be discussed in this talk, Alzheimer's disease and other neurodegenerative conditions might develop when nerve cells are exposed to both internal and external (environmental) sources of Type-2 conjugated alkenes. Furthermore, ACR and other conjugated alkenes produce cumulative neurotoxicity. Therefore, low-level, lifetime exposure to these chemicals and the resulting subtle neuronal damage might be involved in the "normal" aging process.

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NEUROINFLAMMATION AND NEURODEGENERATIVE CONDITIONS. Sue Griffin, *Dept. of Geriatrics, UAMS; Geriatric Research Education Clinical Center, VAMC, Little Rock, AR, 72212*

The central hypothesis of our work is that brain-derived proinflammatory cytokines such as IL-1 and S100B promote, through a variety of mechanisms, incessant neuronal compromise and death. These, together with predisposing genetic factors, are the basis for the progressive nature of Alzheimer's disease (AD) and related disorders, as well as the neurodegenerative consequences of neural insults. A corollary of this hypothesis is that a variety of insults to the brain—direct or indirect, genetic, or simple wear and tear of time—engenders neuroinflammatory processes and promotes neurodegenerative cascades. We seek to elucidate specific mechanisms underlying these neuroinflammatory cytokine-driven neurodegenerative cascades, including specific signal transduction pathways, in human conditions, in animal models and in cell and slice cultures. Toward this focus, we have studied the impact of ApoE and IL-1 genotype on glial–neuronal interactions *i*) in conditions that confer risk for development of AD (e.g. head injury and neurotoxicants), favor precocious development of AD-like pathology (e.g. epilepsy), or that may predispose to, or be associated with, further development of neurofibrillary changes and neuritic plaques (e.g. Parkinson's and AD with Lewy bodies); *ii*) in animal models; and *iii*) in neural cell culture systems. In this way we have directly identified basic mechanisms, which are critical to a more complete understanding of neuroinflammatory processes that give rise to neuropathogenic cascades, as well as potential or putative therapeutic agents. Supported in part by AG12411 and HD37989.

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IS POLYCHLORINATED BIPHENYL EXPOSURE A RISK FACTOR FOR PARKINSON'S DISEASE? Lisa A. Opanashuk; *Department of Environmental Medicine, University of Rochester, Rochester, NY.*

A potential link between polychlorinated biphenyl (PCB) exposure and neurodegeneration was suggested in a previous study that reported elevated PCB levels in the brains of Parkinson's disease (PD) patients. Although PCB exposure has been associated with abnormal locomotor function, the molecular mechanism of neurotoxicity remains unclear. Dopaminergic (DAergic) pathways regulate motor behavior and are putative targets for PCB-mediated damage. Our studies tested the hypothesis that PCB accumulation leads to oxidative stress, which produces DAergic neuronal injury and disrupts motor function following Aroclor 1254 (A1254) exposure. Adult male mice were exposed to 0-25 ppm A1254 for 28 days, and then given two weeks to recover. Locomotor activity was assessed after 2 and 4 weeks of PCB exposure, then following the recovery period. PCB exposure was associated with increased motor activity levels, which persisted throughout the recovery period. Results showed that neuronal cell loss was evident in both the substantia nigra and ventral tegmental areas of the midbrain. Moreover, there was reduced expression of tyrosine hydroxylase and dopamine transporter in the striatum following PCB exposure. These observations were accompanied by elevated expression of oxidative stress related proteins (MnSOD, CuZnSOD, HO-1) and increases in oxidatively modified lipids and proteins in the striatum. Additionally, atomic absorption spectroscopy analyses revealed that Fe levels were elevated in the striatum and midbrain. *In vitro* studies demonstrated that A1254-induced DAergic cell injury was mediated by reactive oxygen species production and Fe. PCB exposure was also shown to activate a signaling cascade that included increased HO-1 catalytic activity. Whereas over-expression of HO-1 predisposed MN9D DAergic cells to enhanced ROS production and cell death in response to A1254 exposure, antisense inhibition of HO-1 expression prevented PCB-induce oxidative cell injury. These observations support the possibility that HO-1 protein induction and concurrent increases in enzyme activity are sources of Fe and ROS that lead to DAergic cell injury and abnormal motor function following PCB exposure. Interestingly, both HO-1 and Fe have been connected to neurodegenerative disorders such as PD. Therefore, understanding the role of HO and Fe in PCB induced DAergic neuronal injury could have global implications for the pathophysiology of Parkinsonism. Supported by NIH ES00375, ES01247, and ES07026.

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USE OF CA^{2+} MODELING AS A PREDICTIVE MEASURE OF NEUROTOXICITY. R Barhoumi and E Tiffany-Castiglioni. *Department of Integrative Biosciences, College of Veterinary Medicine, Texas A&M University, College Station, Tx, USA*

Calcium, a second messenger, controls a wide range of cellular functions such as contraction, neurotransmitter and hormone release, metabolism, cell division and differentiation. Because calcium literally controls life and death and is involved in so many aspects of cellular function, its role in signaling is very complex and the benefits would be enormous if we could identify the individual pathways targeted during toxicant exposure, developmental neurotoxicity or even disease progression. If such individual pathways are identified, they could be used as predictive measure of toxicity as well as for development of new methods for prevention or reversal of injury. The purpose of this study was to begin developing an in vitro Ca^{2+} model that would be suitable for early toxicity screening of potentially neurotoxic drugs using non invasive imaging tools. The effects of propofol, a commonly used short-acting general anesthetic, upon cell growth and Ca^{2+} signaling in a human astrocytic cell line (CCF-STTG1) were examined. Exposure of cells for 1 to 3 days to different concentrations of propofol resulted in a dose dependent decrease in cell number with an inhibitory concentration of cell growth (IC50) of approximately 31.7 ± 1.2 µM. To evaluate the changes in intracellular Ca^{2+} induced by propofol, cytoplasmic and mitochondrial Ca^{2+} were measured with Fluo-4 and Rhod-2, respectively. Mitochondrial Ca^{2+} increased significantly at a propofol concentration lower than the IC50 (10 µM for 24h, 1 µM for 72h), while cytoplasmic Ca^{2+}decreased significantly at the same concentration. In addition, propofol diminished the Ca^{2+} response induced by direct addition of 10% fetal bovine serum (FBS) to propofol-treated cells. To determine the source of the Ca^{2+} alterations induced by propofol, several pharmacologic agents that target intracellular Ca^{2+} homeostasis mechanisms were used. Glyburide (a K^+-ATP channel blocker) used for 1 hr directly before FBS addition restored the FBS response in propofol treated cells to the control cell response. However, estrogen, known to activate K^+ channels, decreased the FBS response of control cells to a level similar to that of propofol-treated cells. Diazoxide (a selective mitochondria K^+-ATP channel activator) increased mitochondrial Ca^{2+} in control cells to a level comparable to propofol treated cells, suggesting the activation of these channels by propofol treatment. Addition of 1 µM RU-360 (a selective and potent mitochondrial calcium uniporter blocker) for 30 min prior to propofol treatment had no significant effect on control cells but decreased the mitochondrial Ca^{2+} level in propofol-treated cells to control levels. These data suggest that mitochondrial Ca^{2+} and ATP-dependent potassium channels may be targets of propofol action in CCF cells and reversal of these actions may occur using K^+-ATP channels and mitochondrial calcium uniporter blocker.

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WHAT IS THAT IN RAT DAYS? A WEB BASED APPROACH TO THE TRANSLATION OF NEURODEVELOPMENTAL TIME ACROSS MAMMALIAN SPECIES Barbara Clancy, PhD. *University of Central Arkansas, and University of Arkansas for Medical Sciences*

A central goal of translational medicine is to find a comprehensive way to equate neurodevelopmental research across a variety of experimental species and extrapolate these data to developing humans. We report that this can be done using a "bioinformatics" approach because underlying the principles that drive brain evolution is a remarkable similarity in the timing and sequence of events that occur during brain development, particularly in highly related mammalian species. This similarity of developmental schedules allows us to use regression theory applied to a highly detailed database via the Internet http://www.translatingtime.net/. Here a user can access detailed "translations" of the timing of neurodevelopmental events across hamsters, mice, rats, rabbits, spiny mice, guinea pigs, ferrets, cats, rhesus monkeys, and humans.

Roundtable Discussion:

Topic:

"Translational Research in Neurotoxicology"

Facilitator: Evelyn Tiffany-Castiglioni, PhD

This Roundtable will address strategies to integrate data from in vitro models, animal models and epidemiologic studies to understand the mechanisms by which toxicants and host responses to these agents during brain maturation and aging may act as triggers or amplifying factors in the pathogenesis of some neurodevelopmental and neuropsychiatric conditions. Strategies include: 1) the use of findings from in vitro systems to identify mechanisms of cell damage and possible biomarkers, 2) use of animal models to sharpen the focus of investigations in human cohorts, creating the basis for translation into novel biomarkers and intervention strategies, and 3) rigorous testing in animal models with differing genetic susceptibilities of hypotheses generated from epidemiologic studies.

Panel Discussants: *Session Speakers plus:*

David C. Bellinger, Ph.D., MSc ~ *Harvard Medical School*
Edgar Garcia-Rill, PhD ~ *Univ. of Arkansas Medical School*
Sue Griffin, PhD ~ *University of Arkansas Medical School*
Mady Hornig, MD ~ *Columbia University*
Annette Kirshner, PhD ~ *NIEHS/NIH*
Don Schmechel, M.D. ~ *Duke University Medical School*

Questions to Invited Experts:

- How do reductionist models, including –omics, cell culture, and animal models, relate to translational research?
- How do epidemiologic studies relate to translational research?
- How can the "disease first" approach advocated by NIEHS lead to more rapid knowledge that would benefit human health?
- How can we prospectively identify interdisciplinary opportunities in environmental health studies that will most substantially improve human health?

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TRANSLATIONAL RESEARCH IN NEUROSCIENCE. E. Garcia-Rill, PhD, *Director, Center for Translational Neuroscience, University of Arkansas for Medical Sciences, Little Rock, AR*

Research at the Center for Translational Neuroscience is aimed at two definitions of translational neuroscience, 1) bringing basic science findings to the bedside and back, and 2) using biomedical knowledge and research to solve real problems in the community.

1) We found that hyperreflexia, a common symptom after spinal cord injury (SCI), was induced after spinal transection in the rat. Passive exercise of the hindlimbs was found to normalize reflexes within 30 days of motorized bicycle exercise training (MBET). We developed a MBET for humans and found that excessive reflexes were normalized in 10-12 weeks of MBET. Further studies in the lab discovered that expression of connexin 36 decreased after spinal cord injury, and an agent that increased electrotonic coupling was found to normalize reflexes without the need for MBET. We are now testing this agent for its potential use in abating or blocking the onset of hyperreflexia after SCI.

2) Low birth weight (LBW) births are a leading cause of death and disability in infants, major sources of stress for parents, and an economic burden for private and public health insurance programs. Delivery at hospitals offering specialized pre- and perinatal care is thought to be effective in improving birth outcomes and perhaps reducing medical costs. However, women residing in rural and underserved areas typically lack access to these centers and to maternal and fetal medicine subspecialists who can assist community physicians with diagnosis and management of high-risk conditions. The CTN set up a Core Facility that will employ a statewide network of 15 interactive compressed video sites, a centralized 24-hour call center, and a statewide physician education and guideline development initiative to enable women and their community physicians to consult with academic subspecialists, and facilitate the evidence-based referral of high-risk women to regional perinatal centers for delivery. In addition to these educational activities, the CTN Core Facility will begin to gather research data on birth outcomes. Supported by USPHS grant RR020146.

Poster Session

SESSION 8: GENERAL POSTER SESSION

SEE PAGES 30 - 45 for abstracts presented from Poster. Poster abstracts are numbered from P-83 to P125.

The poster session is a highlight of this conference series and provides an ideal opportunity for one-on-one personal exchange of research information and ideas in an informal setting with a unique consortium of participants expert in various aspects of the theme and neurotoxicology in general. The General Poster Session has proven to be a wonderful venue for informal, in-depth discussion, collaboration building, and mentoring of young scientists. It is an important networking opportunity for students. Judging and selection of Pre – and Post-Doctoral Student Awardees will be made during the session.

STUDENT AWARD COMPETITION:

Competing students are expected to give an overview of their work in 2-3 minutes 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.

Judging of Pre- and Postdoctoral Student Awardees will be made from 7:00 – 8:00 PM

POSTER GROUP 1:
Health Effects / Imaging / Human Studies

P-83

ENVIRONMENTAL EXPOSURES AND HEALTH EFFECTS IN ADOLESCENT AND ADULT AGRICULTURAL WORKERS. DS Rohlman[1], M Lasarev[1], J Muniz[2], WK Anger[1], L McCauley[2]. [1]*Center for Research on Occupational and Environmental Toxicology, Oregon Health & Science University, Portland, OR, USA;* [2]*University of Pennsylvania, Philadelphia, PA, USA*

There are many occupational hazards associated with working in agriculture including risk of injury and exposure to pesticides. Agricultural workers range in age from children in their teens to adults in there sixties. Adolescents who work in agriculture are vulnerable to the same risks of exposures as adult workers. Environmental exposures and health effects in children have been poorly characterized. A study examining biomarkers of exposure to organophosphate pesticides (OP) and fungicides was conducted in a sample of Hispanic adolescents and adults. We measured urinary biomarkers of OP pesticides and fungicide in urine samples from 186 Hispanic adolescents and adults, obtained information on agricultural work practices and administered a battery of neurobehavioral

tests. Years working in agriculture and experience handling pesticides were associated with deficits in neurobehavioral performance. Differences in metabolite levels were found between the adult agricultural workers and non-agricultural workers. Higher levels of THPI biomarker were associated with pesticide use. Multiple regression methods found no association between neurobehavioral performance and levels of urinary metabolites when controlling for other factors known to influence performance on these tests. These data provide information on environmental exposures and health effects in adolescent and adult agricultural workers. Keywords: Neurobehavioral, Adolescent/Adult Farmworkers, Biomarkers

P-84

QUANTITATIVE MAGNETIC RESONANCE BRAIN IMAGING OF US ARMY VETERANS WITH PRESUMPTIVE EXPOSURES TO SARIN AND CYCLOSARIN DURING THE 1991 GULF WAR. KJ Heaton[1,2,3], CL Palumbo[1,4], SP Proctor [1,2,3], RJ Killiany[4,5,6], DA Yurgelun-Todd[6,7], RF White[1,2,4]. *[1]United States Army Research Institute of Environmental Medicine, Military Performance Division, Natick, MA; [2]Boston Environmental Hazards Research Center, VA Boston Healthcare System, Boston, MA; [3]Boston University School of Public Health, Department of Environmental Health, Boston, MA; Boston University School of Medicine, Departments of [4]Neurology and [5]Anatomy & Neurobiology, Boston, MA; [6]Harvard Medical School, Department of Psychiatry, Boston, MA; [7]Neuroimaging and Neuropsychology, Brain Imaging Center, McLean Hospital, Belmont, MA*

In March of 1991, more than 100,000 US troops serving in the Gulf War (GW) were presumably exposed to low levels of the organophosphate nerve agents sarin (GB) and cyclosarin (GF) following the destruction of a munitions storage facility at Khamisiyah, Iraq. Some research has indicated the presence of subtle, long-term neurobehavioral and neurochemical changes in individuals exposed to GB/GF at levels insufficient to produce obvious clinical symptoms. However, the neuroanatomical underpinnings of such changes are unclear. This study examined the association between estimated GB/GF exposure dosage and volumetric measurements of gross neuroanatomical structures in magnetic resonance images (MRIs) obtained from GW veterans. Participants were 26 GW-deployed veterans with varying degrees of presumed GB/GF exposure recruited from the Devens Cohort Study. Volumetric measures of white matter (WM), gray matter, right and left lateral ventricles (LV), and cerebrospinal fluid were obtained from MRIs and were analyzed in relation to GB/GF exposure dosage estimates. Results indicated a significant dose-dependent relationship between estimated GB/GF exposure and 1) reductions in % volume of WM (adjusted parameter estimate = -4.640, $p < 0.0001$) and 2) increases in % volume of the LVs (right LV adjusted parameter estimate = .114, $p = 0.0288$; left LV adjusted parameter estimate = .131, $p < 0.0001$) (volumes adjusted for intracranial space). This study, which to our knowledge is the first to examine brain volumetric measurements in GB/GF exposed GW veterans, provides evidence of a significant association between low levels of GB/GF exposure and subtle, structural CNS pathology in humans. Keywords: Sarin/Cyclosarin, Brain, Gulf War.

P-85 *Pre-Doc Award Competition*

CHEMICAL INTOLERANCE IN BRAZIL: BEYOND THE DICHOTOMY. Collares CF, Trevisol-Bittencourt, PC. *Sao Paulo Poison Control Center. Federal University of Santa Catarina. Brazil.*

Chemical intolerance (CI), also known as multiple chemical sensitivity (MCS), is still a poorly understood condition in Brazil. Occupational and environmental exposures leading to CI symptoms are common in this country, where there is no bias attributable to media sensationalism. It commonly appears in judicial litigations described as "cross-reactive allergy", probably due to its still controversial physiopathology and the ongoing debate about its existence as a distinct nosological entity. Most of the cases are simply diagnosed as psychiatric, leading these people to an exhausting journey through different specialists, which are mostly incapable of putting an end to their suffering. Many different denominations for CI have been adopted in part because of the various proposed pathological mechanisms. Neuropsychiatric disorders, including vaious forms of somatoform disorders, may occur, difficulting the establishment of causal nexus. Despite the general acceptance of the occurrence of neurobiological phenomena in CI, the dispute about distinguishing psychiatric and toxicological etiologies remains critical. Recent research on neuroimmunomodulation, neuroimmunotoxicology, ecogenetics, epigenetics and endocrine disruptors suggest that various complex relationships may be related to the deflagration and/or perpetuation of CI. This situation raises a myriad of questions including: doctor-patient relationship; translational thinking; conflict of interests involving government, industry and researchers; socioenvironmental responsibility of health professionals; risk communication and "mass hysteria" episodes; attribution of iatrogenic properties to neuropsychological testing; globalization and its consequences on Environmental Health and the ideological usage of psychogenic hypotheses for CI pathogeny. The social perception of toxic risks associated to the increasing number of chemical agents must be properly faced through effective integration of healthcare, surveillance and intervention. Systematic interdisciplinary and interinstitutional programs may allow increased scientific production as well as properly informed descion-making. Keywords: Chemical intolerance, Toxicology, Psychology.

P-86

A CASE OF ACUTE ORGANOTIN POISONING. Cl Yoo[1)], J Kim[2)], Y Endo[3)], Y Kim[1)]. *Department of Occupational and Environmental Medicine[1)], Department of Neurology[2)], Ulsan University Hospital, University of Ulsan College of Medicine, Ulsan, South Korea. Research Center for Occupational Poisoning, Tokyo Rosai Hospital, Japan[3)]*

We report a case of acute organic tin poisoning. A 43 year old male worker who cleared a reactor producing dimethyltin dichloride for 4 days complained a dizziness, disorientation, memory loss, mental change. The first MRI before admission showed normal findings. The result of spinal tapping came out negative but on the 4th day of the admission along with metabolic acidosis and severe hypokalemia he progressed to a state of coma. His urinalysis showed a very high concentration of dimethyl tin

and trimethyl tin. The second MRI at the hospital day 10 showed extensive symmetric high signal intensity lesion in the subcortical white matters. We diagnosed him as a acute organic tin poisoning. After the conservative treatment and chelation therapy, the patient somewhat improved clinically but the neurological defects continued after the cessation of organic tin exposure. But the issue of methylation of organic tin in the human body remains to be solved. Keywords: Organic Tin, White Matter, Intoxication.

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PALLIDAL SIGNALS IN PATIENTS WITH BILE DUCT OBSTRUCTION. SJ Bang[1)], SH Choi[2)], Y Kim[3)]. *[1)]Department of Internal Medicine [2)]Department of Radiology [3)]Department of Occupational and Environmental Medicine Ulsan University Hospital, University of Ulsan College of Medicine, Ulsan, South Korea.*

It has been reported that high signal intensities in globus pallidus in T1-weighted MRI are highly prevalent in liver cirrhosis. However, whether the increase in pallidal signal are due to portal systemic shunt or obstruction of biliary excretion is not yet determined. We studied pallidal signals in 15 cancer patients with bile duct obstruction and marked jaundice (>10 mg/dL). Patient who had fever, leukocytosis or liver cirrhosis were excluded to ensure that jaundice was developed by bile duct obstruction. Brain MRI and blood manganese level was checked. All patients showed dilated intrahepatic duct on CT scan. Pallidal high signals were very rare in the present patients whereas the high signals were highly prevalent in liver cirrhosis. Our findings suggest that the increase in pallidal signal in chronic liver disease might result from the portal systemic shunt rather than the obstruction of biliary excretion. Keywords: Portal Systemic Shunt, Biliary Obstruction, High Signal.

P-88 *Post-Doc Award Competition*

A PROXY MEASURE OF PRENATAL TESTOSTERONE EXPOSURE IS RELATED TO GRAY MATTER VOLUME IN HUMAN NEONATES Rebecca C. Knickmeyer[1], Y. Sampath K. Vetsa[2], Bradley Moore[2], Guido Gerig[1,2] and John Gilmore[1] *[1]Dept. of Psychiatry, University of North Carolina, Chapel Hill, NC 27599, [2]Dept. of Computer Science, University of North Carolina, Chapel Hill, NC 27599.*

There is growing concern that endocrine-active compounds (EACs) in the environment may exert toxicological effects on the developing human brain. Experiments in animals have routinely demonstrated that manipulation of testosterone levels during early life and exposure to EACs that alter the production, action, and conversion of testosterone, have profound effects on brain structure and subsequent behavior. In contrast, information on whether testosterone is related to individual differences in brain development in human beings is extremely limited, especially at early ages. We report the first study to link variation in an index of prenatal testosterone exposure (2D:4D ratio, the ratio of the 2nd to the 4rth digit) to structural brain development assessed via high-resolution MRI at 2 weeks after birth. 31 female and 33 male neonates were scanned unsedated on a Siemens head-only 3T scanner (Allegra, Siemens Medical System Inc., Erlangen, Germany). Tissue segmentation was accomplished using a novel automated approach developed at UNC for neonatal MRIs. 2D:4D was calculated from photocopies of the infants' hands. We focused on left 2D:4D as this showed the greatest sex difference in our sample. Left 2D:4D was significantly correlated with gray matter volume in males (k = -0.347, p < 0.05); males with higher 2D:4D (i.e. lower testosterone exposure) had smaller gray matter volumes. There was also a trend relating left 2D:4D to total intracranial volume (k= -0.267, p = 0.1). No relationship was seen between 2D:4D and volume of unmyelinated white matter. No relationships were seen within females. These results may have important implications for predicting the effects of exposure to EACs on the developing human brain. Keywords: Testosterone, Brain Development, MRI.

P-89

BODY BURDENS OF NEUROTOXIC HEAVY METALS AMONG A COHORT OF AFRICAN-AMERICAN NEONATES: IMPLICATIONS FOR NEUROLOGICAL DEVELOPMENT Bryan L. Williams, PhD; Melina S. Magsumbol, MA; Ramasubbareddy Dhaniredy, MD[1] , Dana Barr, PhD[2], Brian Buckley, PhD[3]. *[1]University of Tennessee Health Science Center Department of Pediatrics, [2]Centers for Disease Control and Prevention, Division of Laboratory Sciences, [3]Rutgers University; Environmental Occupational Health Sciences Institute*

A child's developing brain and nervous system are vulnerable to damage from environmental exposures. The neurotoxic effects of metals on children are well documented in the literature (Benes et al. 2003; Carpenter 2005; Castoldi et al. 2003; Chen et al. 2005; Costa et al. 2004; Fido and Al-Saad 2005; Jarup 2003; Mutter et al. 2005; Oken et al. 2005; Trasande et al. 2005). The fetus is especially susceptible to exposure to metals (e.g., Pb) in utero through transplacental transport (Goyer 1990; Perera et al. 2003; Rogan et al. 1986; Zaitseva et al. 2004). Xenobiotics can impede brain development during critical periods, thereby impacting sensory, motor, and cognitive function (Koger et al. 2005). Biomonitoring of neonates is greatly needed to elucidate exposure effects, quantify individual-level exposures during critical periods and explain inherent susceptibility. Such studies are sorely lacking.

The Department of Pediatrics and Division Neonatology have begun a large 3 year birth cohort study of neonates. The purpose of this study is to estimate the extent of prenatal and perinatal exposure to heavy metals among African-American children living in the inner city of Memphis, TN. We have begun a process by which we will examine the persistent and delayed neurotoxic effects of individual and aggregate exposure to As, Pb, Cd, and Hg. We are studying the relationship between a neonatal body burdens and early birth outcomes. Umbilical cord blood samples were obtained from 105 infants born at Regional Medical Center over the past 4 months. We used Inductively Coupled Plasma - Mass Spectrometry (ICPMS) to determine blood concentrations of As, Pb, Cd, and Hg. Findings from the first phase of this study suggest that neonatal body burdens of Hg and Pb are disproportionately high when compared to comparable birth cohorts. This study will help us identify factors for fetal exposure that can be targeted for reduction during prenatal care or other

interventions. The findings should help fill a major gap in knowledge in the exposure sciences. Keywords: Exposure, Neonates, Neurotoxins.

P-90

NEONATAL ABSTINENCE SYNDROME AFTER INTRAUTERINE EXPOSURE TO FLUOXETINE CASE REPORT AND LITERATURE REVIEW. A H Khan, R Ranganna, R Kadalraja and R Mehta, *Department of Paediatrics and Child Health, Bedford Hospital NHS Trust, Bedford, MK42 9DJ, UK*

OBJECTIVE: Prevalence of Neonatal Abstinence Syndrome after intrauterine exposure to Fluoxetine: Case Report and Literature Review. DESIGN: Case Report. SETTING: Department of Paediatrics and Child Health, Bedford Hospital NHS Trust, UK. *CASE REPORT:* The term infant born to a mother on fluoxetine since last 5 weeks had high Finnegan score. RESULTS: Baby's Fluoxetine level was 70 microg /l and Norfluoxetine level was 140 microg/l which were therapeutic serum levels of the drug, so a diagnosis of Fluoxetine withdrawal syndrome was made. LITERATURE REVIEW: The search revealed 7 references. Wherein, Fluoxetine level was done in only 2 studies CONCLUSIONS: It is probable that the prevalence of Neonatal Withdrawal Syndrome due to maternal ingestion of fluoxetine might be more than what has been reported in published medical literature. Keywords: Neonatal, Abstinence, Syndrome.

P-91 *Post-Doc Award Competition*

ACUTE DELIBERATE ORGANOPHOSPHATE (COUMAPHOS) POISONING WITH INTERMEDIATE SYNDROME IN A ONE YEAR OLD CHILD. Kiat, WK, *Toxicology Unit, St. Luke's Medical Center, Quezon City, Philippines*

Mentor: Dr. Irma Makalinao

Objective: To report a case of acute organophosphate poisoning in a one year old child and development of intermediate syndrome in just eight hours after exposure. Case Report: This is a case of a one year old child who came in the emergency room because of sudden onset of difficulty of breathing, cyanosis, excessive oral secretions, one episode of diarrhea and weakness noted three hours earlier after ingesting allegedly contaminated powdered milk formula. Vital signs showed a blood pressure of 90/60, cardiac rate of 112 per minute, respiratory rate of 12 cycles per minute and axillary temperature of 35.4 degrees Celsius. Arterial blood gas analysis showed respiratory acidosis. Endotracheal intubation was immediately done and was hooked on mechanical ventilation. The toxidrome of the patient is compatible with acute cholinergic excess; hence a trial dose of atropine was given. Improvements were noted, however we cannot totally rule out the possibility of other common pediatric illnesses because of non-specificity of the clinical manifestations. Eight hours later, neurological examination showed absence of deep tendon reflexes, no spontaneous respiration, no response to pain, flaccid muscle tone, no neck rigidity and lateralizing signs but with spontaneous eye opening. Glasgow Coma Scale of 6. Referral to a Pediatric Neurologist ruled out the possibility of CNS infection or trauma. RBC cholinesterase determination using Michel's method showed a result of 0.057 delta pH/hr, which is significantly depressed. Atropine was given at 0.02 milligrams per kilogram intravenously until full atropinization was achieved. We have no available Pralidoxime or Obidoxime in our country. Twenty-four hours later, patient was noted to have response to painful stimuli and spontaneous respiration. A repeat RBC cholinesterase determination showed a result of 0.25 delta pH/hr. Atropine was continued until the patient regained full consciousness and restoration of full motor and sensory function. Test was done on the allegedly contaminated milk using GC-MS, it is positive for Coumaphos. The patient was extubated on the fourth hospital day but was started on antibiotic secondary to pulmonary infiltrates seen on chest x-ray. The rest of hospital stay was unremarkable and was discharge after seven days. Atropine was continued for six weeks. Conclusion: Intermediate syndrome usually develops within 48-96 hours after acute cholinergic crisis due to prolonged inhibition of cholinesterases(1). There is probably a difference with regards to time of occurrence of Intermediate Syndrome or maybe a difference in Acetylcholinesterase function between a very young child and adult. References: (1) Benslama A, et al. The Intermediate Syndrome during Organophosphate Poisoning, Ann Fr Anesth Reanim. 2004 Apr;(4):353-6. Keywords: Organophosphate, Intermediate Syndrome, Very Young Child.

P-92

NEUROTOXIC EFFECTS DURING ARSENIC EXPOSURE. Ligia Fat[1], L. Gyorffy[2], [1] *Institute of Public Health, Cluj-Napoca, Romania,* [2] *District Hospital, Baia-Mare, Romania*

Introduction: The scientific literature shows that in the acute and chronic exposure to arsenic the most common neurological manifestation is the peripheral neuropathy, initial with symmetrical stocking-and-glove paresthesias and pain accompanied by distal weakness and then with proximally progression of sensory and motor deficits. The objective of this study was to analyze the presence of the neurotoxic effects on workers chronically exposed to arsenic. *Methods:* We investigated biotoxicological and neurological a group of 83 exposed workers from the melting sector of a non-ferrous metallurgical plant where the concentration of arsenic at the workplaces exceeded 7-15 times the admissible concentration. The workers' mean age was 31.8±8.1 years and their mean time of exposure 9.03±6.6 years. We made clinical and complete neurological exams and biotoxicological investigations: urine, hair and nail arsenic. A group with minor signs of clinical neuropathy was selected for electromyography (EMG) and nerve conduction velocity registering. *Results:* Values of urine and hair-nail arsenic over the physiological values were found in 69.87% workers; 13.25% had polyneuropathy with symmetrical stocking and glove distribution; 50% were alcohol drinkers. The nerve conduction study showed a delayed motor conduction velocity in 3 cases both on the ulnar and sciatic popliteal extern nerves (SPE). The detection exam showed a gradual record that suggests a pathological troncular neurological compensated registration. *Conclusion:* Workers with greatest and long-term arsenic exposure had the most frequently neurological signs and symptoms. All the selected cases with neurological complaints proved to be real sensitive toxic polyneuropathy, more or less

severe, in connections either with the length of the toxic exposure or the alcohol consumption. Electromyography being able to detect subclinical polyneuropathy, we could extend this examination to exposed persons without clinical manifestations. Keywords: Arsenic Exposure, Neurotoxic Polyneuropathy, Electromyography.

POSTER GROUP 2
Behavioral Assessment / Animal Studies

P-93

METHAMPHETAMINE EXPOSURE IN FEMALE C57BL/6 MICE: NEUROBEHAVIORAL CONSEQUENCES. T. J. Zarcone, L. A. Opanashuk, M. Tubbs, A. Shapiro, S.A. Notter, B. Weiss; *Department of Environmental Medicine and Environmental Health Sciences Center, University of Rochester School of Medicine and Dentistry, Rochester, NY.*

Hyperactivity and stereotypy in rodents are often employed as indices of neurobehavioral and neurodegenerative disorders arising from exposure to drugs or environmental chemicals. Methamphetamine (METH) is known to induce such responses, so these indices have been used to study some of the consequences of METH exposure as they may relate to patterns of METH abuse. Typical methods for assessing hyperactivity and stereotypy rely on functional observation batteries, open-field behaviors, and photo beam activity monitors, all of which provide limited quantitative capabilities. The current study aimed to quantify stereotypy and motor activity produced by a neurotoxic binge-dosing regimen of METH by exploiting a novel approach. It is based on digital video recording equipment and force-plate actometers from which behavioral measures were calculated and correlated with concurrent neurochemical measures. Hyperactivity and stereotypy were induced by 4 s.c. injections of 10 mg/kg every 2 hrs in female C57BL/6 mice followed four days later with another 2 hr force-plate session, prior to collection of biological samples. The binge-METH protocol induced phases of "focused" and "circular" sniffing following an initial phase of hyperactivity. Striatal tyrosine hydroxylase and dopamine transporter reductions corresponded to increases in total activity and altered spatial distribution patterns by METH-exposed mice. Measures of METH-induced stereotypic sniffing appear to be temporally related to dopamine levels measured in microdialysis experiments. The force-plate actometer and associated software offers more sensitive and quantitative measurements than current methods for delineating effects of exposure to neurotoxic agents such as METH that typically have resisted precise quantification. (Supported in part by NIEHS Center Grant ES01247). Keywords: Drug Abuse, Rodents, Activity.

P-94 *Post-Doc Award Competition*

VALIDATION OF THE DELAYED-NON-MATCH-TO-POSITION 8-ARM RADIAL MAZE (8ARM) TASK WITH SCOPOLAMINE, NICOTINE, AND ETHANOL. RI Erickson, EB Defensor, DR Middaugh, LL Rausch, JC Mirsalis, and KL Steinmetz. *SRI International, Menlo Park, CA,USA.*

Mentor: Karen Steinmetz, PhD, DABT

The delayed non-match-to-position 8-ARM paradigm is used to evaluate the potential of compounds to affect spatial navigation, attention, learning, memory, and motivation by counting Working and Reference Memory Errors (WRE and RME, respectively) and elapsed time to complete the task during one testing session. WME were counted as the number of re-visits to any arm. RME were counted as the first visit to an un-baited arm. We tested three compounds, including scopolamine (0.3 and 0.6 mg/kg), ethanol (500, 1000, and 2000 mg/kg) and nicotine (0.4 mg/kg), in adult male Sprague-Dawley rats. Rats treated with scopolamine (SCO), a specific muscarinic (M1) receptor antagonist, required an average of almost 4 min longer to complete the task and made an average of 6 errors above controls. When evaluated by error type, SCO-treated rats made WME 7.5 times more frequently than controls. In contrast, rats treated with nicotine (NIC) completed the task in 65 sec less time than controls. While NIC-treated rats made the same numbers of RME, they made 3-fold fewer WME compared with controls. Rats treated with ethanol (ETH) at doses up to 2000 mg/kg did not show impairment in any parameter of task performance. These results demonstrate that SCO impairs and NIC enhances behavioral elements required to successfully complete the 8-ARM, a complicated spatial navigation task. Future studies will investigate higher dose levels of ETH and other drugs believed to enhance or impair spatial navigation behaviors. This study was supported by NIAID Contract N01-AI-05417. Keywords: Learning, Memory, Maze.

P-95

BEHAVIORAL EFFECTS OF ACRYLAMIDE IN RATS EXPOSED TO DAILY LOW DOSES FROM GESTATION DAY 6 THROUGH 6 MONTHS OF AGE. J Garey, SA Ferguson and MG Paule. *Division of Neurotoxicology, National Center for Toxicological Research/FDA, Jefferson, AR, USA*

While the neurotoxic effects of high-dose acrylamide (ACR) exposure are well known, less is known about the potential behavioral outcomes of chronic, low-dose ACR exposure. ACR contamination occurs when carbohydrate-containing foods are cooked at high temperature. Here, plug-positive Fischer 344 rats were gavaged with ACR daily (0, 0.1, 0.3, 1.0 or 5.0 mg/kg/day) beginning on gestation day 6. Litters (8-10 per treatment group) were gavaged with the same dose as their dams (dams no longer dosed) beginning on postnatal day (PND) 1. From weaning (PND 23) onward, offspring continued to receive their same doses via drinking water. One male and one female per litter were tested in a range of behavioral tasks from 3-6 months of age, with ACR exposures continuing throughout. No effects of ACR were observed in open field activity levels (PNDs 153-154) nor on learning and swim speed in the Morris water maze (PNDs 157-161). Significant effects on acoustic startle responses (PND 151) were observed (5.0 mg/kg/day), while no effect was observed on prepulse inhibition (PND 178). ACR exposure did not affect forelimb grip strength or hindlimb landing foot splay at 6 months. Thus, in 3- to 6-month-old rats at the doses provided here, toxicity is limited to certain auditory-mediated behavioral reactions. Supported by ORISE (to

JG) and NTP 224-93-0001. Keywords: Neurotoxicology, Development, Behavior.

P-96

EFFECTS OF CHRONIC LOW-DOSE ACRYLAMIDE EXPOSURE ON PROGRESSIVE RATIO PERFORMANCE IN RATS. J. Garey, S.A. Ferguson and M.G. Paule. *Division of Neurotoxicology, National Center for Toxicological Research/FDA, Jefferson, AR*, USA

Acrylamide (ACR) is a neurotoxicant known to produce peripheral neuropathy in rats and humans, but little is known of its potential for producing either cognitive or motivational alterations. Chronic exposure to low doses of ACR as a food contaminant is known to occur widely in humans. This research evaluated the effects of daily ACR exposure on food-motivated behavior, with exposures beginning prenatally on gestation day 6 and continuing through approximately postnatal day (PND) 85. Plug-positive Fischer 344 dams (9-10 per dose group) were gavaged daily with 0, 0.1, 0.3, 1.0 or 5.0 mg/kg/day ACR. On PNDs 1-22, pups were gavaged with the same dose their dam had received. On PND 22, pups were weaned and pair-housed with a same-sex littermate and ACR exposure continued at the same doses as before via drinking water. Rats were food-deprived to maintain them at approximately 90% of historical free-feeding weights. One male and one female pup per litter were behaviorally tested in an operant chamber using progressive ratio (PR) performance from approximately six to twelve weeks of age. The PR schedule required that each rat increase the number of lever presses by one for each additional 45 mg food pellet reinforcer, with the first reinforcer being delivered after one lever press. Each PR session was 10 min in duration and occurred every other weekday. Results from the first 14 test sessions indicated a marginal treatment effect of ACR on number of reinforcers earned ($P=0.05$; repeated measures ANOVA), with the Tukey HSD post-hoc tests revealing significantly fewer reinforcers earned in the 5.0 mg/kg/day dose group (5.5 ± 0.2) than in controls (7.2 ± 0.2). A significant effect of ACR on response rate (defined as the total number of lever presses divided by the total session time) was observed ($P<0.05$; repeated measures ANOVA), with the Tukey HSD post-hoc tests revealing a significantly lower response rate in the 5.0 mg/kg/day group (0.041 ± 0.002 responses/sec) than in controls (0.065 ± 0.003 responses/sec). There also appears to be a linear dose-response for ACR effects on this measure. No effects of ACR were observed on post-reinforcement pause (the average time in seconds from reinforcer delivery to the next lever press). These data suggest that daily low-level ACR exposure can produce measurable decrements on aspects of food-motivated behavior. Supported by ORISE (JG) and NTP 224-93-0001. Keywords: Neurotoxicology, Development, Behavior.

P-97

DEVELOPMENTAL NEUROTOXICITY ASSESSMENT OF LOW-LEVEL ACRYLAMIDE EXPOSURE IN FISCHER 344 RATS. MG Paule, SA Ferguson and J Garey. *Division of Neurotoxicology, National Center for Toxicological Research/FDA, Jefferson, Arkansas, USA*

Acrylamide (ACR) is a low-level contaminant of starchy foods cooked at high temperatures. Relatively high doses (> 10 mg/kg/day) produce well-documented neurotoxic effects. Here, rats (8-10 litters per treatment group) were treated daily with ACR (0, 0.1, 0.3, 1.0 and 5.0 mg/kg/day) from gestation day 6 through approximately 3 months of age. Over postnatal days (PNDs) 4-22, male and female pups were tested for righting reflex, negative geotaxis (incline test), forelimb hang, open field and Rotarod performance. Postweaning Rotarod coordination (PNDs 88-89) and running wheel motor activity (PNDs 89-101) were also assessed in one male and one female per litter. Preliminary analyses suggested that early developmental landmarks were largely unaffected by ACR, but a significant delay in vaginal opening ($P < 0.05$) was observed at 5.0 mg/kg/day (~6 day delay versus controls). Rotarod coordination at PNDs 21-22 was significantly altered in rats treated with 5.0 mg/kg/day; however, no differences were observed on retest at PNDs 88-89. No effects of ACR were observed on running wheel activity. Significant differences in both brain and body weight were detectable at sacrifice at PND 90. These data suggest that daily exposure to relatively low levels of ACR throughout early life may produce hormonal disruption in female rats, but otherwise produces few observable neurotoxic effects at the doses provided. *Supported by NTP 224-93-0001 and ORISE (to JG).* Keywords: Neurotoxicology, Development, Behavior.

P-97 *Pre-Doc Award Competition*

AGE-DEPENDENT IMPAIRMENTS OF CHRONIC ESTROGEN EXPOSURE ON DSA AND DRL OPERANT TASKS. VC Wang and SL Schantz. *Neuroscience Program, University of Illinois, Urbana-Champaign, Urbana, IL*

Mentor: Susan Schantz, PhD

Both exposure to estrogen and the aging process have been shown to significantly modulate performance on learning and memory tasks in animals. We investigated the effects of estrogen and age on tests of executive function including working memory and response inhibition by implanting ovariectomized rats in three different age groups (3 mo.; young, 12 mo.; middle-aged, and 18 mo.; old) with subdermal Silastic capsules containing either a low (5%) or a high (10%) physiologic dose of 17-beta estradiol mixed in cholesterol. A third group of ovariectomized animals for every age group received cholesterol-only implants. Young, middle-aged, and old ovariectomized animals receiving the 10% estradiol implants performed more poorly than their age-matched controls on a delayed spatial alternation (DSA) working memory task. Middle-aged animals receiving the 5% estradiol implants also did not perform as well as their middle-age controls. Compared across age groups, animals receiving the cholesterol only implants at all three ages performed similarly on the DSA task. We also tested the animals' ability to withhold responses using a differential reinforcement of low rates (DRL) operant schedule. Young animals receiving 10% estradiol were not able to withhold responses as well as animals receiving the cholesterol only implants when comparing the ratio of reinforced to non-reinforced lever presses. Middle-aged animals receiving either 5 or 10% estradiol did not perform as well as middle-age controls. In contrast, old animals receiving either estradiol treatment did not show the same

impairment seen in the other age groups relative to age-matched controls. As with the DSA task, animals receiving cholesterol-only implants performed similarly on the DRL task across age groups. From these results, it appears that chronic estrogen administration may have detrimental effects on several aspects of executive function including working memory and response inhibition. In contrast, advanced age did not appear to impair either working memory or response inhibition as measured on the DSA and DRL tasks. Supported by AG024387 from NIA. Keywords: Estrogen, Aging, Memory.

P-98

DEVELOPMENTAL EFFECTS OF IN UTERO AND LACTATIONAL EXPOSURE TO A MIXTURE OF PERSISTENT ORGANIC POLLUTANTS. S. Gill [1], H. Marrif[1], W. Bowers[2], J. Nakai[2], R Mueller[1] and O Pulido[1]. [1]*Toxicology Research Division, Bureau of Chemical Safety, Health Products and Foods Branch, Health Canada.* [2]*Environmental Health Sciences Bureau, Healthy Environments and Consumer Safety Branch, Health Canada.*

The neurotoxic effects of exposure to chemicals (14 PCBs, 12 organochlorine and methylmercury) were investigated in *Sprague-Dawley* rats. The mixture was given orally from the first day of pregnancy until weaning postnatal day (PND) 23 at doses of 0 mg/kg, 0.05 mg/kg, 0.5 mg/kg, 5 mg/kg, and 15mg/kg. A positive control group was dosed with 15 mg/kg Aroclor 1254. Animals were sacrificed on PND 35 and 70. Brains were perfused and sections stained with H&E, Fluoro-Jade B, Luxol Fast Blue/Cresyl Violet (LFB/CV) and by glial fibrillary protein, blood brain barrier and tryptase immunohistochemistry. H&E slides of PND 35 showed delays in brain development in the groups treated with 0.5 mg/kg/day and 5.0 mg/kg/day. LFB/CV slides showed delayed myelination in several brain areas at PND 70 and a significant increase of mast cells in treated animals at PND 35 and 70. Mast cells showed positive staining with tryptase and preferentially localized in the thalamus near blood vessels. A significant increase in numbers of blood vessels were observed in the thalamus, particularly in the 5 mg/kg group. Fluoro Jade B stained sections did not reveal compound-related effects. The changes observed in brain development, angiogenesis and mast cells population suggest a possible association between chemical exposures and brain inflammation. The mechanisms associated with these changes warrant further investigation. Keywords: Developmental Neuropathology, Mast Cells, Persistent Organic Pollutants.

POSTER GROUP 3:
Testing / Verification / Methodologies

P-99

EXAMINING DATABASES USED TO EVALUATE TRENDS IN NEURO DEVELOPMENT DISORDERS. A ter Schure and J Yager. *Air Quality Health & Risk Assessment, Environment, Electric Power Research Institute (EPRI), Palo Alto, California, USA.*

A recent study[1] used publicly available databases (VAERS and CDDS) to evaluate trends in diagnosis of new Neurodevelopment Disordesr (NDs), i.e. autism, in children in relation to Thimerosal-Containing Vaccines (TCVs). The study concluded that the number of newly reported autism cases was related to TCVs usage over time. Here the suitability of these databases for such trend evaluations is examined, using the same search and age criteriaas put forward in the study, though applying a more rigorous approach. Among other inconsistencies, it was found that 1) the CDDS's reported numbers represent net change in number of autism cases quarter by quarter and does not necessarily represent new autism cases; 2) the study used CDDS data for patients between 0 and >62yrs old, whereas the objective was to address trends in children ≤5yrs old, born in the 1990's and assuming a 3- to 4 year lag time between birth and diagnosis of NDs; 3) contrary to the study's results, no significant relationships between the numbers of reported autism cases by CDDS for children aged 2 to 4 years and reporting quarters was observed; 4) for any reported event in VAERS no cause and effect relationship has been established; 5) for patients ≤5yrs old, over 60% of VAERS's reported autism cases used in the study are of age <18 months, whereas an autism diagnosis is currently made at the earliest at 18 months of age; and 6) when the VAERS cases of autism age ≥18 months to ≤5yrs are plotted againsttheir reporting date, "trends" similar to those published appear; an increase after 1998 until 2002 and a decrease after 2002. It is concluded that both databases are unsutable for assessing trends in new autism cases in relation to TCVs, and in assessing cause and effect relationships between autism and TCVs. In regard to the study's objective incorrect data and analyses were applied to reach conclusions. Recommendations are given to better investigate possible causes of autism and the recent sharp increase in reported cases. 1) Greier DA, Greier MR. Early downward trends in neurodevelopmental disordersfollowing removal of thimerosal-containing vaccines. J Am Phys SUrg 2006;11:8-13. Keywords: Autism, Thimerosal, Database-Analysis.

P-99

A TOOL FOR REGULATORY SAFETY TESTING: INTERVAL SCALE FOR DEVELOPMENT OF RATS AGED 0-70 DAYS. Didima de Groot[1], Gert Jacobusse[2], Jan Lammers[1], André Wolterbeek[1], Stef van Buuren[2] *TNO Quality of Life, Zeist [1] / Leiden [2] , The Netherlands*

To protect children against adverse exposure to drugs and chemicals in the environment (including food chain) safety rules have been developed and documented in a number of test guidelines, including those required for experimental testing of potential toxic effects on neuro-development [1, 2]. A number of neuro-physical, sensory, motor and cognitive endpoints for rats of different age are included in these test guidelines. Such regulatory animal studies with extensive behavioural testing are logistically complex, labour intensive, time consuming and involve hundreds of animals. Unfortunately, clear guidance on adequate and rational integration of the test results is lacking, mainly because the test scores lack a common metric that allows comparison of developmental scores across age.

Inspired by an interval scale for development of children aged 0-2 years [3] we are developing a quantitative score (so called *D*-score) with improved measurement characteristics to estimate normal development in rats, with the intention to ultimately use the *D*-score and interval scale in (regulatory) developmental and juvenile neurotoxicity testing. The basic assumption of the *D*-score is the existence of a common continuous scale for development. The strength of the proposed model is that the definition of the *D*-scores is not specific to age, so the *D*-score of a measured individual can be compared to the *D*-score of another individual of different age. Difference scores between sessions can be used to evaluate development on the individual level.

This implies that, once such an interval scale for rats is available, the number of tests can be limited by selecting those tests that are most informative to detect delayed/disturbed development. When not limited by strain differences, control groups need not be further included. For regulatory testing this allows a reduction of labour-intensive testing and reduction and refinement of animal use. [1] Guidelines EPA, OPPTS 870.6300/8600. [2] Guidelines OECD 426. [3] Jacobusse et al. (2006) *Stat Med.* 25:2272-83. Keywords: Rat Serial Neurobehavioral Endpoints, Rat *D*-Scores Interval Scale, (Regulatory) Developmental Neurotoxicity Testing.

P-100

TOWARDS AN ES-D3 STEM CELL ASSAY FOR QUANTITATIVE *IN VITRO* DEVELOPMENTAL NEUROTOXICITY TESTING. Didima de Groot[1], Johan van Burgsteden[1], Andreas Freidig[1], Laura Hondebrink[1], Jan Lammers[1], Andrea Seiler[2], Henk Vijverberg[3], Remco Westerink[3], Andre Wolterbeek[1] *[1]TNO Quality of Life, Zeist, NL; [2]ZEBET at the BfR, Berlin, FRG; [3]IRAS, Utrecht, NL*

Effects of chemicals on any developmental process like proliferation, migration, differentiaton or apoptosis will likely have neurotoxic consequences for the CNS as an organ. Current *in vitro* models mimic only part of the in vivo development and, as a consequence, only detect chemicals that interfere with the *in vivo* process that is mimicked by the model. Recent developments in stem cell technology may help to solve this problem, for stem cells can be forced to undergo differentiation into a mixed culture of neurons, astrocytes and oligodendrocytes through processes like proliferation, migration and differentiation. We hypothesize that the *in vitro* neuronal development from stem cells mimics the *in vivo* differentiation to such extent that chemicals that interfere with any process in *in vivo* neuronal development also interfere with the neuronal development in this *in vitro* system. We have developed an assay in which ES-D3 stem cells differentiate into neurons, astrocytes and oligodendrocytes. Cell types are characterized by immunohistochemical staining. Treatment of the test system with the thyroid hormone T3 leads to an increase in oligodendrocytes. However, quantitative analysis of results needs further effort in order to overcome misinterpretation of observed differences in cell-ratios. For that purpose, the use of the computer aided stereological toolbox (CAST) system in *in vivo* developmental neurotoxicity testing and the translation to it's possible use in quantification of *in vitro* results is explained. Further, we aim to optimize characterization of the present *in vitro* test and to test a selection of chemicals for their effects on individual developmental processes in relation to a shift in neuronal cell types that arise after occurrence of these effects. By selection of compounds whose *in vivo* characteristics are already known we will also look for possible relations between the *in vivo* effects and the effects found on the individual processes and/or shift in cell types *in vitro*. Keywords: *in vitro* developmental neurotoxicity, ES-D3 mouse stem cells, neuronal development.

POSTER GROUP 4:
Autism / LDDI / ASA / AAMR / LDAA

P-101

THE LEARNING AND DEVELOPMENTAL DISABILITIES INITIATIVE: PREVENTING TOXIC THREATS TO CHILD DEVELOPMENT. Elise Miller, MEd, *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 over 250 organizational and individual members nationally. Members of LDDI include scientists, health-care providers, and LDD groups (including the Learning Disabilities Association of America, the American Association for Intellectual and Developmental Disabilities, and the Autism Society of America) as well as environmental health and justice advocates. 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, National Collaboration.

P-102

TOXIC EXPOSURES IN HOMES OF CHILDREN WITH DEVELOPMENTAL DISABILITIES (DD). Michele (Gagnon) Wagner, *Director, Environmental Health Initiative, American Association on Mental Retardation, 444 North Capitol Street, NW, Suite 846, Washington, DC 20001*

This poster presentation identifies the unique characteristics of the DD community that predisposes them to nuerotoxic exposures. This exposure may lead to

disruptions in normal brain development or further compromise limited development. These unique characteristics include behavioral, communication limitations, motor limitations, nutritional, health disparities and socio-economic status. An argument will be made that special consideration and additional steps need to be taken to reduce toxic exposures for this population to ensure optimal health and prevention of further neurological insult. Keywords: Developmental Disabilities, Neurotoxic Exposures, Environmental Justice.

P-103

THE HEALTHY CHILDREN PROJECT OF THE LEARNING DISABILITIES ASSOCIATION OF AMERICA. Kathy Lawson, *Healthy Children Project, Learning Disabilities Association of America, 4156 Library Road, Suite One, Pittsburgh, PA 15234-1349*

The overall mission of the Healthy Children Project (HCP) is to reduce human exposure to environmental neurotoxins to promote healthy child development and reduce learning disabilities. Assuming knowledge is the important first step in behavior change, the goal of the Healthy Children Project is to significantly increase public awareness of known preventable precursors to developmental disabilities, focusing primarily on Learning Disabilities. Believing personal relevance stimulates change, the target audience for the Healthy Children Project is women and men of reproductive age, their children and families, and the health care professionals who serve them. Once this audience is informed and convinced of the premise that our health and the health of our children can be directly related to untested chemical exposure, the goal is for those individuals to become advocates, demanding and discerning consumers, and engaged citizens working towards societal change on a local, national, and global level. This poster depicts the structure and method by which The Healthy Children Project accomplishes its mission and goals.
Keywords: Collaboration, Prevention, Learning Disabilities.

P-104

GENETIC SUSCEPTIBILITY IN AUTISM. SE Owens[1], ML Summar[2], JL Haines[2] and M Aschner[1]. [1]*Department of Pediatrics, Vanderbilt University School of Medicine, Nashville, TN, USA.* [2]*Center for Human Genetics Research, Vanderbilt University School of Medicine, Nashville, TN, USA.*

Autism is a common neurodevelopmental disorder with both genetic and environmental components. Genetic susceptibility to abnormal mercury metabolism is recognized as a plausible explanation for autism in a subset of children. It is assumed that mercury toxicity occurs when a "safe" level of exposure has been surpassed. However, even normal mercury levels could be implicated in the etiology of autism due to genetic susceptibility that alters metabolism or intracellular compartmentalization. To identify genetic polymorphisms associated with autism that influence the extent of individual susceptibility to mercury neurotoxicity, we are conducting a thorough search for polymorphisms in four genes involved in mercury transport and clearance (MT1a, DMT1, LAT1 and MTF1) in the general population and in autistic individuals and assessing their frequency and association to the disorder. Using a sample pool of 24 unrelated individuals from both the general and autistic populations, we screened all of the exons, 5` and 3` UTR, 1000 bp upstream of the message start, and the exonic bordering regions for genetic variations. We have identified and characterized a number of polymorphisms in both MT1a and DMT1. Two of the MT1a polymorphisms are nonsynonymous, Thr to Asn at amino acid position 27, and Lys to Arg at amino acid position 51. Variations in MT1a, DMT1, LAT1 and MTF1 might increase susceptibility to mercury toxicity by altering the transport, binding, efflux, or tissue distribution of mercury in the body. Exposure to mercury might then act synergistically with these genetic susceptibility factors to manifest the behavioral and developmental deficits present in autism. Keywords: Genetics, Mercury, Autism.

P-105

MICROARRAY ANALYSIS OF TNFRSF AND OTHER CYTOKINE MRNAS DIFFERENTIALLY REGULATED IN AUTISTIC, EBV-IMMORTALIZED B-LYMPHOCYTES. JT Graves and SJ Walker. *Department of Physiology and Pharmacology, Wake Forest University School of Medicine, Winston-Salem, NC 27101*

Given the central role of tumor necrosis factor alpha (TNF-α) and related superfamily (TNFSF) members in inflammatory processes, juxtaposed with the putative role of inflammation in the pathogenesis of autism, we investigated the expression of these cytokines and their associated receptors in autistic-sourced lymphoid cell lines. Microarray analysis of total RNA isolated from resting, Epstein-Barr Virus (EBV)-immortalized B lymphocytes derived from autistic children and non-affected siblings revealed differential expression of several cytokines and cytokine receptors, including TNFSF members Lymphotoxin beta, CD30L and CD40L, and TNFSF receptors TNFR1 and TNFR2, in the autistic population compared to their non-autistic siblings. In total, we evaluated the expression of 277 cytokine and cytokine receptor genes and found significant (≥1.2 fold increase; $p<0.05$) up-regulation in the mRNA of 84 genes, and down-regulation (≤0.83 fold decrease) in 61 genes. Post hoc semi-quantitative PCR is currently underway to validate interesting targets among the TNFRSF members, and Western analysis has given preliminary evidence of a correlated gene/protein expression of sTNFR2. Keywords: autism, cell culture, gene expression.

P-106

PEPTIDURIA IN AUTISM AND RELATED DISORDERS: AN EXPLORATORY STUDY. S.G. Kahler[1,2], E. Cooper[2], D. Gaylor[3]. *1) University of Arkansas for Medical Sciences, Little Rock, AR; 2) Murdoch Childrens Research Institute Parkville, VIC, Australia; 3) Gaylor and Associates Eureka Springs, AR.*

Excessive amounts of small peptides, some with opioid activity, derived from gluten and casein, have been found in the urine of many autistic children. Identification has been made by co-chromatography with synthetic standards, and immunological techniques. The peaks attributed to casein and gluten disappear with dietary

elimination of their sources, and there is often amelioration of autistic symptoms. We undertook to replicate work by Reichelt, Cade, Friedman, Shattock, and others, toward the goal of confirming the identity of the peptides by mass spectrometry (MS), and quantifying them. Urine samples from children ages 1-12 years old (diagnosed with autism (N=121), developmental or intellectual delay (89), speech delay (30)), and healthy children ages 3-12 (39) were eluted with TFA and acetonitrile by HPLC from a C-18 reverse-phase column, and analyzed with dual UV detectors at 215 and 280nM. Peptiduria was quantitated as the total area under the curve (AUC) of all peaks after hippurate (~30 min) till the end of the peptide region (~68min). Retention time was expressed in relation to hippurate. Twenty-six peaks were recognized. Putative identifications include cis-indolylacryloylglycine (cis-IAG), casomorphin (CM) A5, beta(b)CM, bCM 1-4 amide, trans-IAG, alpha-gliadin, bCM 1-7, bCM 1-8. The identification of bCM 1-4 was confirmed by MS. There was notable skewing of the data from the patient groups because of high values and outliers. Statistical analysis after log transformation showed autistic children had higher total AUC ($p < 0.001$, Welch's modified t-test). Comparison of the frequency distributions showed autistic children had a significantly greater proportion of samples with values above the 75th centile for tIAG and bCM1-4 amide. Increased amounts of peptides in the urine of some children with autism/developmental problems may reflect altered intestinal function, shared etiopathogenesis, or perhaps dietary differences. Further characterization of the peaks and their significance is needed. Supported by the Murdoch Childrens Research Institute.

POSTER GROUP 5:
Mechanisms / Genomics

P-107
TRANSPORT OF ETHYLMERCURY AND METHYLMERCURY IN AN IN VITRO BLOOD-BRAIN BARRIER MODEL. SJ Walker, A Nelson, M Blaxill and M Aschner. *Department of Physiology and Pharmacology, Wake Forest University School of Medicine, Winston-Salem, NC 27101*

Methylmercury (MeHg) is a ubiquitous environmental contaminant known to cause characteristic toxic effects, and is especially dangerous to the central nervous system (CNS) in man. It has long been understood that MeHg readily crosses cell membrane barriers to reach target tissues, including the brain, via transport as a complex on L-type large neutral amino acid transporters (LATs). Recently, interest has focused on ethylmercury, a related mercury-containing compound which is a major constituent of the preservative thimerosal. Thimerosal had been, up until recently, widely used in childhood vaccines to prevent contamination within the multi-dose vials. It has been hypothesized that some children, perhaps uniquely susceptible to heavy metal toxicity, may have suffered neurological damage as a result of ethylmercury exposure from repeated vaccinations. Ethylmercury, while similar in structure to methylmercury, has not been extensively studied. It is therefore of great importance to compare parameters related to transport and toxicity of thimerosal with that of methymercury since the guidelines for mercury toxicity are based on laboratory and epidemiological studies on methylmercury toxicity. The central focus of this study is to determine if ethylmercury (EtHg) is transported through the blood-brain barrier (bbb) in similar fashion to MeHg, i.e. via the human isoforms of the large neutral amino acid transporters (LNAA) or by some other active means. LAT1 and LAT2 are the LNAAs specific to human endothelial cells, and share a strong functional homology with other mammalian LNAA transporters. To investigate these parameters we have begun to evaluate a human retinal pigment epithelial cell line (ARPE-19) that shares barrier properties with other established cell culture models of the bbb. Characterizing the transport parameters of EtHg across the blood-brain barrier may help to determine the plausibility of the previously stated hypothesis and suggest therapeutic options for EtHg removal from the brain. Keywords: autism, blood brain barrier, ethylmercury.

P-108
EFFECTS OF LOW-LEVEL FORMALDEHYDE EXPOSURE ON APOPTOSIS-RELATED MOLECULES IN THE HIPPOCAMPUS OF MICE. S Tsukahara[1], S Yamamoto[1], Tin-Tin-Win-Shwe[1], S Ahmed[1], N Kunugita[2] and H Fujimaki[1]. *1: National Institute for Environmental Studies, Tsukuba, Japan; 2: University of Occupational and Environmental Health, Fukuoka, Japan*

Our recent study showed that low-level formaldehyde (FA) exposure increases the production of nerve growth factor, involving the survival and maintenance of neurons, in the hippocampus of immunized mice. In the present study, we examined the effects of FA on apoptotic mechanisms regulating survival and death of cells and on NMDA receptors related to hippocampal functions. In the hippocampus of mice exposed to 0 or 400 ppb of FA with or without ovalbumin-immunization, Bcl-2, Bax and NMDA receptor subtypes (NR2A and NR2B) were examined by Western blot analyses. Immunohistochemical analysis for active caspase-3 was also performed. The ratio of Bcl-2 to Bax expression levels significantly increased with FA-exposure in immunized mice but not in mice without immunization, although differences in each protein level were not significant. The number of active caspase-3-immunoreactive cells was not affected by FA-exposure and immunization. NR2A and NR2B expression levels of FA-exposed mice were sustained at comparative levels to those for the control mice. These results indicate that the change in the Bcl-2/Bax expression ratio, which occurs with FA-exposure and immunization, exerts protective effects against toxicity of FA. Keywords: Formaldehyde, Hippocampus, Apoptosis.

P-109
VAST DIFFERENCE IN GENE EXPRESSION FOR NEURONAL AND HEPATIC CELLS FOLLOWING MICROMOLAR PCB EXPOSURES HIGHLIGHT THE NEED FOR ORGAN-SPECIFIC DATA IN RISK ASSESSMENT. MS Maier, WH Hanneman, and ME Legare. ~ *Colorado State University, Boulder, CO*

Using genome-wide oligonucleotide DNA microarrays and RT-PCR, changes in gene expression profiles resulting from exposure to PCB 126 were characterized in cells derived from xenobiotic-metabolizing liver and brain

tissues. Under maximal inducing conditions for cytochrome P450 1A1 (CYP1A1) in H4IIE rat hepatoma cells, H4IIE and C6 rat glioma cells were exposed for 24 hours to 2.5 x 10^{-7} M PCB 126 or DMSO vehicle. Gene expression profiles for approximately 28,000 gene probes were computationally analyzed and compared. As expected, PCB 126 strongly induced *CYP1A1* transcription in hepatoma cells. Surprisingly, no induction of *CYP1A1* transcription was observed in glioma cells. PCB 126-treated hepatoma and glioma cells had uniquely different transcriptional profiles sharing only three activated genes. PCB 126 substantially activated overall transcription in C6 cells, including transcripts for *LGALS1* associated with histone deacetylase activity, synaptic plasticity modulator *S100B*, and numerous ribosomal proteins. In hepatoma cells, PCB 126 caused significant transcriptional activation of key genes associated with progression of hepatocellular carcinoma (*EIF3S3*) and mitogenesis (*EREG, AREG*). Numerous transcription factors and cytoskeleton-associated genes were suppressed in H4IIE cells. Taken together, these findings indicate unique changes in gene expression caused by PCB 126 in H4IIE hepatoma and C6 glioma cells, and differentially expressed genes for each cell-type have plausible mechanistic linkage to effects of PCB 126 in representative organs. These unique responses by different cell-types highlight the importance of collecting organ-specific toxicological data for risk assessment. Keywords: Gene Expression, Risk Assessment, PCB.

P-110 *Post-Doc Award Competition*

ARE NONENZYMATIC FUNCTIONS OF ACETYLCHOLINESTERASE (ACHE) INVOLVED IN THE DEVELOPMENTAL NEUROTOXICITY OF ORGANOPHOSPHATES? EFFECTS OF CHLORPYRIFOS AND DIAZINON ON EXPRESSION OF ACHE SPLICE VARIANTS IN VITRO AND IN VIVO. RR Jameson, FJ Seidler and TA Slotkin. *Department of Pharmacology & Cancer Biology, Duke University Medical Center, Durham, NC, USA.*
Mentor: Ted Slotkin, PhD

Organophosphate (OP) pesticides affect mammalian brain development through mechanisms independent of the cholinergic hyperstimulation that results from the inhibition of AChE enzymatic activity. In the brain, AChE occurs as two catalytically similar splice variants that have distinct functions in development and repair. The rare, read-through isoform, AChE-R, is preferentially induced by injury and appears to promote repair and to protect against neurodegeneration. The more abundant, synaptic isoform, AChE-S, instead enhances neurotoxicity. We exposed differentiating neuronotypic PC12 cells to 30 μM chlorpyrifos (CPF) or diazinon (DZN), or to CPF oxon, the active metabolite that irreversibly inhibits AChE enzymatic activity. After 48 hr of exposure, all three agents enhanced gene expression for AChE-R by about 20%, whereas CPF and DZN, but not CPF oxon, increased AChE-S expression by 20-40%. Thus, despite the fact that CPF oxon is a much more potent AChE inhibitor, it is the native compound (CPF) that induces expression of the neurotoxic AChE-S isoform. We then administered CPF or DZN to neonatal rats on postnatal days 1-4, using daily doses that spanned the threshold for barely-detectable AChE inhibition (0-20%), evaluating gene expression in forebrain and brainstem on postnatal day 5. Whereas 1 mg/kg of CPF had little or no effect, 0.5, or 2 mg/kg of DZN induced both AChE-R and AChE-S, with a greater effect in males. Our results support the idea that AChE splice variants are involved in the mechanisms for the developmental neurotoxicity of OPs. (Supported by NIH ES10356 and 07031). Keywords: Acetylcholinesterase, Chlorpyrifos, Development.

P-111

EVALUATION OF HIPPOCAMPAL GENE EXPRESSION CHANGES ASSOCIATED WITH CHRONIC KETAMINE OR REMACEMIDE EXPOSURE IN RATS. LKM Wright[2,1], TA Patterson[1], E Pearson[3], T Hammond[3] and MG Paule[1,2]. *[1]Division of Neurotoxicology, National Center for Toxicological Research, Jefferson, AR, USA, [2]Department of Pharmacology and Toxicology, University of Arkansas for Medical Sciences, Little Rock, AR, USA and [3]AstraZeneca Safety Assessment, Loughborough, England (UK).*

Our study has shown the chronic ketamine or remacemide exposure impairs the acquisition/performance of several operant behaviors. Ketamine is an N-methyl-D-aspartate (NMDA) receptor antagonist, whereas remacemide has both NMDA receptor antagonist and sodium channel blocking properties. Beginning on postnatal day (PND) 23 and continuing until PND 257, rats were gavaged daily with ketamine (100 mg/kg/day), remacemide (150 mg/kg/day) or water. Ketamine disrupted motivation to perform for food reinforcers and delayed the acquisition of auditory discrimination for a brief period midway through treatment. Remacemide, on the other hand, had no effect on appetitive-motivation and delayed the acquisition of auditory discrimination until late in treatment. These findings suggest that ketamine and remacemide have selective behavioral effects and that tolerance to the effects of ketamine on auditory discrimination develops more rapidly than tolerance to the effects of remacemide. In order to document changes in gene expression associated with chronic ketamine or remacemide exposure, rats were sacrificed 98 days after their last treatment on PND 386 and mRNA from the CA1 region of the hippocampus was isolated for microarray analysis. Both ketamine and remacemide significantly decreased the expression of genes involved in a number of cellular processes including cell-to-cell signaling, amino acid metabolism and inflammation. Ketamine also decreased the expression of genes involved in feeding behavior and nervous system development. These observations suggest important differences in the cellular response to chronic ketamine or remacemide exposure, some of which may underlie the noted behavioral differences. Keywords: Gene Expression, Operant Behavior, NMDA Receptor.

P-112 *Post-Doc Award Competition*

T CELL-MEDIATED NEUROPROTECTIVE RESPONSE IN TOXIC CHEMICAL INDUCED MEMORY-RELATED GENE EXPRESSIONS IN A MOUSE HIPPOCAMPUS. Tin-Tin-Win-Shwe[1], S. Ahmed[1], S. Tsukahara[2], S. Yamamoto[2], M. Kakeyama[1], D. Nakajima[2], S. Goto[2], T. Kobayashi[1], H. Fujimaki[2] *[1]Environmental Health Sciences Division, National Institute for Environmental Studies, 16-2*

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Mentor: Dr. Hidekazu Fujimaki

The amino acid glutamate is the principal excitatory neurotransmitter in the brain, where it participates in learning and memory processes. However, at abnormally high concentrations it becomes neurotoxic. The neurotoxic effect of glutamate is widely thought to be exerted predominantly by the N-methyl-D-aspartate (NMDA) subtype of glutamate receptor. Toluene is a volatile organic solvent commonly found in a variety of commercial, industrial and household products. It has been suggested that toluene may produce some of its effects by directly inhibiting NMDA receptor function in a subunit-selective manner. To investigate the possible involvement of T cells in toluene-induced memory-related gene expressions in mouse hippocampus, we exposed BALB/c and nude mice to toluene at 0 ppm and 9 ppm in nose-only exposure chamber for 30 min in 3 consecutive days followed by once a week for 4 weeks. Four hours after the last challenge, we collected the hippocampus and examined the mRNA expression of NMDA receptor, protein kinase and transcriptional factors, which are potentially involved in memory functions, and then chemokines by a quantitative real-time PCR method. Our data clearly demonstrates that NMDA receptor NR2A, Ca2+/calmodulin-dependent kinase, CaMKIV, and transcriptional factor cAMP response element-binding protein, CREB-1, CCL2 and CCL3 mRNA expressions increased in the hippocampus of BALB/c mice exposed to 9 ppm toluene compared to that of 0 ppm. There were no alterations of memory-related gene expressions observed in nude mice. This is the first study to show that the T cell-mediated neuroprotective mechanisms are activated in glutamate toxicity induced by toluene in BALB/c mice, but not in nude mice. Keywords: Glutamate Toxicity, T Cells, Neuroprotection.

P-113 *Post-Doc Award Competition*
GASEOUS ANESTHETIC DRUG COMBINATIONS INDUCE DEVELOPMENTAL NEURO-APOPTOSIS IN THE RAT FRONTAL CORTEX. X Zou, N Sadovova, AC Scallet, B Divine, C Hotchkiss, TA Patterson, MG Paule, W Slikker and C Wang. *Division of Neurotoxicology, NCTR/FDA, Jefferson, Arkansas, 72079*
Mentor: Cheng Wang, MD, PhD

Recently published data have demonstrated that anesthetic drugs can cause widespread and dose-dependent neurotoxicity during the brain growth spurt in the developing rat. It is well known that most widely used general anesthetics are either NMDA receptor antagonists or GABA agonists. The specific aims of this study were: 1) to screen for and evaluate possible neurotoxicity from exposure of the developing rat brain to general gaseous anesthetics such as nitrous oxide or isoflurane; 2) to determine whether a combination of nitrous oxide and isoflurane will potentiate or attenuate neurotoxicity; and 3) to investigate the underlying mechanisms associated with gaseous anesthetic-induced neuro-apoptosis. Postnatal day (PND)-7 rat pups were exposed to nitrous oxide (75-vol %) or isoflurane (0.75-vol %) alone, or in combination (75% nitrous oxide + 0.75% isoflurane) for a period of 2, 4, 6 or 8 hours. Six hours after withdrawal, rats were perfused and serial frozen brain sections (10 µm) throughout the entire frontal cortical levels were cut using a cryostat. No significant apoptotic effects were observed in either nitrous oxide- or isoflurane-treated rat brains after any of the exposure times (2-8 hours). However, the combination of nitrous oxide with isoflurane produced significant neuro-apoptosis as indicated by an increased number of caspase-3 immunostained neurons, as well as an increase in Silver-positive and Fluoro-Jade C-positive cells in the frontal cortical regions (layers II and III). Maximal apoptotic neurodegeneration was observed in the 6-8 hour-exposure groups, compared with only mild damage in the 4-hour group and no significant effects in the 2-hour group. These data suggest that prolonged exposures to a combination of commonly used general gaseous anesthetics (nitrous oxide plus isoflurane) produce apoptotic neurotoxicity in the developing rat brain. Supported by the National Toxicological Program E-2155 and ORISE. Keywords: Gaseous Anesthetics, Neurodegeneration, Rat.

P-114
THE RECOVERY OF DOPAMINERGIC INNERVATION AND THE REPAIR OF NEURODEGENERATION IS NEARLY COMPLETE IN THE BASAL GANGLIA WITHIN 6 MONTHS AFTER A SEVERE ACUTE NEUROTOXIC EXPOSURE TO AMPHETAMINE. John F. Bowyer. *Division of Neurotoxicology, National Center for Toxicological Research/ FDA, Jefferson, AR 72079-9502.*

The recovery of structures within the basal ganglia and brain regions associated with its function were determined over a six month period after a severe acute amphetamine (AMPH) neurotoxic insult. Animals were dose subcutaneously with four doses of D-amphetamine consisting of 5, 7.5, 10 and 10 mg/kg (2hr intervals between each dose) at an environmental temperature of 23 to 24°C to enhance the possibility that severe hyperthermia occurred from the AMPH exposure. When severe hyperthermia occurred, over an 80 % loss of tyrosine hydroxylase (TH) immunoreactivity within the caudate/putamen (CPu) from 1 to 14 days post AMPH consistently resulted. Also, significant neurodegeneration occurred within 3 days of exposure in regions associated with the basal ganglia including layers III and IV of the parietal cortex and the intralaminar, ventrolateral and ventromedial nuclei of the thalamus (as determined by Fluoro-Jade C labeling of degenerating neurons and isolectin B4 labeling of phagocytic microglia). Fluoro-Jade C labeling of degenerating neurons and isolectin B4 labeling of phagocytic microglia were no longer present 2 weeks post AMPH in any brain region except the ventrolateral and ventromedial thalamus where they persisted for at least 1 month. One month after AMPH exposure, dopaminergic reinnervation in the CPu appeared underway by the partial recovery of TH immunoreactive fibers within the CPu, and the TH immunoreactivity within the CPu had almost recovered except for the ventrolateral region within3 months post AMPH. By 6 months, TH immunoreactivity had recovered throughout the CPu including the ventrolateral area. The mechanisms by which the damaged and degenerated dopaminergic axons and terminals are regenerated within this 6 month period

are now being examined through analysis of changes in gene expression that occur within the CPu and the substantia nigra. In addition, a determination of whether neurodegeneration continues in the thalamus for 1 month post AMPH or whether the degenerated neurons within this region are all produced within 3 days of AMPH exposure and just take much longer to be scavenged by the microglia is being determined. Keywords: Basal Ganglia, Dopamine, Axonal Regeneration.

P-115 *Post-Doc Award Competition*

GENE EXPRESSION PROFILING OF MPP^+-TREATED MN9D CELLS: A MECHANISM OF TOXICITY STUDY. Jianyong Wang, Zengjun Xu, Hong Fang, Helen M. Duhart, Tucker A. Patterson, and Syed F. Ali. *Neurochemistry Laboratory, Division of Neurotoxicology, National Center for Toxicological Research, Jefferson, Arkansas, USA*

Mentor: Syed F. Ali, PhD

Parkinson's disease (PD) is a neurodegenerative disease characterized by progressive loss of midbrain dopaminergic neurons with unknown etiology. MPP^+ (1-methyl-4-phenylpyridinium) is the active metabolite of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which can induce parkinsonian syndromes in humans and animals. MPTP/MPP^+ treatment can produce selective dopaminergic neuronal degeneration, and for this reason these agents are commonly used to study the pathogenesis of PD. However, the mechanisms of their toxicity have not been elucidated. In order to gain insights into MPP^+-induced neurotoxicity, a gene expression microarray study was performed using a midbrain-derived dopaminergic neuron cell line, MN9D. Utilizing a two-color reference design, Agilent mouse oligonucleotide 22K microarrays were used to examine relative gene expression changes in MN9D cells treated with 40 μM MPP^+ compared to controls. Bioinformatics tools were used for data evaluation. Briefly, raw data were input into the NCTR ArrayTrack database, normalized using a Lowess method, and data quality was assessed. The Student's *t*-test was used to determine significant changes in gene expression ($p < 0.05$, fold change > 1.5). GOFFA (Gene Ontology for Function Analysis) and Ingenuity Pathway Analysis were employed to analyze the functions and roles of significant genes in the biological processes. Of the 51 significant genes identified, 44 were present in the GOFFA or Ingenuity database. These data indicate that multiple pathways are involved in the underlying mechanisms of MPP^+-induced neurotoxicity, including apoptosis, oxidative stress, iron binding, cellular metabolism, and signal transduction. Several toxicity markers were also identified and further pathway analysis will be conducted to explore the molecular mechanisms of MPP^+-induced neurotoxicity as well as the pathogenesis of PD. Keywords: MPP+, MN9D Cell, Gene Expression.

P-116 *Pre-Doc Award Competition*

AGING ACCELERATES THE PROGRESSION AND MANIFESTATION OF SEIZURES IN POST-TRAUMATIC MODEL OF EPILEPSY. Amar Jyoti, Pallavi Sethi and Deepak Sharma. *Neurobiology Laboratory, School of Life Sciences, Jawaharlal Nehru University, New Delhi-67, India.*

Mentor: Dr. Deepak Sharma

Experimental epilepsy induced by an intracortical injection of $FeCl_3$/ $FeCl_2$ in rat model's human post-traumatic epilepsy. This model has been useful for studying the basic mechanisms involved in the pathogenesis of post-traumatic epilepsy. In the present work, we studied the age-related vulnerability of $FeCl_3$-induced epileptogenesis. We employed Video-EEG monitoring for 5 months to look for the electrobehavioral changes resulting from $FeCl_3$ induction of epilepsy. $FeCl_3$ was stereotaxically injected in the sensorimotor cortex in both young (4 months) and old (22 months) rats. We found that both the onset and spread of spike discharges were faster in older rats as compared to young ones. Broad-spectrum limbic and severe sustained seizures in all of the treated rats were noticed. Young rats exhibited limbic and sustained seizures after 4 months post induction of epilepsy while in old rats, myoclonic seizures (bilateral clonic movements) started appearing at the 11^{th} –12^{th} week after $FeCl_3$ injection. Behavioral seizures severity was assessed offline by using Racine's classification. In young rats, discharges of partial seizures begin at focus (ipsilateral side), spread to contralateral sides and the hippocampal CA3 field within one month of iron injection. During first two months partial seizures were accompanied with steadfast posture and facial movements. In the third month, both frequency and duration of seizures considerably increased with mild head nodding, appearance of clonic movement in the EMG recordings. In the fourth month myoclonic seizures were observed interspersed with other seizures. Old animals also exhibited similar but rapid electrobehavioral progression. Our results show that old brain in particular is more vulnerable to post-traumatic epilepsy because of faster seizure spread and lower latency in the generalization of electroclinical seizure activity. Keywords: Aging, Epilepsy, Electrophysiology.

P-117 *Pre-Doc Award Competition*

UTILIZATION OF BEHAVIORAL TESTING, ATOMIC ABSORPTION SPECTROSCOPY (AAS) AND ISOPROSTANE ANALYSIS TO DETERMINE THE EFFECTS OF BRAIN MANGANESE (MN) ACCUMULATION IN MALE C57BL/6J MICE. LA Miyatake[1], V Fitsanakis[1], D Milatovic[1], J Anderson[2], KM Erikson[2], M McDonald[3], and M Aschner[1, 4, 5]. [1]*Department of Pediatrics, Vanderbilt University School of Medicine, Nashville, TN.* [2]*Department of Nutrition, University of North Carolina – Greensboro, Greensboro North Carolina.* [3]*Department of Pharmacology, Vanderbilt University, Nashville, TN.* [4]*Department of Pharmacology and the* [5]*Kennedy Center, Vanderbilt University Medical Center, Nashville, TN.*

Mentor: Dr. Vanessa Fitsanakis

Manganese (Mn) is an important and essential metal required by both animals and humans. Both Mn in excess and deficiency can lead to serious health problems. Manganism is a disease associated with elevated levels of Mn in the brain. It progresses from a psychological to a physically debilitating disease. In this study, male C57BL/6J mice received food with either normal (10mg Mn/kg) or high (100mg Mn/kg) Mn levels for a total of 22 weeks. Animals were weighed weekly as a measure of

general health. During the last eight weeks of the study, the mice underwent a battery of behavioral experiments, including tests for anxiety, learning and memory, motor and sensorimotor abilities. At the end of the study, blood was collected and the brains were removed. One hemisphere was used to determine levels of oxidative stress as measured by isoprostanes and neuroprostanes levels. The second hemisphere was dissected into cerebellum, brain stem, midbrain, hippocampus, striatum or cortex to determine Mn accumulation via atomic absorption spectroscopy. AAS results indicate a statistically significant increase in striatal Mn ($p = 0.014$), but not Fe, levels in the absence of increased isoprostane or neuroprostane levels in each hemisphere. Interestingly, the elevated plus maze test suggests that treated animals may be more anxious than control animals. This data is useful in characterizing the relationship between brain Mn accumulation and potential behavioral correlates. Keywords: Manganese, Neurotoxicology, Behavioral Testing.

P-118 *Pre-Doc Award Competition*
EQUATING NEURODEVELOPMENT ACROSS MAMMALIAN SPECIES USING NEUROINFORMATICS.
Brandon M. Kersh, James R. Hyde, Barbara Clancy. *University of Central Arkansas, Conway AR /INBRE, University of Arkansas for Medical Sciences, Little Rock, AR*
Mentor: Barbara Clancy, PhD

We present a web-based approach to characterize and equate the timing of mammalian neurodevelopment in an accessible, user-friendly format. Because the scientific literature reports developmental data from multiple species, clinicians and researchers currently struggle to equate the timing of neurodevelopment across experimental species and extrapolate to humans. Although "rules of thumb," are often used, many conversions are vague, unable to account for species variations, and limited to rat/human comparisons. But because development occurs with evolutionary-based constraints on the sequence and timing of distinct neural events, we are able to use standard regression methods to identify similarities and differences that occur within and across mammalian species, including humans. Using hundreds of data points taken from ten mammalian species, we produce a database-driven website that allows for real-time, comparative neurodevelopmental conversions, including predicted dates of neural events for which empirical data are currently unavailable. Data are stored in a Structured Query Language (SQL) database and client-side scripts written in Hypertext Preprocessor Language (PHP) to predict post conception (PC) dates transformed to the mathematical term Y as $Y = \ln(PC\ days - k)$. Our ultimate goal is to maintain a user-friendly cross-species database where researchers and clinicians worldwide can add new species and new developmental events such that increasingly more precise predictions and broader applications can be produced and accessed from a web-based portal. *Grant Support: P20 RR-16460 IDeA (INBRE) Program of NCRR.*

POSTER GROUP 6:
Redox State / Oxidative Stress

P-119
COMPARING THE RELATIVE IMPORTANCE OF GLUTATHIONE V. ASCORBATE IN PROTECTING THE DEVELOPING BRAIN.
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Ascorbate and glutathione represent two major antioxidants in the brain, and depletion of either can lead to neurological deficits. In the developing brain, oxidative stress can permanently alter brain function. There are known polymorphisms in human genes related to glutathione synthesis and utilization that can moderately or severely reduce glutathione levels. Humans are also at risk of ascorbate deficiency as one of the few mammals unable to synthesize ascorbate de novo. To better understand the relative importance of these antioxidants in protecting the developing brain, we are studying mice genetically engineered to have lowered levels of GSH and ascorbate: *Gclm(-/-)* and *Gulo(-/-)* mice, respectively. *Gclm (-/-)* mice lack the modifier subunit for synthesis of □-glutamyl cysteine, the rate-limiting step in glutathione synthesis. These mice have significantly decreased tissue glutathione levels (10-40% of wild-type levels). In the brain, GSH levels are 0.27± 0.02 μmol/g in *Gclm (-/-)* mice compared with 0.69 ± 0.03 μmol/g in wild-type mice. *Gulo (-/-)* mice are unable to synthesize ascorbate. These mice can be rescued from scurvy with 220ppm ascorbate in drinking water, but still show dramatically decreased ascorbate levels in brain compared with wild type (0.88 ± 0.04 μmol/g v. 3.8 ± 0.16 μmol/g). Preliminary results indicate that both Gulo (-/-) and Gclm (-/-) mice show serotonin depletion in the hippocampus. Both the parent compound 5-HT and its metabolite 5-HIAA were decreased in the knockout lines. In addition, Gclm (-/-) mice show significantly higher levels of dopamine and its metabolite DOPAC in the hypothalamus compared with wild type and Gulo (-/-) mice. No differences were found in striatal neurotransmitter levels of dopamine, serotonin or their metabolites.

P-120
IMPAIRMENT IN OXIDATIVE BIOTRANSFORMATION OF A REACTIVE INTERMEDIATE OF DOPAMINE METABOLISM.
JA Doorn, VR Florang, JN Rees, DG Anderson, and NK Brogden. *Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa City, Iowa, USA.*

Dopamine (DA) undergoes monoamine oxidase (MAO) catalyzed oxidative deamination to 3,4-dihydroxyphenylacetaldehyde (DOPAL), which is metabolized primarily to 3,4-dihydroxyphenylacetic acid (DOPAC) via mitochondrial aldehyde dehydrogenase (ALDH). Previous studies demonstrated DOPAL to be neurotoxic and suggested the aldehyde intermediate to be involved in pathogenesis of Parkinson's disease (PD). Oxidative stress and resulting products, e.g., the lipid aldehyde 4-hydroxy-2-nonenal (4HNE), are thought to play a role in the mechanism of PD and could represent a pathway for generation of DOPAL via impairment of ALDH; furthermore, earlier work demonstrated protein reactivity of the DA-derived aldehyde, indicating a mechanism for toxicity. Aims of this study include determining

mechanisms for inhibition of DOPAL metabolism and measuring the reactivity of the DA-derived aldehyde toward protein amines. It was found that 4HNE (at uM) inhibited oxidation of DOPAL to DOPAC in rat brain synaptosomes. Furthermore, treatment of rat brain mitochondria with the organochlorine dieldrin, known to induce oxidative stress, lead to impairment of DOPAL oxidation. The DA-derived aldehyde was found to be reactive toward Lys and Lys-containing peptides. Novel methods were developed to detect DOPAL-protein adducts. These data indicate that DOPAL metabolism is sensitive to a product of oxidative stress and an environmental agent and that the DA-derived aldehyde is reactive toward protein amines. Future work includes elucidation of cellular protein targets of DOPAL. Dopamine, 3,4-Dihydroxyphenylacetaldehyde, Parkinson's Disease.

P-121

KETAMINE PRODUCES OXIDATIVE DNA DAMAGE AND LOSS OF MONKEY FRONTAL CORTICAL NEURONS IN CULTURE. C. Wang, N. Sadovova, X. Zou, A.C. Scallet, C. Hotchkiss, T.A. Patterson, J. Hanig, M.G. Paule and W. Slikker. *Division of Neurotoxicology, NCTR, FDA, Jefferson, Arkansas, 72079*

Previous studies from our laboratory have demonstrated that administration of ketamine, a pediatric anesthetic and an N-methyl-D-aspartate (NMDA) antagonist, produces a dose-related increase in neurotoxicity. To help elucidate the neurotoxic effects of ketamine, this study was designed to determine whether ketamine-induced neuronal cell death correlates with oxidative DNA damage and influences expression of polysialic acid neural cell adhesion molecule (PSA-NCAM). Frontal cortical cultures were incubated for 24 hrs with 10 μM ketamine or normal culture medium (control). After washout of ketamine, cultures were kept in serum and glutamate-containing medium. In this study, 8-oxoguanine [a modification of guanine induced by endogenous and extrogenous reactive oxygen species (ROS) to nucleic acids] was used as a biomarker for oxidative DNA damage. PSA-NCAM, a neuronal specific marker, was applied to determine the cell types of ketamine-induced cell death. Double-immunostaining data indicated that 8-oxoguanine is primarily localized in the nucleus and mitochondria, in both neurons and glial cells. Ketamine administration significantly upregulated 8-oxoguanine expression and decreased endonuclease Ape1/ref-1 expression (preliminary data) that is responsible for repair of alkylation and oxidative DNA damage. Ketamine also caused a marked reduction in immunostaining for PSA-NCAM, a substantial increase of lactate dehydrogenase (LDH) release, increased TUNEL-positive cells, and a reduction in mitochondrial metabolism of 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT). Western analysis demonstrated that ketamine produces a decrease in PSA-NCAM expression. These data suggest that ketamine-induced neuronal loss in the developing monkey is most likely both apoptotic and necrotic in nature. ROS have been associated with ketamine-induced oxidative DNA damage as indicated by increased 8-oxoguanine expression and decrease DNA repair enzyme (Ape1) expression. Supported by FDA/NCTR E-7189, and NICHD. Keywords: Ketamine, DNA Damage, DNA-Repair.

P-122 *Pre-Doc Award Competition*

1-METHYL-4-PHENYLPYRIDINIUM-INDUCED ALTERATIONS OF GLUTATHIONE AND REDOX ENVIRONMENT IN IMMORTALIZED RAT DOPAMINERGIC NEURONS. DA Drechsel, LP Liang, and M Patel. *Department of Pharmaceutical Sciences, University of Colorado at Denver and Health Sciences Center, Denver, CO, USA.*

Several neurodegenerative disorders, including Parkinson's Disease (PD) are characterized by decreased glutathione (GSH) levels and increased oxidative stress. GSH is the most abundant intracellular protein thiol and thus is a major determinant of cellular redox environment. Previous studies have demonstrated that neurotoxins can cause changes in both reduced and oxidative GSH levels, however information regarding the regulation of these molecules in such cases remains unexplored. The goal of this study was to characterize changes in cellular GSH levels and regulatory enzymes in a rat dopaminergic cell line (N27) following treatment with the Parkinsonian toxin, 1-methyl-4-phenylpyridinium (MPP+). MPP+ produced a time- and concentration-dependent cell death in N27 cells with a maximal release of lactate dehydrogenase occurring at 48 hours. Cellular GSH levels were initially significantly decreased at 12 hrs of treatment, but recovered to values greater than controls by 24 hrs. However, oxidized glutathione (GSSG) levels were increased at 24 hrs, concomitant with a decrease in GSH/GSSG ratio prior to overt cell death. In accordance with these changes, ROS levels were also increased, confirming the presence of oxidative stress in this model. Decreases in enzymatic activities of glutathione reductase and glutamate-cysteine ligase by approximately 20-25% were observed at early timepoints and partly account for such changes in GSH levels upon MPP+ exposure. Additionally, glutathione peroxidase activity was increased at 24 hrs. MPP+-induced efflux of glutathione into the extracellular medium was not observed and does not account for the observed changes in this cell model. These data indicate that changes in regulatory enzymes parallel, and are partly responsible for the observed effects on steady state glutathione levels and cellular redox environment upon MPP+ treatment in the N27 dopaminergic cell model of PD. This study funded by NIHRO1NS045748 (M.P.). Keywords: Glutathione, MPP+, Parkinson's Disease

P-123

INCREASED INTRACELLULAR FREE RADICAL PRODUCTION AND DECREASED GLUTATHIONE REDOX RATIO IN LYMPHOBLASTOID CELL LINES FROM AUTISTIC CHILDREN.

Stepan Melnyk, Stefanie Jernigan, Alena Savenka, and S. Jill James. *Arkansas Children's Hospital Research Institute, University of Arkansas for Medical Sciences, Little Rock, AR*

Autism is a behaviorally-defined neurodevelopmental disorder diagnosed in early childhood that is characterized by impairment in reciprocal communication and speech, repetitive behaviors, and social withdrawal. Although the 10-fold increase in autism spectrum disorder diagnosis in the last decade has raised great public health concern, the pathogenesis remains poorly defined. Both genetic and

environmental factors are thought to be involved yet none have been reproducibly identified. Recently we demonstrated that many children with autism exhibit a significant decrease in plasma glutathione redox ratio (GSH/GSSG) suggesting the presence of chronic oxidative stress. The purpose of the present study was to determine whether lymphoblastoid cells derived from autistic children are more sensitive to pro-oxidant exposures than cells from unaffected control individuals. Autistic and control cells were exposed to increasing doses (from 50 nmol/L to 2.5 µmol/L) of Thimerosal as a pro-oxidant. Measurements of oxidative stress were made at baseline and after 1-3 hours exposure to Thimerosal, a source of ethyl mercury. The relative rate of free radical generation was quantified by dichlorofluorescene (DCF) fluorescence and the intracellular levels of reduced and oxidized glutathione (GSH/GSSG) and cysteine as a source for glutathione synthesis were measured by HPLC with electrochemical detection. A commercially available kit was used to measure caspase 3-mediated apoptosis. The results at baseline (without Thimerosal) indicated that the rate of free radical production and caspase-3 activation was significantly greater in the autistic compared to the control cells and the baseline glutathione redox ratio was significantly decreased. With increasing doses of Thimerosal, the autistic cells consistently exhibited higher levels of free radical-induced DCF fluorescence and reduced levels of cysteine and glutathione redox ratio. These results suggest that lymphoblastoid cells from autistic children have lower glutathione-mediated antioxidant capacity and may be genetically more sensitive to environmental pro-oxidant stressors. Keywords: Autism, Oxidative Stress, Thimerosal.

P-124

METHYLMERCURY CAUSES GLIAL OXIDATIVE STRESS AND IL-6 SECRETION. JY Chang *Department of Neurobiology and Developmental Sciences, University of Arkansas for Medical Sciences, Little Rock, Arkansas.*

Mercury, especially methylmercury (MeHg), is an environmental toxin that causes severe neurological disorders in humans and experimental animals. Failure of glia to perform normal functions when poisoned with MeHg contributes to MeHg-induced neuronal death. This study focused on the effects of MeHg on glia by using rat and human glial cell lines. MeHg caused dose-dependent cytotoxicity in rat C6 glioma cells. This cytotoxicity was partially dependent on cell density and the volume of MeHg solution used in the experimental system. MeHg also caused reactive oxygen species (ROS) generation. The ROS generated by 10 µM MeHg was approximately the same as that by 25 µM H_2O_2 or 25 µM t-butylhydroperoxide. A significant mitochondrial depolarization was observed when cells were treated with 10 µM MeHg for one hour. MeHg greatly increased glial IL-6 secretion, a phenomenon observed in rat C6 glioma, human U251HF glioma and human ARPE-19 cells. ROS generation by MeHg did not appear to contribute to IL-6 secretion. On the other hand, IL-6 generation was associated with MeHg cytotoxicity such that only moderately toxic concentrations of MeHg (causing 30-40% cell death) can lead to IL-6 secretion. Because this cytokine can be beneficial or harmful to surrounding neurons, there is a possibility that modulation of glial cytokine production is a reasonable approach for treating MeHg toxicity. Keywords: Methylmercury, Oxidative Stress, Interleukin-6.

P-125

EFFECTS OF ETHANOL ON OXIDATIVE STRESS IN THE NEONATAL RAT CEREBELLUM. Cynthia J.M. Kane, Jason Y. Chang, Tarun K. Garg, and Lihong Han. *Dept. of Neurobiology & Developmental Sciences, University of Arkansas for Medical Sciences, Little Rock, AR 72205*

Increase in oxidative stress is one of the strong candidate mechanisms of ethanol toxicity in Fetal Alcohol Spectrum Disorder. Some *in vivo* studies demonstrate increased oxidative stress in the cerebellum and neuroprotective effects of antioxidants. But other *in vivo* studies fail to show positive effects of antioxidants. This study employed a unique model to bridge the gap in understanding between the realism of ethanol exposure *in vivo* and the power of quantitative analysis in isolated cells. Rats received ethanol (IE), isocaloric vehicle (IC), or were handled-only without treatment (HC) on postnatal day (P) 4 or P14. Cerebellar granule cells were isolated from fresh tissue 2-24 hr after treatment and immediately assayed in cell suspension for biomarkers of oxidative stress: production of ROS and mitochondrial membrane depolarization. Cell survival was measured after overnight culture of the same cell isolates. The basal level of ROS, mitochondrial depolarization, and cell survival were not different in the HC group at P4 compared to P14. Comparison of IE and IC groups revealed no change in these parameters due to ethanol treatment. Gavage alone increased ROS production and decreased mitochondrial membrane polarization compared to handling alone at P14 but not P4. As a result, both IC and IE were different than HC 12-24 hr after treatment on P14. The results indicate there was no difference in production of ROS or mitochondrial membrane potential in cerebellar granule cells following exposure to ethanol *in vivo*. These findings contribute to understanding the potential role of oxidative stress in ethanol neurotoxicity. Supported by NIH grants AA014645 and AA014888. Keywords: Alcohol, Microglia-Neuron Interactions, Brain Development.