Opening

SESSION I: OPENING AND WELCOME TO THE 26TH INTERNATIONAL NEUROTOXICOLOGY CONFERENCE

Conference Co-Chairs:
Joan M. Cranmer, Kenneth R. Reuhl, Jason R. Richardson

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TWENTY-SIXTH INTERNATIONAL NEUROTOXICOLOGY CONFERENCE: "UNIFYING MECHANISMS OF NEUROLOGICAL DISORDERS: SCIENTIFIC, TRANSLATIONAL, AND CLINICAL IMPLICATIONS." Joan M. Cranmer[1], Kenneth R. Reuhl[2], Jason R. Richardson[2]. [1]*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;* [2]*Department of Environmental and Occupational Medicine, Environmental and Occupational Health Sciences Institute, Robert Wood Johnson Medical School, Piscataway, NJ 08854, USA.*

The twenty-sixth meeting in this *International Neurotoxicology Conference Series* (NEUROTOX 26) convenes June 6-10, 2010 at the Portland Marriott Downtown Waterfront Hotel in Portland, Oregon. The theme of the 2010 conference is *Unifying Mechanisms of Neurological Disorders: Scientific, Translational, and Clinical Implications.* Scientists from around the world will present state-of-the-science research in a variety of venues including Plenary Sessions, Symposia, Roundtable Discussion, Platform Sessions, Workshops, Poster Session with Pre-doctoral Student and Post-doctoral Trainee Research and Travel Award Competitions, and focused Panel Discussions. NEUROTOX 26 will wrap up with a Roundtable Discussion on "Unifying Mechanisms of Neurological Disorders: Scientific, Translational, and Clinical Implications: Summary and Recommendations."

Background on the Conference Series: The *International Neurotoxicology Conference* is an annual (almost!) event that focuses on a timely theme while providing an opportunity for presenting new data related to the general interdisciplinary field of neurotoxicology. Special emphasis is placed on promoting, nurturing and mentoring students and young investigators in the field; networking with experts is facilitated and encouraged. The Neurotoxicology Conference has convened in either Little Rock or Hot Springs, Arkansas, the majority of the time. Other cities in which the conference has convened are Chicago (x2), Colorado Springs (x2), Honolulu, Research Triangle Park, San Antonio and Rochester, NY. This is the first time the conference has convened on the West Coast. Each year the conference focuses on a timely theme that is identified by leaders in the field of neurotoxicology.

Significance of the Theme: Over the past several years, research into neurological diseases and disorders has evolved to recognize that clinical presentation and disease severity can vary widely within a given disease or disorder and that there are often overlapping similarities in seemingly different disease states. For example, protein aggregation is a pivotal component of Alzheimer's, Parkinson's, Tauopathies, and Huntington's disease. Deficits in executive function are observed in disorders such as ADHD, schizophrenia, and autism.

Determination of the underlying mechanisms responsible for these pathological processes is likely to prove critical to understanding how to treat these devastating diseases. Although genetic factors have long been a primary focus in neurological disease, identification of a single gene that can be associated with a significant number of cases in a variety of neurological diseases is exceedingly rare. This rarity has led to the emerging recognition that environmental factors or gene-environment interactions are likely to play a pivotal role in a variety of neurological diseases and disorders.

Exposure to a variety of neurotoxicants has been associated with characteristic derangements of nervous system function, some fleeting in nature and others virtually permanent. Furthermore, exposure to neurotoxicants has been hypothesized to play a role in the development of many neurological diseases and disorders. NEUROTOX 26 will focus on common mechanisms in neurological disease and the role of environmental exposures and gene-environment interactions in the development of neurological disease and disorders.

Specific Aims. The purpose of NEUROTOX 26 is to:

Aim 1. Convene the identified international experts and other established and new researchers anywhere in the world to present, discuss and evaluate the most recent research data and information related to causes, mechanisms, diagnosis, treatment, and prevention of environmentally-linked nervous system diseases and disorders.

Aim 2. Encourage and enhance communication, understanding and collaboration between practicing clinicians, basic scientists, clinical researchers, epidemiologists, special interest groups, risk assessors and others working to further our understanding of all aspects of neurological diseases/disorders.

Aim 3. Organize the conference participants, tutorials, invited and free presentations, discussions, debates, demonstrations and interactions to document major new research advances, facilitate identification of specific research gaps, formulate hypotheses and potential experimental designs to answer questions.

Aim 4. Facilitate interdisciplinary collaboration of practitioners and scientist/investigators to help foster or initiate new multidisciplinary research approaches necessary to advance the science related to the theme.

Aim 5. Include promising young scientists as invited speakers in each session in order to promote and foster new researchers in the field. To provide opportunity for late breaking research to be presented in each session by holding open platform time for speakers to be chosen from submitted abstracts.

Aim 6. Rapidly peer-review and disseminate findings to the scientific community. All presenters are invited to submit original research papers of new data presented as well as Reviews or Mini-Reviews of recent findings related to the specific topics. Guest Editors will be selected from the Session Chairs and Invited Experts participating in the conference. Papers will be peer-reviewed and accepted papers will be published in less than one year in *NeuroToxicology,* an international scientific journal that is widely disseminated, indexed, abstracted and available online. This Specific Aim has been accomplished for each of the previous 25 conferences in this series.

Twelve Sessions comprise NEUROTOX 26:

Sunday Afternoon Opening Session
SESSION I: Opening, Introductions, and Welcome to Conference

Sunday Afternoon Symposium Session
SESSION II: Dietary Exposure to Methylmercury from Fish Consumption: New Findings from the Seychelles Child Development Study

Monday Morning
Opening of Plenary Sessions: Rationale for the Theme

Monday Morning Plenary
SESSION III: Mitochondrial Dysfunction in Neurological Disease

Monday Afternoon Platform Session
SESSION IV: Metals and Selected Topics

Monday Evening
SESSION V: Poster Session. Pre- and Post-Doctoral Student Award Competition

Tuesday Morning Plenary
SESSION VI: Protein Aggregation and Misfolding in Neurological Disease

Tuesday Afternoon Platform Session
SESSION VII: Pesticides and Selected Topics

Wednesday Morning Plenary
SESSION VIII: Emerging Areas of Research: Epigenetic Mechanisms in Neurodevelopmental Disorders

Wednesday Afternoon Platform Session
SESSION IX: Neurotoxic Effects of Environmental Exposure to Manganese in Children and Adults

Thursday Morning Keynote "Anchor Talks"
SESSION X: Hot Topic Keynotes: Channelopathies

Thursday Afternoon Keynote "Anchor Talks"
SESSION XI: Hot Topic Keynotes: Gender Differences in Neurological Disorders

Thursday Afternoon Roundtable Discussion
SESSION XII: Unifying Mechanisms of Neurological Disorders: Scientific, Translational, and Clinical Implications: Summary and Recommendations

Symposium

SESSION II: DIETARY EXPOSURE TO METHYL MERCURY FROM FISH CONSUMPTION: NEW FINDINGS FROM THE SEYCHELLES CHILD DEVELOPMENT STUDY

Session Chair: **Philip Davidson**

Theme and Rationale: *The Seychelles Child Development Study (SCDS) is a longitudinal evaluation of prenatal exposure to MeHg from fish consumption. The study began in the mid 1980s and has followed several cohorts of subjects whose mothers consumed a diet high in fish during pregnancy. The subjects themselves also habitually consume a diet high in fish. Initial results indicated that prenatal MeHg exposure was associated with "beneficial" outcomes on specific cognitive domains such as psychomotor performance and discrimination learning. Subsequent studies indicated that prenatal MeHg exposure effects may be modified by simultaneous exposure to long-chain polyunsaturated fatty acids (LCPUFA) which are also present in fish. The net effect of combined exposure to LCPUFA and MeHg on the developing fetal brain appears beneficial for specific neurocognitive and behavioral outcomes. Analyses that include postnatal MeHg exposure have revealed a different pattern of results with possible adverse effects on cognitive functions including impulsivity/response inhibition and social behaviors. These endpoints were not associated with prenatal exposure to MeHg. Additional studies that include exposure to Hg vapor from dental amalgams and selenium, which is present in high concentrations in fish, do not change these effects. Nutrients in fish appear to modify the adverse effects of co-exposure to MeHg in a manner that is related to the time of exposure. The differential risks and benefits of fish consumption with co-exposure to LCPUFA and both prenatal and postnatal MeHg appear to be much more complex than currently understood and the toxicant-nutrient interplay opens a new line of scientific inquiry of substantial public health relevance.*

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ASSOCIATIONS BETWEEN PRENATAL AND POSTNATAL MEHG AND NEUROCOGNITIVE AND BEHAVIORAL ENDPOINTS AT 17 YEARS OF AGE IN THE SEYCHELLES CHILD DEVELOPMENT STUDY. D.A. Cory-Slechta and the SCDS Team, *University of Rochester School of Medicine, Rochester, NY, USA*

Prospective studies have reported both 'beneficial' and adverse associations of prenatal and of postnatal methylmercury (MeHg) exposures with various measures of cognitive functions and behavior, raising questions about potential differential roles of these developmental periods and the specific behavioral functions and achievement measures that may be influenced by each. Based on its prospective longitudinal design and measurement of both prenatal and postnatal MeHg levels, the Seychelles Child Development Study (SCDS) offers an opportunity to examine these questions. Tests of learning/reversal learning and perseveration, working memory, and attention and Woodcock Johnson (WJ) achievement were measured in 16-18 year old SCDS subjects and analyzed by linear regression controlling for sex, socioeconomic status, maternal IQ and age. Inverse associations of prenatal MeHg with numbers of trials on the Intra-Extradimensional Shift Set test (CANTAB) and higher WJ math calculation scores were found, findings consistent with beneficial influences on learning functions that may reflect the influence of nutrients in fish, as similarly reported in our previous studies. In contrast, recent postnatal MeHg was associated with lower WJ passage comprehension scores, marginal deficits in working memory in delayed matching-to-sample performance, and, in boys, an increased false alarm rate in the CANTAB Rapid Visual Processing test, findings suggestive of recent postnatal MeHg influences on working memory and attention domains. These adverse associations may reflect a lack of any beneficial over-riding effect of nutrients in fish postnatally, and/or a reflection of cumulative toxicity, but should be interpreted with caution since the SCDS was not designed to address postnatal exposure, and there is yet no agreement on its measurement. Collectively, these findings underscore the need to determine the generality of these outcomes, and to further evaluate differential influences of prenatal and postnatal MeHg and the interactions of each exposure period with dietary nutrients.

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SELENIUM STATUS AND ITS RELATION TO CHILD DEVELOPMENT IN THE SEYCHELLES CHILD DEVELOPMENT NUTRITION STUDY. Gary J. Myers[1], Abbie Stokes-Riner[1], Gene Watson[1], Sally W. Thurston[1], Conrad F. Shamlaye[2], J. J. Strain[3], Nicholas Ralston[4],

Philip W. Davidson[1]. [1]*University of Rochester School of Medicine, Rochester, NY, USA;* [2]*Ministry of Health, Republic of Seychelles;* [3]*Northern Ireland Centre for Food and Health, University of Ulster, Coleraine, BT52 1SA;* [4]*University of North Dakota Energy & Environmental Research Center, Grand Forks, ND, USA*

Fish consumption results in exposure to the neurotoxicant methyl mercury (MeHg). Fish also contain nutrients important to normal brain development including selenium (Se) and long chain polyunsaturated fatty acids (LCPUFA). Currently the interrelationship of these toxicant and nutrient exposures and their impact on the developing brain is not well understood, but evidence is accumulating that the benefits may equal or exceed any adverse effects. In a population of 300 mothers consuming fish daily we studied the association of Se, LCPUFA, and MeHg exposure. We measured Se, Hg, and LCPUFA in maternal whole blood, Se in maternal serum, and Se and Hg in cord whole blood and then evaluated the children at ages 9 and 30 months using the Bayley Scales of Infant Development II (BSID II). Cord and maternal whole blood Se, Hg, and LCPUFA were available on 174 mother-infant pairs. The interrelationship of these exposures and the results of these analyses will be discussed.

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BENEFICIAL BENCHMARK CONCENTRATIONS FOR Ω-3 LONG CHAIN POLY-UNSATURATED FATTY ACIDS (LCPUFAs) OBTAINED FROM THE SEYCHELLES CHILD DEVELOPMENT NUTRITION STUDY. E van Wijngaarden[1], C Beck[1], D Harrington[1], SW Thurston[1], GJ Myers[1], CF Shamlaye[2], JJ Strain[3], PW Davidson[1]. [1]*University of Rochester School of Medicine, Rochester, NY, USA;* [2]*Ministry of Health, Republic of Seychelles;* [3]*Northern Ireland Centre for Food and Health, University of Ulster, Coleraine, BT52 1SA*

Fish is an important source of nutrition worldwide, and is naturally rich in long chain poly-unsaturated fatty acids (LCPUFAs) and other nutrients essential to the fetus for growth and development of the brain. However, fish is also a dietary source of heavy metals such as methyl mercury (MeHg). MeHg is toxic to the developing nervous system at high doses, which raises the question of whether children whose mothers consume fish contaminated with background levels during pregnancy are at an increased risk of impaired neurological function. Some governmental advisory bodies have recommended limiting consumption of fish during pregnancy, particularly those fish that may contain higher levels of MeHg. However, recent studies have suggested that the benefits of fish consumption may balance or outweigh the potential adverse consequences of MeHg on child development. Previously, we conducted analyses of 26 cognitive and behavioral endpoints collected on the Seychelles main cohort at 9 years of age to determine benchmark doses (BMD) for risk based on the MeHg level in maternal hair. Here, we expand on these analyses by determining the benchmark doses (BMD) for benefit associated with fish intake in a cohort of 229 Seychellois children (the Seychelles Child Development Nutrition Study). Specifically, we conducted a benchmark analysis of Bayley Scales of Infant Development-II (BSID-II) – administered at 9 and 30 months of age – in relation to Ω-3 LCPUFA measured in maternal serum samples taken at 28 weeks of pregnancy and at delivery. Our dose–response models applied to these continuous BSID-II endpoints adjusted for various covariates encompassing socio-demographic, developmental and environmental characteristics. We estimated the BMD associated with a 5 and 10 percent increase in the proportion of children performing better than the median score on the BSID-II. In other words, we provide BMD estimates at which there is a beneficial 5 or 10 percent shift in the population distribution of acceptable performance on the BSID-II. These analyses were conducted with and without controlling for MeHg level in the statistical models to explore the risk-benefit issue regarding fish intake. The resulting estimates of maternal serum Ω-3 LCPUFA level at which there is a meaningful beneficial effect on child development in the presence and absence of MeHg intake may guide policy development and consumer education regarding the potential harms and benefits associated with fish consumption.

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STABLE ISOTOPE ESTIMATES OF MERCURY BURDEN FROM DENTAL AMALGAM. GE Watson[1], JD Blum[2], MW Johnson[2], G Zareba[1], ML Lynch[1], SW Thurston[1], SA McKinlay[3], and TW Clarkson[1]. [1]*University of Rochester, Rochester, NY, USA;* [2]*University of Michigan, Ann Arbor, MI, USA;* [3]*New England Research Institutes, Watertown, MA, USA*

Dental amalgam restorations continuously emit mercury (Hg) vapor, which is subsequently inhaled and effectively absorbed via the lungs. Exposure to elevated levels of Hg vapor, as well as to other forms of Hg, has potential toxicological ramifications. Because Hg in the

body can derive from multiple sources, the precise contribution of chronic Hg vapor exposure from dental amalgam restorations to the total Hg body burden remains controversial, but of clear health significance due to amalgam's widespread use. In this study we explored the novel use of stable, isotopically enriched Hg in dental amalgams placed in humans (n = 5), as an unambiguous tracer of the origin of Hg excreted in urine. Over a 12 month exposure period, the buildup of tracer Hg in urine progressed in a time and dose dependent fashion, whereas total Hg exhibited significant fluctuations. At presumed steady state, Hg in urine attributable to dental amalgam averaged 0.10 to 0.11 mcg Hg/g creatinine/occlusal amalgam surface, and represented between 20 to 55% of the total Hg burden of subjects' urine. Based on one compartment, continuous infusion, first-order kinetics models, the elimination rate for tracer Hg in urine was estimated to be approximately 1%-1.5% per day, yielding half times of 46.5-71.5 days (average 59 days). This tracer strategy shows great promise as a means to precisely quantify absorption, distribution, and elimination of Hg vapor from dental amalgams.

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DIETARY DETERMINANTS OF MATERNAL LONG-CHAIN POLYUNSATURATED FATTY ACID STATUS IN THE SEYCHELLES CHILD DEVELOPMENT NUTRITION STUDY.

JJ Strain[1], MS Barnes[1], AJ McAfee[1], MP Bonham[1], EM McSorley[1], JMW Wallace[1], CF Shamlaye[2], SW Thurston[3], J Sloane-Reeves[3], GS Myers[3], TW Clarkson[3] and PW Davidson[3]. [1]*Northern Ireland Centre for Food and Health, University of Ulster, Coleraine, BT52 1SA,* [2] *Ministry of Health, Seychelles and* [3]*University of Rochester, School of Medicine and Dentistry, United States*

We have previously shown in the Seychelles Child Development Nutrition Study (SCDNS) that at 9 months of age, the psychomotor developmental index (PDI) of the Bayley Scales of Infant Development-II (BSID-II) was positively associated with total n-3 long-chain polyunsaturated fatty acids (LCPUFA) and negatively associated with the ratio of n-6/n-3 LCPUFA. When the infants were 30 months of age the PDI was negatively associated with prenatal methylmercury (MeHg) exposure, but only when maternal LCPUFA measures were included in the regression analysis. These findings suggest that LCPUFA from fish and the total diet were influencing relationships between prenatal MeHg exposure and developmental outcomes in this high fish-eating population. In the current study we used dietary intake data which were previously measured in these pregnant women (*n*= 229) by 4 day food diaries and analysed using WISP v. 3.0 (Tinuviel Software, Warrington, UK), to investigate the correlation between mean intake of 21 food groups and fatty acid status (SPSS v.17.0). Among the significant findings from this exploratory analysis were that total n-3 PUFA was positively correlated with white meat consumption (β=0.147, p=0.03) while the n-6/n-3 ratio was positively associated with fatty fish consumption (β=0.136, p=0.04). Mean intakes of white meat and fatty fish were 62.18 g/d and 75.12 g/d respectively. These findings further emphasise the complex interrelationships between nutrition and any adverse effects of prenatal MeHg exposure in longitudinal observational studies.

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METHYLMERCURY IRREVERSIBLY INHIBITS SELENOENZYMES NVC Ralston. *University of North Dakota Energy & Environmental Research Center, 15 North 23rd Street, Grand Forks, ND, 58202 USA*

Methylmercury (MeHg) is a highly specific, irreversible inhibitor of selenium (Se)-dependent enzymes. Since selenocysteine (Sec) is required at the active sites of genetically unique and elite enzymes that prevent and/or reverse oxidative damage in brain and neuroendocrine tissues, the toxic effects of MeHg are consistent with the effects expected to accompany interruption of these enzyme activities. Binding affinities between the Hg of MeHg and Se are a million times greater than between Hg and sulfur thus overcoming the ~105 higher abundance of sulfur in tissues. The latency effect that characterizes toxic exposures to MeHg or dimethy-Hg appear to be the result of the slow kinetics, but inexorable thermodynamics of the Hg-Se binding interaction and the remarkably effective Se-homeostatic control mechanisms. Enzyme inhibition reactions are of necessity bimolecular. Therefore, it is not surprising that the accuracy and reliability of prior MeHg exposure risk assessments have suffered as a result of mistaken assumptions that first-order or pseudo-first-order reaction mechanisms were occurring. The Se-sequestration molecular mechanism of MeHg toxicity may explain why whale or shark consumption has been shown to cause harm, but ocean fish consumption has repeatedly been shown to prevent rather than contribute to causing MeHg toxicity. In summation, the

abundance of Se in fish needs to be considered when evaluating risks associated with MeHg exposures.

Keywords: Methylmercury, Selenium, Selenoenzymes

Opening

CONFERENCE INTRODUCTION AND OVERVIEW OF THE THEME

Conference Co-Chairs: **Kenneth Reuhl,** *UMDNJ/EOHSI*
Jason Richardson, *UMDNJ/EOHSI*

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OVERVIEW: UNIFYING MECHANISMS OF NEUROLOGICAL DISORDERS: SCIENTIFIC, TRANSLATIONAL, AND CLINICAL IMPLICATIONS. Jason R. Richardson and Kenneth R. Reuhl, *EOHSI, UMDNJ and Rutgers University*

Through this conference, we have brought together experts in clinical medicine, basic science, toxicology, and translational research to explore common mechanisms of neurological disease with the goal of facilitating interactions and the transfer of knowledge *between groups that have not historically interacted to a significant degree*. We have chosen to focus on **two major common mechanisms** associated with a number of neurological disease states and three emerging areas of research that have the potential to benefit from increased focus. First, we will explore the role of **mitochondrial dysfunction**, which is a hallmark of several devastating neurological disease such as Parkinson's, Alzheimer's, Huntington's, and ALS. The salient questions relating to mitochondrial function in disease to be assessed in this session will include the role of mitochondrial dysfunction in pathogenesis, the role of environmental factors and gene-environment interactions, and how mitochondrial function can be targeted for therapeutic intervention. The second major common mechanism of focus will be on the role of **protein aggregation and misfolding** in neurological disease. Protein aggregation and misfolding have been linked to a variety of neurological disorders, both genetic and idiopathic in origin. However, there is currently controversy as to whether this process is a cause or consequence of the disease process. This session will bring together experts in neuropathology, clinical medicine, and basic researchers to evaluate the role of protein aggregation and misfolding in neurological disease and what information relating to pathogenesis and treatment that can be gleaned from examining common mechanisms affecting these pathways.

The **three emerging areas** that we have chosen for focus include epigenetics, channelopathies, and gender. First we will explore the role of **epigenetic alterations** in neurological disorders. Emerging evidence suggests that epigenetic alterations resulting from environmental exposures during critical periods of development may play a significant role in neurodevelopmental disorders. This session is designed to assess the current state of knowledge in this emerging area and identify common mechanisms involving epigenetic alterations that may provide insight into disease pathogenesis and ultimately, therapeutic intervention. Next we will address **channelopathies** and their importance in neurotoxicology. Channelopathies are thought to play an important role in epilepsy, chronic pain, and migraine. Although most of the focus has been on the role of genetic alterations in channel proteins, a variety of environmental toxicants target ion channels, which suggests the potential for environmental exposures to contribute to channelopathies. Finally, we will explore **gender differences in neurological disease**. The IOM report, "Does Sex Matter" (2001) emphasized the need for examining differences between male and female responses in the research enterprise because of wide gaps between males and females in disease patterns and in response to therapeutic practices and drugs. This symposium is designed to exemplify both current knowledge of sex differences in environmental chemical vulnerability and to propose insight into the relationship between the environmental exposures, gender, and the development of neurological disease.

We will wrap up the conference with Roundtable Discussion focused on **Unifying Mechanisms of Neurological Disorders: Scientific, Translational, and Clinical Implications: Summary and Recommendations.**

Plenary Session

SESSION III: MITOCHONDRIAL DYSFUNCTION IN NEUROLOGICAL DISEASE

Session Chair: **Cindy Lawler**
Co-Chair: **Flint Beal**

Theme and Rationale: *Emerging evidence has linked alteration of mitochondrial function to a variety of neurological disorders. Some of these alterations arise*

from mitochondrial DNA mutation. However, exposure to environmental toxicants that target the mitochondria directly or indirectly have been linked to disease etiology. This session seeks to bring together clinicians, basic researchers, and those involved in translational research to evaluate the etiology and pathogenesis of neurological diseases using mitochondrial dysfunction as a unifying feature. The session will provide a state of the art overview of what is currently known about mitochondrial dysfunction in neurological disease and continue with speakers performing cutting edge research on mitochondrial function and the interaction of genetic and environmental factors in mitochondrial dysfunction. Finally, we will end with clinical observations and a discussion on how the information discussed can be used to develop translational research aimed at clinical interventions.

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INTRODUCTION AND STATE OF THE ART OVERVIEW: MITOCHONDRIAL DYSFUNCTION. Tim Greenamyre, *Pitt and PIND*

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MEF2 AS AN INDICATOR OF MITOCHONDRIAL OXIDATIVE STRESS. Hua She Postdoctoral fellow, Qian Yang Postdoctoral fellow, and Zixu Mao Advisor. *Departments of Pharmacology and Neurology, School of Medicine, Emory University, Atlanta, GA, USA*

Transcription factor myocyte enhancer factor 2 (MEF2) is composed of four members, MEF2A-D. Various MEF2 isoforms are known to promote neuronal survival in several experimental paradigms. Our previous studies showed that enhancing MEF2 activity in substantial nigral protects dopaminergic neurons from 1-methy-4-phenyl 1,2,3,6, tetrahydropyridine (MPTP)-induced toxicity in mice. Both MEF2 activity and its protein level are highly regulated in neurons. Several key signaling pathways including p38MAPK and Cdk5 are involved in modulating MEF2 function in response to either trophic/survival or stress/death signals. Our studies indicate that oxidative stress can activate negative regulators of MEF2s such as Cdk5, leading to inhibitory phosphorylation of MEF2. Several neurotoxins used to induce Parkinson's disease in model animals are known to target mitochondrial complex I and to induce oxidative stress. Our recent studies suggest that these toxins modulate MEF2 activity. MEF2 is sensitive to and modified by toxin-induced oxidative stress. We will discuss these findings in the context of pathogenic process of Parkinson's disease.

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MITOCHONDRIAL DYSFUNCTION IN AN APP/PS1 MOUSE MODEL OF ALZHEIMER'S DISEASE. Rosemary Schuh[1,2], Anna Schlappal[3], and Paul Fishman[1,2]. *[1]Research and Development Service, VA Maryland Health Care System, Baltimore, MD; [2]Department of Neurology, University of Maryland School of Medicine, Baltimore, MD. [3]Neuroscience and Cognitive Sciences Program, University of Maryland, College Park, MD*

An intensively investigated hypothesis of human neurodegenerative disease involves the role of mitochondrial dysfunction. The mitochondrial electron transport chain (ETC) complexes have been determined to be sensitive targets in these debilitating diseases: Complex I dysfunction with Parkinson's disease, Complex II with Huntington's disease and Complex IV with Alzheimer's disease (AD). In AD, over-expression of human mutant forms of amyloid precursor protein (APP) and presenilin-1 (PS1) in mouse models have been used to examine indices leading to disease pathology. In the present study, oxygen consumption rates were assessed in non-synaptic mitochondria and synaptosomes isolated from APP/PS1 and wild type mouse brain (3-9 months) using either a Clark electrode or the Seahorse Bioscience XF24 flux analyzer. Levels of ETC, APP and PS1 protein levels were determined using western blotting. Amyloid plaque formation and cell viability in the hippocampal regions were assessed with congo red and cresyl violet staining. Additionally, organotypic hippocampal slice cultures (OHC) from APP/PS1 and wild type litter mates were exposed to specific ETC inhibitors to assess the potential utility of an in vitro model of AD. Non-synaptic mitochondria from APP/PS1 mouse brain have decreased respiratory rates and ETC protein levels compared to wild types and are present at time points prior to the appearance of increased amyloid plaque formation. OHC from APP/PS1 mice exposed to ETC respiratory inhibitors have increased sensitivity to cell death. Further study into mitochondrial dysfunction and its temporal correlation with plaque formation may provide new therapeutic strategies for amelioration of AD.

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CLINICAL OBSERVATIONS AND TRANSLATIONAL OPPORTUNITIES FOR TARGETING MITOCHONDRIAL DYSFUNCTION IN NEUROLOGICAL DISEASE. M. Flint Beal, *Weill Cornell Medical College*

There is increasing evidence that both mitochondrial dysfunction and oxidative damage play a critical role in the pathogenesis of neurodegenerative diseases. This appears to be the case in Alzheimer's Disease (AD), ALS, Huntington's Disease (HD), Parkinson's Disease (PD) and Friedreich's Ataxia. In these diseases, either animals models with toxins or causative mutations in genes encoding mitochondrial proteins have been linked to the disease pathogenesis. We have investigated a number of approaches to treating mitochondrial dysfunction. We have studied the effects of dietary supplements such as Coenzyme Q10 (CoQ10), creatine and resveratrol. We found neuroprotective effects of CoQ10 in transgenic mouse models of AD and HD and in the MPTP model of PD. Similarly, creatine has been shown to produce neuroportective effects in transgenic mouse models of ALS and HD as well as toxin induced models using 3-nitropropionic acid (3-NP) and MPTP. We showed that resveratrol is effective in preventing amyloid deposition in a transgenic mouse model of AD. Studies with both CoQ10 and creatine have moved into phase III clinical trials in PD, HD and Friedreich Ataxia. There is also promising evidence for CoQ in progressive supranculear palsy. We have also been studying agents, which can increase the transcription of genes, which encode antioxidant enzymes. We have carried out a number of studies using triterpenoids, which activate the Nrf2/ARE pathway. We found that these compounds are neuroprotective against 3-NP and MPTP neurotoxicity. We also showed that they are effective in transgenic mouse models of ALS, AD and HD. We also showed that small peptides termed the SS peptides are protective against the mitochondrial permeability transition and protect against MPTP neurotoxicity. These studies provide strong evidence that agents which target mitochondrial dysfunction or oxidative damage will produce neuroprotective effects *in vivo*, and a number of these approaches are now entering the clinic, with initial promising results.

Panel Discussion

Moderators

- Tim Greenamyre, *University of Pittsburgh*
- Cindy Lawler, *NIEHS*

Platform Session

SESSION IV: METALS AND SELECTED TOPICS

Session Chair: **Michael Aschner**
Co-Chairs: **Didima de Groot**
Laura Raymond

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NOVEL MECHANISMS OF MANGANESE TRANSPORT. C Au[1], A Benedetto[1], Z Yin[1], AB Bowman[2], M Aschner[1]. *Departments of Pediatrics[1] and Neurology[2], Vanderbilt University Medical Center, Nashville, TN, USA*.

Although manganese (Mn) is an essential trace element for human development and growth, chronic exposure to excessive Mn levels can result in psychiatric and motor disturbances, referred to as manganism. Little is known on mechanisms of Mn uptake and even less on its efflux. Recently, we have characterized the role of divalent metal transporter (DMT1)-like proteins in the nematode *C. elegans* in maintaining Mn homeostasis. We identified three DMT1-like genes in *C. elegans*: *smf-1*, *smf-2* and *smf-3.* Deletion of *smf-1* or *smf-3* led to increased Mn tolerance, and the loss of *smf-2* led to increased Mn sensitivity. Translational GFP-fusions established that SMF-1 and SMF-3 strongly localize to the gut epithelium, suggesting a differential role for SMF-1 and SMF-3 in Mn nutritional intake. In contrast, SMF-2 localized predominantly to the marginal pharyngeal epithelium. In additional studies, we tested the hypothesis that the cytoplasmic iron (Fe) exporter ferroportin (Fpn) also serves to transport Mn. Using an inducible human embryonic kidney (HEK293T) cell model, we examined the influence of Fpn expression on Mn-induced cytotoxicity and intracellular Mn concentrations. Induction of Fpn was cytoprotective against several measures of Mn toxicity and cytoprotection correlated with decreased Mn accumulation. In sum, we unraveled a complex interplay of transcriptional and post-translational regulations of 3 DMT1-like transporters in two adjacent tissues, which regulate metal-content in *C. elegans* and established a role for Fpn in regulating the uptake/efflux balance of Mn transport into HEK293T cells. (Supported by RO1ES010563, RO1ES07731, RO1ES016931 and W81XWH-05-0239).

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FUNCTIONAL MRI FINDINGS IN WELDERS EXPOSED TO MANGANESE. Yongmin Chang[1], Hui-Jin Song[1], Joon-Ho Ahn[2], Sin-Jae Park[2], Yangho Kim[3]. *[1]Department of Molecular Medicine, Kyungpook National University College of Medicines, Daegu, South Korea. [2]Department of Psychiatry, Ulsan University College of Medicines, Ulsan, South Korea. [3]Department of Occupational and Environmental Medicine, Ulsan University College of Medicines, Ulsan, South Korea*

We performed the first-ever functional magnetic resonance imaging (fMRI) experiment with sequential finger-tapping to investigate the behavioral significance of additionally recruited brain regions and with 2-back memory task to assess the neural correlates of manganese (Mn)-induced memory impairment in response to a subclinical dysfunction of working memory network in welders with chronic Mn exposure.

During the finger tapping task, activation of the bilateral primary sensorimotor cortex, bilateral supplementary motor area, bilateral dorsolateral premotor cortex, bilateral superior parietal cortex and ipsilateral dentate nucleus was higher in welding group than in control group. Our findings suggest that increased brain activation results from the compensational activation of ancillary cortical pathways, which ensures adequate motor function.

Our within and between group analysis revealed that welders with chronic Mn exposure showed increased brain activity in the working memory networks during the 2-back verbal working memory task compared to healthy controls. Our fMRI findings therefore implicated that welders might need more neural resources in working memory network to compensate the subtle working memory deficit and an altered working memory process even they performed the task at the same level as normal control subjects.

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HIGH-THROUGHPUT ASSAY TO ASSESS MANGANESE TRANSPORT KINETICS IN A STRIATAL CELL MODEL OF HUNTINGTON'S DISEASE. GF Kwakye, D Li, AB Bowman. *Dept. of Neurology, Vanderbilt Kennedy Center. Vanderbilt University Medical Center. Nashville, TN, USA.*

(SEE ABSTRACT IN POSTER SESSION ON PAGE 39 #P-83)

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PRENATAL METHYLMERCURY EXPOSURE INDUCES IN RATS STRUCTURAL BRAIN DEFICITS AT WEANING AND FUNCTIONAL IMPAIRMENT AT ADULT AGE. D. de Groot[1], M. Radonjic[1], M. Bogaard[1], N. Cappaert[3], R. Dierckx[2], F. Kuper[1], R. Nederlof[1], M. Verwei[1], B. Voet[1], E. de Vries[2], A. van Waarde[2], W. Wadman[3], A. Wolterbeek[1], R. Stierum[1]. *[1]TNO Quality of Life, Zeist, The Netherlands; [2]University Medical Center Groningen, PET Center Groningen, The Netherlands; [3]University of Amsterdam, the Netherlands*

Studies in man and animals have shown that certain developmental factors, e.g chemical exposure or (maternal) nutrition, may reprogram (neuro)physiological set points and have lasting consequences for health later in life. The aim of present research was to study effects of early diet focussing especially on 1) body weight (BW), 2) glucose tolerance and 3) vulnerability to early methylmercury (MeHg) exposure.

Three groups of female Wistar rats (n=24) were kept on a semi-synthetic control diet (n=24), a high-caloric diet (15% butter oil, n=48) or a caloric restricted (deficient) diet (75% of control diet, n=38) during 6 weeks premating, mating (1 week) and gestation (3 weeks) period. Pups were cross-fostered at birth to obtain 5 diet groups, i.e. (gestation/LACTATION): cont/CONT; high/HIGH; def/DEF; def/HIGH and high/DEF. A selection of male (n=100) and female (n=100) pups was additionally dosed sc. with saline (control) or MeHg (3 mg/kg BW) from PN2 to 21. BW and food intake were measured, behavior tested and neuropathology survey carried out, as well as glucose tolerance and fat tests and measurement of some (satiety) hormones.

The results showed that specifically the reprogrammed def/HIGH group showed 'catch-up growth' during lactation, persistently increased body weight at adulthood, and increased response in the glucose tolerance test, suggesting an increased prevalence for obesity and diabetes. Moreover, an increased vulnerability to MeHg was observed in the def/HIGH group exposed to MeHg, demonstrated by neural impairment in auditory startle response which was not observed by diet or MeHg exposure alone.

Speculating on the mechanisms underlying the findings in the def/HIGH group it is hypothesized that early dietary reprogramming affects the Hypothalamus

Pituitary Adrenal (HPA)-axis, leading to an increase in adipose tissue and increased levels of glucocorticoids. The latter, in turn might have caused a reduction in the threshold of reactivity to toxicity.

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SINGLE POSTNATAL METHYLMERCURY (MEHG) EXPOSURE INITIATES ACUTE STEM CELL DEATH IN HIPPOCAMPUS LEADING TO SUBSEQUENT NEURONAL DEFICITS AND LEARNING IMPAIRMENT. K. Sokolowski[1,2] Kelsey Robinson[1] and E. DiCicco-Bloom[1,2] *Joint Graduate Program in Toxicology, Rutgers[1]; Neurosci & Cell Biol, RWJMS, Piscataway, NJ[2]*

(SEE ABSTRACT IN POSTER SESSION ON PAGE 40 #P-87)

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SELENIUM PREVENTS AND REVERSES MERCURY TOXICITY LJ Raymond and NVC Ralston. *University of North Dakota Energy & Environmental Research Center, 15 North 23rd Street, Grand Forks, ND 58202 USA*

Since dietary selenium (Se) influences sensitivity to methylmercury (MeHg) toxicity, these studies examined the protective and therapeutic effects of dietary Se intakes at levels that reflect the nutritionally relevant dietary range. Torula yeast-based diets that were nutritionally complete but low in Se (0.1 µmol/kg; ~0.01 ppm), were used as the basal diet. Diets were supplemented with sodium selenite to either normal (1.0 µmol/kg) or rich (10 µmol/kg) levels or with Se from fish. Each diet was prepared without added MeHg (analyzed content; 0.5 µmol Hg/kg) or with MeHg added at 50 µmol MeHg/kg (10 ppm). Weanling male Long Evans rats were fed these diets ad libitum and monitored for effects on food consumption, growth, and motor function in a 3 × 2 feeding study. In the absence of added dietary MeHg, all rats grew normally and without statistically significant differences. High MeHg exposures were not consistently associated with depressed weight gains or neurotoxicity, but were uniformly proportional to Hg:Se molar ratios. Rescue of MeHg-intoxicated rats with dietary Se resulted in rapid recovery of growth and gradual resolution of neurotoxic effects. Recovery rates were dependent on dietary Se, but were only slightly affected by high MeHg maintained in the diets. In summary, dietary Se protects against and effectively treats MeHg toxicity.

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THE EFFECT OF ALUMINUM CHLORIDE ON COGNITIVE ABILITY AND RELATED PROTEIN IN apoE KNOCK OUT MICE. ZHANG Qin-li[1], LI Mei-qin[1], GUO Wei-li[1], Ce Zhang[2] and NIU Qiao[*1]. *Ministry of Education Key Laboratory, [1]Department of Occupational Health, Shanxi Medical University, Taiyuan 030001 P.R. China; Ministry of Education Key Laboratory, [2]Department of Physiology, Shanxi Medical University, Taiyuan 030001 P.R. China*

The aim of this study is to investigate the effects of aluminum chloride ($AlCl_3$),which was administered by lateral cerebral ventricle injection ,on spacial learning and memory and the expression of metabotropic glutamate receptor-subtype 1(mGluR1) in apoE knockout mice. After being successfully trained by Step through test, Step down test and Morris Water Maze (MWM), 16 mouse were evenly and randomly divided into 2 groups: control group and 5% $AlCl_3$ treated group. The $AlCl_3$-exposed group was exposed to $AlCl_3$ by lateral ventricle injection at a dose of 3µl/d for 5 days, while the controls were treated with NS in the same volume as the $AlCl_3$-exposed group. Cognitive ability was evaluated with Step through test, Step down test and MWM on the 15th day after Al treatment for 5 days. Cerebral cortex was token from the mice on 20th day after Al treatment for 5 days. mGluR1 in cytoplasm was detected by Western-blot, images were collected respectively by gelatum analyzing system and scanner. Data were analyzed by the gelatum analyzing system. The results were: Latent period of step down test and step through test were significantly decreased ($P<0.05$); it was significantly increased in the second day ($P<0.05$) compared with the control group . In MWM the time of place navigation significantly increase ($P<0.05$), the time of spatial probe was significantly shortened ($P<0.05$) compared with the control group. The expression of mGluR1 was significantly decreased in the 5% $AlCl_3$ treated group compared with the control group ($P<0.05$). In conclusion, The learning and memory ability and the expression of mGluR1 in apoE knockout mice were decreased obviously by $AlCl_3$ lateral ventricle injection.

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THE EFFECT OF ALUMINUM CHLORIDE ON COGNITIVE ABILITY OF APP/PS1 DOUBLE TRANSGENIC MICE AND EXPRESSION OF mGluR1. ZHANG Qin-Li[1], JIAO Xia[1], JI Xiu-liang[1],Ce Zhang[2] and NIU Qiao[*1]. *Ministry of Education Key Laboratory, [1]Department of Occupational*

Health, Shanxi Medical University, Taiyuan 030001 P.R. China; Ministry of Education Key Laboratory, [2]Department of Physiology, Shanxi Medical University, Taiyuan 030001 P.R. China

(SEE ABSTRACT IN POSTER SESSION ON PAGE 28 #P-61)

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ARSENIC INHIBITS NEURITE OUTGROWTH BY INHIBITING LKB1-AMPK SIGNALING PATHWAY. Xin Wang[1], Dan Meng[2], Qingshan Chang[1], Jingju Pan[1], Zhuo Zhang[1], Gang Chen[3], Zunji Ke[2], Jia Luo[3], and Xianglin Shi[1*]. *[1]Graduate Center for Toxicology, University of Kentucky, Lexington, KY 40536 USA [2]Key Laboratory of Nutrition and Metabolism Institute, Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, 200031, P.R. China [3]Department of Internal Medicine, University of Kentucky, Lexington, KY 40536 USA*

(SEE ABSTRACT IN POSTER SESSION ON PAGE 28 #P-60)

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IN VITRO NEUROTOXIC POTENTIAL OF AFGHANISTAN SAND EXTRACT. Krishnan Prabhakaran, Shawn McInturf, Michael Stockelman, Palur Gunasekar. *Naval Health Research Center Detachment/Environmental Health Effects Laboratory, WPAFB, Dayton, OH*

U.S. troops deployed to the Middle East are often exposed to dust storms with particulate matter (PM) levels above the military exposure guideline of 150 $\mu g/m^3$. High prevalence of adverse health effects among troops have been reported. Numerous metals (Ca, Si, Fe, Al, Mg, K, Ni, Mn, Cr) and pathogens (diphtheroids, streptococci) have been detected in the Middle East sand. Sand PM2.5 can damage alveolar macrophages and causes cytotoxicity/cell death leading to respitory ailments. It is proposed that fine and ultra fine particles may be translocated by circulation to other organs including brain. Soluble components of atmospheric dust may contribute to sand-induced neurotoxicity. Metals known to cause neurotoxicity (e.g., Mn) following inhalation are present in Afghanistan sand. Present study was undertaken to characterize the neurotoxic potential of the soluble components of Afghanistan sand extract using neuronal cell culture model. Sterilized Afghanistan sand samples (100% W/V) were extracted overnight in serum free media. Different concentrations of extracted samples (100-500 mg of sand/ml) were added to the MES 23.5 neuronal cells for 24 h and cytotoxicity assessed using cellular release of lactate dehydrogenase (LDH), and MTT assay. Dose-dependent increase in LDH release with significant increase at high concentration (500 mg/ml), and decreased MTT metabolism were seen. In addition, exposure to sand extract resulted in significant reduction in total antioxidant capacity (TAC) with an elevation of reactive oxygen species (ROS) and DNA damage as assessed by Comet assay. The antioxidant N-Acetyl Cystein (NAC) pretreated cells significantly decreased sand-induced ROS generation and cytotoxicity, indicating that Afghanistan sand produced neurotoxicity through oxidative stress mediated mechanism. Further, the toxicity was associated with mitochondrial dysfunction (reduced membrane potential) followed by release of cytochrome c and increased caspase activity. Our results demonstrate that soluble components of Afghanistan sand can be neurotoxic at sufficiently high concentrations from environmental exposure.

SESSION V: POSTER SESSION

Co-Chairs: **Kent Anger**
Richard Seegal
Dongren Yang

The Poster Session is a highlight of this conference series. It has proven to be an effective venue for informal, in-depth discussion and collaboration building -- as well as an important networking opportunity for all participants. Papers on any aspect of neuroscience, toxicology, children's environmental health, public health & policy are welcome! Posters may be put up as early as 9:00 AM on Sunday and should remain up for the conference duration for maximum exposure.

Poster abstracts are numbered from P-58 to P-100 and can be found on pages 22 - 40.

STUDENT AWARD COMPETITION

Co-Chairs: **Anumantha Kanthasamy**
Isaac Pessah

The Student Award Competition is divided into two groups: one for post-doctoral and one for pre-doctoral competition. Awards will consist of a cash prize, plaque or certificate, plus a one year subscription to the international journal NeuroToxicology. A winner(s) will be chosen from each group. Competing students are

asked 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 in competition for the Student Awards must be presented from poster. Judging will be done during this time.

Plenary Session

SESSION VI: PROTEIN AGGREGATION AND MISFOLDING IN NEUROLOGICAL DISEASE

Session Chair: **Kenneth Reuhl**
Co-Chair: **Peter Spencer**

Theme and Rationale: *Several neurological diseases including Alzheimer's, Parkinson's, and Creutzfeldt-Jakob disease share common features involving aggregation and deposition of misfolded proteins. However, there is currently controversy as to whether this process is a cause or consequence of the disease process. This session will bring together experts in neuropathology, clinical medicine, and basic researchers to evaluate the role of protein aggregation and misfolding in neurological disease and what information that can be gleaned from examining common mechanisms affecting these pathways. Finally, we will end with clinical observations and a discussion on how the information discussed can be used to develop translational research aimed at clinical interventions.*

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INTRODUCTION AND STATE OF THE ART OVERVIEW: PROTEIN AGGREGATION. Kenneth Reuhl[1] and Peter Spencer[2]. [1] *Environmental and Occupational Health Sciences Institute, UMDNJ and Rutgers University, Piscataway, New Jersey;* [2]*Oregon Health & Sciences University, Portland, Oregon*

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ALPHA-SYNUCLEIN, LRRK2 AND ENVIRONMENTAL FACTORS IN THE PATHOGENESIS OF PARKINSON DISEASE AND RELATED NEURODEGENERATIVES DISORDERS. BI Giasson. Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA.

The presence of protein aggregates comprised of various distinct polypeptides is a general theme characteristic of most neurodegenerative diseases. For example, α-synuclein can aberrantly polymerize into fibrils with typical amyloidogenic properties, and these fibrils are the major component of many types of pathological inclusions, including Lewy bodies. These lesions and other types of α-synuclein inclusions are associated with various neurodegenerative diseases, such as Parkinson disease. Although the function of α-synuclein is still not completely elucidated, genetic studies have unequivocally demonstrated that duplications, triplications or missence mutations of the *α-synuclein* (*SCNA*) gene can cause Parkinson disease or the related disorder dementia with Lewy bodies. Although there is substantial evidence supporting the toxic nature of α-synuclein inclusions, other modes of toxicity such as oligomers have been proposed. Several additional alterations in many other genes have been established to either cause or increase the risk of Parkinson disease. More specifically, autosomal dominant missense mutations in the gene for *leucine-rich repeat kinase 2* (*LRRK2/PARK8*) are the most common known cause of Parkinson disease. Various experimental studies, including the characterization of the effects of environments factors on genetic products involved in causing Parkinson disease, provide strong evidence indicating that environmental factors can have an important role in influencing disease presentation and progression. A better fundamental understanding of the interactions between various genetic and environments factors should provide disease modifying opportunities.

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DOES EARLY-LIFE GENOTOXIN EXPOSURE TRIGGER PROTOTYPICAL NEURODEGENERATIVE DISEASE BY INDUCING PERSISTENT GENOMIC DYSREGULATION? QUERYING DNA REPAIR-DEFICIENT MICE. MIT-OHSU-UMDNJ-UNC-UW Star Project Toxicogenomics Team, presented by PS Spencer. *Oregon Health & Science University, Portland.*

Three genetically distinct populations in Asia-Oceania have experienced very high rates of amyotrophic lateral sclerosis (ALS), parkinsonism (P) and/or dementia (D). Changing clinical patterns, declining rates, and increasing age at diagnosis, suggest an etiology dominated by a disappearing xenobiotic common to the three affected populations. The leading etiologic candidate is the cycad plant, the neurotoxic seed of which has been used by disease-affected patients as an oral tonic (Honshu Island), poultice (Guam/Rota, New Guinea), and foodstuff/confection

(Guam/Rota). In Guam, there is a strong association between historical disease rates among indigenous Chamorros and their flour content of cycasin, the glucoside of methylazoxymethanol (MAM); this is a potent cycad genotoxin that perturbs brain development and, as a metabolite of azoxymethane, induces malignant colonic tumors in rodents. In the azoxymethane model, MAM-induced DNA lesions (i.e., O^6-methylguanine, O^6-mG) cause mutations and hypomethylation that affect key cell signaling pathways and precipitate uncontrolled cell proliferation. We explored the effects of MAM-induced genomic instability on brain transcription in 11-week C57BL6 wild-type mice and age-matched O^6-mG methyltransferase knock-outs (*Mgmt*$^{-/-}$). Animal age corresponded to 20-40 year-old humans who normally have low brain Mgmt activity and, for Chamorros, are at high risk for cycad-associated P-D and D. Brain gene expression patterns were determined 6h, 24h, 48h, or 7 d after a single i.p. treatment with MAM (20 mg /kg)or vehicle (0.05% acetic acid). Genes modulated by MAM were anchored to O^6-mG levels and compared by genotype and treatment. MAM induced changes in cell signaling pathways linked to neurodevelopment, neurodegeneration, epigenesis, and cancer. These data suggest an overlap between mechanisms of MAM-induced malignancy and neurotoxicity. Supported by NIEHS Toxicogenomics Research Consortium.

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DOES EARLY-LIFE GENOTOXIN EXPOSURE TRIGGER PROTOTYPICAL NEURODEGENERATIVE DISEASE BY INDUCING PERSISTENT GENOMIC DYSREGULATION? QUERYING HUMAN TAU OVEREXPRESSING MICE. GE Kisby, P Renslow, T Park, R Woltjer. *Oregon Health & Science University, Portland, OR, USA.*

One of the best-studied neurodegenerative disorders with an environmental etiology is western Pacific ALS/PDC. Individuals with ALS/PDC develop neurofibrillary tangles (NFTs) that are biochemically and morphologically indistinguishable from Alzheimer disease (AD) suggesting a common pathogenic mechanism. Exposure of children, adolescents or young adults to cycad chemicals is a strong etiological factor for Guam dementia (GD) and PDC (Borenstein *et al.,* 2007). These studies suggest that early life exposure to cycads, a plant containing the genotoxicant methylazoxymethanol (MAM), is possibly associated with a reported inherent dysregulation of normal human tau (Sieh *et al.,* 2009) to cause the tau pathology in GD and PDC. To test this hypothesis, we examined the influence of MAM on normal human tau in an established animal model of tauopathy: the hau mouse. htau mice develop neuronal loss and tau pathology in a time course and distribution that are comparable to those in GD and PDC. Young (PND3) htau mice were treated once with MAM and the brain examined 1-day, 3 wks, or 3 mos later for DNA damage (i.e., O^6-mG), tau isoforms, and the accumulation of pathogenic forms of tau (i.e., tau oligomers, truncated tau, and phosphorylated tau). DNA damage is an early event in the brain of MAM-treated htau mice, whereas the accumulation of tau isoforms and pathogenic forms of tau are latent events. MAM also activated cellular enzymes (tau kinases) that phosphorylate tau. These findings strongly suggest that the genomic dysregulation produced by MAM during early brain development perturbed neuronal processes that regulate the synthesis, structure and phosphorylation of normal human tau. Such mechanisms might explain how cycad chemical exposure, acting in combination with the inherent tau dysregulation, contributes to the NFTs in GD and PDC. Related tauopathies, notably AD, should be investigated for exposure to substances that mimic the action of MAM (e.g., nitrosamines). Supported by Oregon Partnership for Alzheimer's Research (OPAR).

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ROLE OF METALS IN PRION PROTEIN UPREGULATION AND AGGREGATION. Anumantha G. Kanthasamy, *Iowa Center for Advanced Neurotoxicology, Biomedical Sciences, Iowa State University, Ames, IA, USA*

The key biochemical event in the pathogenesis of prion diseases is the conversion of normal cellular prion proteins (PrP^C) to the proteinase K (PK) resistant, abnormal form (PrP^{SC}); however, the cellular mechanisms underlying the conversion remain enigmatic. Binding of divalent cations such as copper to the octapeptide repeat regions of PrP has been shown to be important for the stability of the protein. Nevertheless, the roles of other divalent cations in the normal processing of cellular PrP^C are not well understood. Recently, we showed that prion protein interact with divalent metals and attenuates manganese-induced neurotoxicity. We also showed that cellular prion protein attenuates oxidative stress induced activation of proapoptotic kinase PKCdelta. Additionally, we examined the role of transition metals

(Mn^{2+} and Cu^{2+}) on PrP^C expression and degradation in cell culture and brain slice models. Neuronal cells expressing mouse prion proteins with a genetically altered novel epitope (mAb 3F4) and brain slices were exposed to Mn^{2+} and Cu^{2+} over 24hr. Levels of PrP^c protein and mRNA were measured. Limited proteolysis, mRNA stability, proteasomal activity and pulse-chase experiments were conducted. Metal treatment increased PrP^C levels in both cytosolic and membrane-rich fractions in a time-dependent manner. However, metal treatment neither increased PrP mRNA transcripts nor altered the mRNA stability, indicating that Mn may act at the post-translation and/or degradation levels of PrP^c. Additionally, metal exposure did not alter the proteasomal or lysosomal degradation pathway. Pulse-chase analysis showed that the PrP^c turnover rate was significantly decreased with metal treatment. Limited digestion with PK also revealed that metal treatment decreased the digestion rate of PrP^c. These data suggest that divalent metals can alter the normal processing of PrP^c, resulting in the accumulation of PrP^c with altered susceptibility to PK. Mn treatment also increased the formation of prion oligomers. Additionally, exposure of manganese to an infectious prion cell model, mouse RML-infected CAD5 cells, significantly increased prion protein levels. Collectively, these data suggest that divalent metals can alter the normal processing of PrP^c, resulting in the accumulation of PrP^c with altered susceptibility to PK. These findings may provide critical mechanistic insights into the pivotal role of metals in the pathological progression of prion disease and other proteinopathies, including Parkinson's disease. (supported by DoD/MHRP grant W81XWH-05-10239 and RO1ES10586)

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CONTRIBUTIONS OF GLYCOLIPID-PROTEIN INTERACTIONS TO NEURONAL VULNERABILITY. A.B. Manning-Boğ. *SRI International, Menlo Park, CA 94025 USA*

Clinical, genetic and neuropathological analyses have provided evidence for a link between Gaucher disease and alpha-synucleinopathies, in particular Parkinson's disease. To better understand the biochemical and pathological substrates that may underlie this association, nigrostriatal alterations were evaluated in a pharmacological model of Gaucher disease. First, we examined changes in alpha-synuclein, associated with diminished glucocerebrosidase activity caused by acute exposure of C57BL6 mice to the irreversible inhibitor conduritol B epoxide (CBE). A single systemic administration of the inhibitor (200 mg/kg, i.p.) revealed enhanced alpha-synuclein in CBE vs. vehicle-treated in neurons and astrocytes in ventral mesencephalon, along with markers of neurodegeneration. In preliminary experiments using a subchronic treatment paradigm, CBE exposure produced a modest but significant decrease in striatal dopamine and metabolite values as compared to vehicle-treated mice with no alteration in dopaminergic or total neuronal cell counts at the level of the substantia nigra. These findings suggest that glucocerebrosidase inhibition affects vulnerability within the nigrostriatal pathway and shared pathogenic mechanisms may contribute to Gaucher disease and parkinsonism. This work is funded by the John A. Blume Foundation and SRI International.

Panel Discussion

Moderators:

- Benoit Giasson, *University of Pennsylvania*
- Kenneth Reuhl, *Environmental and Occupational Health Sciences Institute, UMDNJ & Rutgers University*
- Peter Spencer, *Oregon Health Sciences University*

Platform Session

SESSION VII: PESTICIDES, OR TBA

Session Chair: **Brenda Eskenazi**
Co-Chair: **Charles Timchalk**

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PON1, ORGANOPHOSPHATE EXPOSURE, AND NEURODEVELOPMENT IN TODDLERS LIVING IN AN AGRICULTURAL COMMUNITY: THE CHAMACOS STUDY. Brenda Eskenazi, Amy Marks, Karen Huen, Kim Harley, Asa Bradman, Dana Barr, Nina Holland. *Center for Children's Environmental Health Research, School of Public Health, University of California, Berkeley 94720; Emory University, Atlanta, Georgia.*

We examined the relationship of PON1 genotype and enzyme activity in relation to neurobehavioral measures in two year old children whose mothers were enrolled during pregnancy in the CHAMACOS study. These women were primarily Latinas, recently from Mexico, and living in farmworking households in the

agricultural community of Salinas, California. We previously reported that women's levels of DAPs metabolites of OP pesticides during pregnancy were related to their 2-year olds' mental development and reported symptoms of pervasive developmental disorder (PDD) (Eskenazi et al. 2007). In the present study, we examine whether the mother and child's PON1 genotype and associated enzyme activity levels are related to these same outcomes and whether genotype and enzyme levels modify the associations with DAPs. We found that the *PON*-$_{108T}$ allele in children was associated with poorer mental development and with somewhat poorer psychomotor development on the Bayley tests. Maternal, cord, and child enzyme levels, although related to genotype, were not significantly related to these neurobehavioral outcomes except that children were less likely to display symptoms of PDD when they or their mothers had higher AREase and when their mothers had higher POase concentrations. Although we have observed a relationship of DAPs and neurobehavioral outcomes and of PON1 genotype/enzyme and these same endpoints, we are less certain as to whether genotype or enzyme modifies the association of DAPs and neurobehavior, that is, whether a subgroup of children by virtue of their genetic make-up are more susceptible to the adverse effects of maternal exposure to OP pesticides during pregnancy. There is a suggestion with mental development that children with *PON*$_{-108T}$ allele are more affected by OP exposure *in utero* but the interaction is not significant. We will also results of PON and attention.

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***PON1* POLYMORPHISMS, ORGANOPHOSPHATE (OP) EXPOSURE, AND PARKINSON'S DISEASE (PD).** F Kamel, S Goldman, D Umbach, J Hoppin, M Richards, M Korell, G Bhudhikanok, C Meng, W Ross, A Blair, W Langston, D Sandler, C Tanner. *NIEHS, NIH, DHHS, Research Triangle Park, NC, USA.*

Pesticide exposure has been associated with PD; some studies implicate OPs. The enzyme paraoxonase may affect OP toxicity by metabolizing OP oxon derivatives. Two coding polymorphisms in the paraoxonase gene *PON1,* Q192R and L55M, may alter enzyme activity toward specific substrates. We studied *PON1* in FAME, a case-control study of PD nested in the Agricultural Health Study (AHS), a cohort of pesticide applicators and their spouses. Movement disorder specialists verified diagnoses of incident PD cases (n=52) who were compared to matched controls (n=370). We collected data on OP use at AHS enrollment and genotyped *PON1* on an Illumina platform. We report odds ratios and 95% confidence intervals from logistic regression models with adjustment for age, sex, and smoking. Of seven specific OPs, PD was positively associated with two [parathion, 1.5 (0.7-3.2); phorate, 1.7 (0.8-3.4)] and inversely associated with one [diazinon, 0.5 (0.2-0.9)]. *PON1* Q192R was weakly associated with PD [RR vs QQ: 1.7 (0.7-4.5)]. Though estimates were imprecise, some but not all OPs appeared more strongly associated with PD in 192R carriers than in QQ homozygotes (fonofos, 2.2 vs 1.3; malathion, 2.4 vs 1.7; phorate, 2.3 vs 1.6; terbufos, 1.8 vs 1.5). *PON1* L55M was not associated with PD nor did it affect the OP-PD relationship. These results suggest that some OPs may increase PD risk and that *PON1* may modify the association of PD with some OPs.

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POTENTIAL DOSIMETRY IMPLICATIONS FROM LOCALIZED BRAIN METABOLISM OF ORGANOPHOSPHORUS INSECTICIDES IN THE PREWEANLING RAT. C. Timchalk. *Biological Monitoring & Modeling, Pacific Northwest National Laboratory, Richland, WA, USA*

The developing brain is uniquely vulnerable to xenobiotic exposures at levels that do not appear to adversely impact adult brain function . Emerging evidence implicates low-level chemical exposures as potential causative factors. Organophosphorus (OP) insecticides are well established neurotoxicants based upon their ability to inhibit acetylcholinesterase (AChE), in both the central and peripheral nervous systems. However, recent studies suggest that OP insecticide neurotoxicity can occur at low-doses, even in the absence of significant brain AChE inhibition. We are testing the hypothesis that low-dose exposures of preweanling rats to OP insecticides will result in differential brain region dosimetry, enhancing localized brain metabolism, potentially resulting in subtle changes in brain chemistry. The implication of localized heterogenous CYP450 brain metabolism has historically not been extensively investigated, but recent research suggests it is of key importance. Comparative in vitro metabolism stUdies were conducted with hepatic and brain microsomes prepared from na'ive adult male rats . In addition, the liver microsomes and brain homogenates were also analyzed for CYP2D6 activity, since this CYP450 is one of several that are of key

importance for OP metabolism. Consistent with the literature the overall brain microsomal metabolism was a fraction (-1 %) of the liver, yet the CYP2D6 was highly expressed. Groups of preweanling pups were orally administered 5 daily doses of the OP insecticide chlorpyrifos (CPF) at 1 and 5 mg/kg/day beginning on post-natal day-10 (PND-10) and individual pups were humanely sacrificed 4 hour after the last dose (PND-15). Brains were dissected into forebrain, midbrain and cerebellum and analyzed for AChE activity, CPF and trichlorpyridinol (TCPy). In addition, levels of CPF and TCPy in whole brains from adult rats and PND-15 pups were also compared. In these preliminary experiments age-, dose-, gender-and brain regional-differences were observed . These are the first experimental results that quantified the brain concentrations of chlorpyrifos and the major metabolite in vivo, and report differences in regional brain deposition and localized metabolism. The importance of localized brain metabolism is highly relevant for lipophilic pesticides that sequester in the lipid rich regions of the brain and can undergo localized metabolic activation to produce neurotoxic effects. This is particularly important in juvenile animals, and children, where there may be a disproportionate deposition of the parent pesticide in the brain. (Supported by: CDC/NIOSH grants R01-0H003629 and R01-0H008173).

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PESTICIDE EXPOSURE AND NEUROBEHAVIORAL PERFORMANCE IN ADOLESCENT PESTICIDE APPLICATORS. DS Rohlman[1], A Ismail[2], O Hendy[3], G AbdelRasoul[2]. *[1]Center for Research on Occupational and Environmental Toxicology, Oregon Health & Science University, Portland OR USA; [2]Public Health and Community Medicine Department, Faculty of Medicine, Menoufia University, Shebin Elkom, Egypt; [3] Liver Institute, Faculty of Medicine, Menoufia University, Shebin Elkom, Egypt.*

Agricultural workers, including both adolescents and adults, are at risk for many occupational hazards including workplace injuries and exposure to pesticides. Adolescents working in agriculture are exposed to the same risks as adults but it is unknown whether their risk is equivalent to or greater than that of adults. Experimental animal studies indicate that the developing brain is more susceptible to the neurotoxic effects of OPs than the adult brain, and low-level exposures to OP pesticides cause significant neurobehavioral deficits in animal research. Although we have evidence of neurobehavioral deficits in adolescents working as pesticide applicators compared to controls, there is no quantitative exposure data available for adolescent applicators. Adolescents in Egypt are hired seasonally to work as pesticide applicators for the cotton crop. The pesticide application to the cotton crop is highly regulated and standardized across Egypt, and has been limited primarily to organophosphorus pesticides, generally chlorpyrifos, in recent years. Adolescent applicators (n=21) and Controls (n=20) completed a battery of neurobehavioral tests, an occupational and symptoms questionnaire and a medical exam. Blood and urine samples were collected to evaluate biomarkers of OP exposure. Applicators had higher levels of pesticide metabolite in their urine (TCPy) and decreased neurobehavioral performance compared to controls.

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BIOMARKERS OF ORGANOPHOSPHORUS (OP) EXPOSURES IN HUMANS. J Marsillach[1,2], RJ Richter[1,2], JH Kim[3], RC Stevens[1,2], MJ MacCoss[2], D Tomazela[2], SM Suzuki[1,2], LM Schopfer[4], O Lockridge[4], CE Furlong[1,2]. *Univ. of Washington, Departments of [1]Medicine (Div. Medical Genetics), [2]Genome Sciences, [3]Anesthesiology, Seattle, WA, [4] University of Nebraska Medical Center, Omaha, NE*

(SEE ABSTRACT IN POSTER SESSION ON PAGE 37 #P-80)

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CAN COMPARISONS OF AGE-RELATED MILESTONES HELP EQUATE MATURATION IN EXPERIMENTAL SPECIES TO HUMAN DEVELOPMENT? Barbara Clancy. *Department of Biology, University of Central Arkansas, Arkansas USA.*

One of the most important challenges in basic and applied neurotoxicology research is finding an optimal, efficient, and economical way to compare developmental stages in model species to those in humans. For a variety of theoretical, evolutionary, and pragmatic reasons, we have developed a statistical model to compare, and when appropriate to equate, a database of developmental milestones across mammalian species. Recently we included postnatal data in our model with some surprising results. Although "sexual maturation" milestones (both male and female) are data-points some researchers routinely use to equate postnatal development across species, we find these events do not fit our model, which now

includes over 220 developmental events in 11 species. However, the onset of some motor behaviors, including crawling and walking, can be statistically related across species.

Keywords: cross-species development; mammals; statistical modeling

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A NEUROLOGICAL ASSESSMENT OF A COMMUNITY WITH GROUND WATER EXPOSURE TO PERCHLOROETHYLENE. JS Rutchik, MD, MPH[1] and R Wittman, MD, MPH[2]. [1]*Neurology, Environmental and Occupational Medicine Associates. Mill Valley, CA and UCSF Division of Occupational Medicine.* [2]*Stanford Hospital and Clinics, Occupational Health Services, Palo Alto, CA.*

Exposure to mixed organic solvents, including PCE (perchloroethylene), has been associated with clinical symptoms and nerve conduction study (NCS) changes consistent with peripheral neuropathy (PN) as well as with color vision (CV) and contrast sensitivity deficits (CS). A physician board certified in both Neurology and Occupational Medicine examined a cohort of 27 community residents ranging from 5 to 85 years of age, referred for medical legal independent evaluations, with documented household well-water contamination with PCE in order to investigate for the presence of NCS abnormalities, CV changes, and CS abnormalities.

The source of these exposures may have been from either or both, abandoned 55 gallon drum tanks, and a local dry cleaning establishment. Cases referents were both children and adults who had resided in homes with detectable PCE in the well water. Exposure validity was based on record review and direct case interview. Peak well-water measurements were reported NCS testing was performed bilaterally in the upper and lower extremities. CV acuity was assessed by Farnsworth D-15 testing and CS was measured by placing Vistech charts (VCTS 6000) in front of the subjects as per protocol.

Of the 27 cases, 22 were determined to have sustained verifiable exposure to the implicated well-water. Residential well-water peak sampling levels ranged from 15.7 to 576 ppb of PCE in this exposed cohort. The mean duration of exposure was 16.7 years. Of these 22 individuals, 17 participated in NCS testing and 19 completed both CV evaluation and CS testing. In these groups, we found no evidence of PN, CV deficits, or CS dysfunction that could be attributed to this exposure.

Exposure to PER has been linked with various disturbances of the central and peripheral nervous system. In this case series, we found no association between chronic PCE well-water exposure and deficits on NCS, CV, and CS testing. It should be noted that we examined this cohort approximately 8 years following cessation of exposure. Although loss of CV and CS dysfunction has been reported following PCE exposures, this deficit has been documented during on-going exposure. While the literature is unclear about the reversibility of these deficits, our negative result may reflect the time lapse since the period of active exposure. Further research into the effects of chronic environmental level exposures is recommended, particularly at a time interval more proximal to the active exposure itself.

Keywords: perchloroethylene; neurotoxicity; nerve conduction studies

Plenary Session

SESSION VIII: EMERGING AREAS OF RESEARCH: EPIGENETIC MECHANISMS IN NEURODEVELOPMENTAL DISORDERS

Session Chair: **Jason Richardson**
Co-Chair: **M. Daniele Fallin**

Theme and Rationale: *Although genetic factors have long been a primary focus in neurodevelopmental disorders, exhaustive candidate gene studies and genome-wide association studies have not identified single genes that account for more than a small proportion of the disease burden in a variety of disorders. Emerging evidence suggests that epigenetic alterations resulting from environmental exposures during critical periods of development may play a significant role in neurodevelopmental disorders. This brainstorming session is designed to assess the current state of knowledge in this emerging area and identify new opportunities and research directions to assess the role of epigenetic alterations in neurological disease. Discussion will focus on the potential for epigenetic alterations to provide insight into disease pathogenesis and ultimately, therapeutic intervention.*

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INTRODUCTION AND STATE OF THE ART OVERVIEW: EPIGENETICS. Helmut Zarbl, *Environmental and*

Occupational Health Sciences Institute, UMDNJ and Rutgers University, Piscataway, New Jersey.

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GENETIC EPIDEMIOLOGY AND EPIGENETICS IN NEUROLOGICAL DISORDERS. M. Daniele Fallin. *Johns Hopkins School of Public Health, Baltimore, Maryland.*

Epigenetic mechanisms, such as methylation, are heritable during cell replication, but not contained in the DNA sequence itself. Epigenetic marks contribute to the regulation of gene expression and are thus an important piece of information when considering the effect of genetic variation on human disease. Further, unlike sequence variation, epigenetic marks are affected by the environment, as well as influenced by genes. Thus, epigenetic measurement is an essential complement to current epidemiologic studies focused on genes and/or environments. In this talk, I will discuss several models for the role of epigenetics in disease risk including as a direct cause of disease, as a gene effect modifier, as a mediator of genetic or environmental causes, or as a biomarker of disease. Each of these models implies a different set of hypotheses and approaches to research regarding the relationship between epigenetics and neurodevelopmental diseases.

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EPIGENETICS IN NEURODEVELOPMENTAL DISORDERS AND NEURTOXICOLOGY. Jason R. Richardson. *Environmental and Occupational Health Sciences Institute, UMDNJ and Rutgers University, Piscataway, New Jersey.*

Recently, interest has turned to epigenetic alterations as contributing factors in neurodevelopmental disorders. One of the first indications that epigenetic alterations were involved in neurodevelopmental disorders came with the discovery that Rett syndrome was the result of X-linked inactivation of the methyl CpG binding protein (MeCP2). MeCP2 mediates transcriptional repression through binding to CpG islands in the promoter regions of genes and through associations with histone deacetylases and histone methyltransferases. DNA methylation is a key player in regulation of gene expression along with histone deacetylation, chromatin modification, and RNA interference. Working in concert, these mechanisms regulate the appropriate expression of genes in a time- and tissue-specific manner throughout development. Thus, alterations of the epigenetic machinery can have a powerful impact on the proper expression of genes, and potentially lead to significant alterations in development that result in disease and dysfunction. Furthermore, evidence has been accumulating that environmental influences during critical periods of development can lead to disease later in life, and that these exposures may mediate their effects through epigenetic mechanisms. An important example of this is the finding that children born during the Dutch Famine have increased risk of schizophrenia and that these children also have decreased DNA methylation in the imprinted IGF2 gene. Additional work in experimental animals has found that exposure to environmental toxicants, such as bisphenol A, lead, methoxyclor, and vinclozin alters DNA methylation and produces epigenetic changes associated with various toxicities later in life. In this talk, I will discuss several findings regarding the potential role of epigenetic alterations in neurodevelopmental disorders and the potential contributions of environmental exposures to disease etiology by acting through epigenetic mechanisms.

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ENVIRONMENTAL NEUROTOXIC PESTICIDE INDUCED HISTONE ACETYLATION AND ITS ROLE IN EPIGENETIC MECHANISMS OF NEURODEGENERATION. Song C, Kanthasamy A, Anantharam V, Sun F, Kanthasamy AG. *Iowa Center for Advanced Neurotoxicology, Department of Biomedical Sciences, Iowa State University, Ames, IA 50011, USA.*

(SEE ABSTRACT IN POSTER SESSION ON PAGE 38 #P-81)

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EARLY CHILDHOOD LEAD EXPOSURE ALTERS THE DNA METHYLATION PATTERN IN CHILDREN. Douglas M. Ruden, *Institute of Environmental Health Sciences, Wayne State University, Detroit, MI, USA.*

In order to comprehensively determine which genes are affected by early childhood lead exposure, we did pilot-feasibility studies of 28 dried blood spots (DBS) from anonymous 3-5 year old children with lead levels that ranged from 3 to 16 μg/dL using the Infinium Assay from Illumina that can measure DNA methylation (mC) levels at 27,578 CpG sites in over 14,000 genes. We identified 423 genes with significant positive correlations and 728 genes with significant negative correlations with lead and mC levels ($p < 0.05$, not corrected for multiple comparisons). Gene Ontology (GO) analyses of these 423 genes with the program

DAVID reveals that there is a five-fold enrichment in the GO category "Disease mutations" containing 49 genes, such as genes involved in retinoblastoma (RB1), breast cancer (BRCA1), and Wilm's tumors (WT1). This data supports the Barker Hypothesis that early life exposures to lead can affect the development of later diseases. In addition to the 423 genes with significant ($P < 0.05$) positive correlations between Pb and DNA methylation, there are also 728 genes with significant negative correlations. The two most significantly negatively correlated CpG methylations were near the genes Cdkn1c ($P = 0.00024$) and Ptprm ($P = 0.000015$). Cdkn1c is a cyclin-dependent kinase inhibitor that is imprinted (OFF on the paternal chromosome and ON on the maternal chromosome). Ptprm encodes a protein tyrosine phosphatase that down-regulates growth factor signaling. The Preliminary identification of Cdkn1c and Ptprm is especially exciting because of similar findings from another laboratory. Le-Niculescu and colleagues recently found that gene expression levels in human blood can be used to identify useful biomarkers for neuropsychiatric disorders, including Ptprm..

Panel Discussion/Brainstorming

Moderators:

- M. Daniele Fallin
- Jason Richardson

Symposium

SESSION IX: NEUROTOXIC EFFECTS OF ENVIRONMENTAL EXPOSURE TO MANGANESE IN CHILDREN AND ADULTS

Session Chair: **Donna Mergler**
Co-Chair: **Roberto Lucchini**

Theme and Rationale: *Since Couper first identified manganese intoxication in 1837, there have been hundreds of reports of neurologic disorders and neurobehavioral deficits due to high levels of occupational exposure to manganese. Since manganese is an essential element and subject to homeostatic control, there has been little concern about possible neurotoxic effects of environmental exposure through air and water. Recent studies with children and adults living in the vicinity of manganese mines and processing plants and with children exposed to manganese through drinking water, suggest that environmental manganese exposure is associated with loss of nervous system function. This is an emerging field and many questions still need to be answered: What are the effects throughout the lifespan? What is the contribution of in utero exposure? What is the most adequate biomarker of exposure? Are current guidelines protective for health? In this session, researchers from several countries present their studies on environmental exposure to manganese.*

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NEUROBEHAVIORAL EFFECTS OF INHALED MANGANESE IN ITALIAN ADOLESCENTS. Roberto Lucchini, *University of Brescia, Occupational Health, Brescia, Italy*

Background and Objective: Increased parkinsonism was observed in Valcamonica, a valley in the province of Brescia, Italy. Prevalence data were higher in the vicinities of ferroalloy plants and associated to the concentration of manganese in deposited dust. The aim of the present study was to assess motor, cognitive and neurosensory functions in adolescents in the exposed area and in a reference area. Methods: Metals were measured in airborne particles collected with 24-hours personal samplers, and in various environmental media. Soil was analyzed at surface and 10cm depth. Adolescents were recruited through the local school system for neurobehavioral examination and assessment of dietary intake of metals. Blood and urine samples were collected for metal analysis. Results: A total of 303 children residing in an exposed area and a reference area participated in the study. Airborne manganese was 84.38±89.65 ng/m3 in the exposed area and 22.79±23.82 ng/m3 in the reference area. Lead, iron, zinc and chromium also showed significantly higher levels. Manganese results were significantly higher also at the surface and at 10 cm depth of soil and in salad. Exposure biomarkers were not significantly different between the two areas. Children in the exposed area showed impairment of motor coordination and odour identification associated with airborne manganese at multivariate analysis. Blood lead was inversely associated with IQ. Conclusion: Environmental exposure to manganese in adolescents is related to deficit in motor and olfactory functions whereas concomitant lead exposure is related to decrease of IQ. Acknowledgement: This work was partially supported by the EU through its Sixth Framework Programme for RTD (contract no FOOD-CT-

2006- 016253). It reflects only the authors' views. The European Community is not liable for any use that may be made of the information contained therein.

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ELEVATED MANGANESE AFFECTS MOTHERS' AND CHILDREN'S COGNITION. José A. Menezes-Filho, Cristiane de O. Novaes, Ciro R. Paes, Josino C. Moreira, Paula N. Sarcinelli & Donna Mergler. *Laboratory of Toxicology, College of Pharmacy, Federal University of Bahia, Salvador, Bahia, Brazil.*

There is growing evidence that excess manganese (Mn) in children is associated to neurobehavioral impairments. In Brazil, hair Mn concentrations among children living in the vicinity of a ferro-manganese alloy production plant, are highest nearest and downwind from the plant. Little is known about the effects of airborne Mn on children's and their parents' cognitive functions. This study sought to investigate the relations between biomarkers of Mn exposure (hair and blood) and children's neurobehavioral performance and to examine the relation between mothers' hair Mn concentrations and their performance on a test of intelligence. In a cross-sectional study, we assessed Mn blood and hair levels, blood lead (PbB) and cognitive performance, using the WISC-III, for 83 children aged 6 to 11 years and 11 months. The Raven Progressive Matrix was administered to the mothers and their hair Mn was determined. An interview administered questionnaire served to collect socio-demographics. Mean blood and hair Mn were 8.2 µg/L (range: 2.7 - 23.4) and 5.83 µg/g (range: 0.1 - 86.68 µg/g), respectively. Mean PbB was 1.43 µg/dL (range: 0.2 - 10.35 µg/dL). Mean maternal MnH was 3.50 µg/g (range = 0.10 - 77.45), and was significantly correlated (rho=0.29, p=0.01) with children's MnH levels. Children's MnH concentrations were significantly and negatively, related to Full-Scale (FS) and Verbal IQ. After adjusting for maternal education, nutritional status the coefficients for Mn were β=-5.78, p=0.03 and β=-6.72, p=0.02 for FS and Verbal IQ, respectively. MnH levels explained 5.6% and 6.8 % of the variance of the respective IQ scores. Maternal MnH was negatively associated with performances on the Raven's score (β=-2.689, p=0.055), after adjusting for education years, family income and age (partial r^2 for Mn=5.6%). The present study confirms that high MnH levels in children are associated with cognitive deficits, especially in the verbal domain. Mother's IQ is likewise associated to Mn exposure, suggesting that, in this situation of environmental airborne exposure to Mn, children's cognition may be doubly affected by exposure to airborne Mn.

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EFFECTS OF ENVIRONMENTAL EXPOSURE TO MANGANESE ON MOTOR FUNCTION OF CHILDREN IN MEXICO. Riojas Rodríguez Horacio, Ph.D. Hernández Bonilla David, Npsic. Schillman Astrid, McSc. *Instituto Nacional de Salud Publica, Mexico.*

Objective: The Molango mining district located in the state of Hidalgo has important manganese (Mn) deposits. An ecosystem health approach has been used to study the factors that determine the population exposure using the interaction between social, environmental and health sciences. The objective of this study is to evaluate the effect of Mn exposure on motor skills in school-aged children. Materials and methods: School-aged children between 7 and 11 years old were selected from two communities in the Molango mining district (100 children) and 95 children from non-exposed communities with similar socioeconomic conditions. The Luria motor scale was applied. The exposure to Mn was measured as blood Mn (MnB) and hair Mn (MnH). Multivariate lineal regression was performed to assess the effect of Mn on the global index of motor function adjusting by relevant covariates. Results: Median MnH and MnB were significantly different in exposed (12.6 mcg/g and 9.5 mcg/L) and not exposed (0.6 mcg/g and 8.0 mcg/L) children. After adjusting for haemoglobin, maternal education, gender, age and blood lead levels, MnB, was associated with a worse performance in the global Luria's test (coefficient 3.2, p=0.022). In a gender stratified analysis the boys execution is was worse compared with the girls. A significant interaction term between MnB and age was found. Conclusions: The younger boys were more susceptible to the adverse effect. The environmental exposure to Mn has an adverse effect on some aspects of motor function in school-aged children.

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METHODOLOGICAL ASPECTS OF AN EPIDEMIOLOGIC STUDY OF ADULTS LIVING NEAR A MN POINT SOURCE*

Rosemarie M. Bowler[1], Y. Kim[2], L. Ngo[3], and H.A Roels[4]
[1]*San Francisco State University, San Francisco, CA,* [2]*Ulsan University Hospital, Ulsan, S Korea,* [3]*Harvard Medical School, Boston, MA,* [4]*Université Catholique de Louvain, Brussels, Belgium*

Background: Exposure to high levels of Mn in occupational studies showed health effects similar to Parkinson's Disease, with neurological and neuropsychological sequelae. Few adult residential studies of Mn exposure report air dispersion modeling, biomarkers, neurological and neuropsychological test results. **Methods:** This multidisciplinary epidemiologic study used a recruitment strategy utilizing property tax and water records for random selection of 200 residents out of ~3939 in both a Mn-exposed and control town. Standardized tests of attention, immediate and delayed memory, cognitive flexibility, emotional function, and the UPDRS ADL and motor scales were studied with regard to biomarkers, Mn in air, scales of symptoms and illnesses, and dietary intake of Mn and Fe. **Results:** Preliminary results show a positive relationship between the neurological UPDRS motor and the neuropsychological tests of Fingertapping, Grooved Pegboard, Dynamometer and parallel lines tremor test. **Conclusion:** Neurological and neuropsychological methods correlate significantly, suggesting that several of the functional tests can be used interchangeably. Environmental Mn exposures are significantly lower than occupational exposures –likely posing a lower risk for clinical illness than occupationally inhaled Mn.

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RELATIONSHIPS BETWEEN MOOD AND NEUROPSYCHOLOGICAL PERFORMANCE IN ADULTS WITH ENVIRONMENTAL EXPOSURE TO MANGANESE.

Harris, M[1].; Bowler, R[2]. *[1]Alliant International University, San Francisco, CA; [2]San Francisco State University, California, USA.*

(SEE ABSTRACT IN POSTER SESSION ON PAGE 30 #P-64)

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INTELLECTUAL IMPAIRMENT IN SCHOOL-AGE CHILDREN WITH LONG-TERM EXPOSURE TO MANGANESE IN DRINKING WATER.

Maryse Bouchard, Sébastien Sauvé, Benoit Barbeau, Marie-Ève Brodeur, Thérèse Bouffard, Elyse Limoges, Melissa Legrand, David C. Bellinger, and Donna Mergler. *Centre for Interdisciplinary Studies in Biology, Health, Society and Environment (CINBIOSE), Université du Québec à Montréal, Canada.*

Background: Manganese (Mn) is an essential nutrient, but in excess, can be a potent neurotoxicant. Little data is available on possible neurotoxic effects of manganese exposure from drinking water, and no study examined children exposed to levels common in aquifers in North America. Methods: We recruited 362 children in elementary schools (age 6–13 years) living in households whose water source is groundwater. We administered the Wechsler Abbreviated Scale of Intelligence, and measured the concentration of manganese in residential tap water. Results: The samples of tap water in children's homes had manganese concentrations ranging from 1 to 2,700 µg/L (mean, 98 µg/L). Eighty-five percent of the children live in the current home, with the same source of water, since more than 12 months. Higher water manganese concentration is associated with lower IQ, before and after adjustment for confounders (including maternal education and intelligence, home stimulation, family income, and other metals in tap water). In adjusted analyses, a 10-fold increase in water manganese concentration is associated with a decrease of 2.4 points in Full Scale IQ (P=0.002), 3.1 points in Performance IQ (P=0.001), and 1.2 points in Verbal IQ (P=0.13). On average, children exposed to the highest quintile of water manganese concentration scored 6.2 IQ points below those in the lowest quintile. Conclusion: Our findings suggest that long-term exposure to manganese from drinking water is associated with significant intellectual impairments in children, at levels common in drinking water of communities relying on groundwater for domestic use.

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BIRTH COHORT STUDY GESTE: COULD CORD BLOOD BIOMARKERS SAY US HOW NEUROTOXIC METALS AFFECT THE FOETAL BRAIN AT LOW DOSE? FIRST DATA ON PROLACTIN AND MONOAMINE OXIDASE.

Nadia Abdelouahab and Larissa Takser. *University of Quebec at Montreal, Canada.*

Neurodevelopmental disabilities affect 3-8% of babies born each year, with known etiology for less than 25% of those disabilities. The potential for environmental contaminants such as lead, manganese, cadmium, PCB, and mercury, to produce neurological, cognitive, or other behaviour deficits as a result of developmental exposure has received increasing attention. The determination of dose-response

relationships in human populations requires the use of sensitive indicators of the effect, or biomarkers, of a wide range of environmental doses in the exposed population. Objective**:** To evaluate the long term neurodevelopmental effects caused by environmental exposure to persistent environmental chemicals from *in utero* life, we have designed this prospective birth cohort using biomarkers of effect for a mixture of known persistent substances. Method: This follow up study was carried was carried out on 800 pregnant women half of whom were recruited before 20 weeks of pregnancy. Mothers and cord blood samples were collected to measure manganese, lead, mercury, cadmium, PCB. Serum prolactin and platelet monoamine oxidase activity were measured in cord blood samples. Socio-demographics data, work and medical history were obtained by questionnaire and from medical records. Children follow up will be carried out at 2, 4, 6 and 10 years old. Results: Cord blood prolactin and monoamine oxidase were correlated with prenatal exposure to metals. Conclusion: Same biomarkers used at birth could indicate early neurotoxic effects of metals. The follow up study will indicate the predictive value of these biomarkers for future psychomotor skills.

Keynote "Anchor Talks"

SESSION X: HOT TOPIC KEYNOTE: CHANNELOPATHIES

Session Chair: **Tim Shafer**
Co-Chair: **April Neal**

Theme and Rationale: *Ion channels are the most basic elements of neuronal function and are key players in the proper function of the nervous system. In order to conduct these functions, channel proteins must exhibit proper structure, localization, and expression. Dysregulation of any of these processes may contribute to pathophysiology and result in what generally are known as channelopathies. The vast majority of research on channelopathies has focused on organ systems such as the heart, with recent focus starting to explore the role of channelopathies in neurological disorders such as epilepsy, chronic pain, and migraine. With this emerging area of research, most of the focus has been on the role of genetic alterations in channel proteins. However, a variety of environmental toxicants target ion channels, which suggests the potential for environmental exposures to contribute to neurological disease associated with alterations in ion channels. This workshop will bring together experts in ion channel structure and function, toxicologists studying environmental toxicant interactions with ion channels, and experts in clinical medicine and translational research to define the state of the science of channelopathies and the potential role of environmental exposures in channelopathies. This session will present an overview of effects of chemicals on ion channels, background about different channelopathy models, and explore the possibility that individuals with channelopathies may or may not be more sensitive to effects of chemicals.*

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INTRODUCTION AND STATE OF THE ART OVERVIEW: CHANNELOPATHIES. Timothy Shafer, *US Environmental Protection Agency, Research Triangle Park, North Carolina, USA.*

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DO ION CHANNELOPATHIES CONSTITUTE A GENE X ENVIRONMENT INTERACTION FOR ENVIRONMENTAL NEUROTOXICANTS? Timothy Shafer, *US Environmental Protection Agency, Research Triangle Park, North Carolina, USA.*

Voltage-and ligand-gated ion channels are key regulators of electrical activity in, and hence proper function of, the nervous system and cardiovascular system. The exquisite control over spatial and temporal changes in membrane function due to its regulation by ion channels make possible the rapid spatial transmission of individual spikes and bursts of neuronal activity that encode information underlying higher neural function. Neuroactive and neurotoxic environmental chemicals, including pesticides, solvents and metals, all disrupt function of various ion channels as critical events underlying their toxicity. Several neurological diseases are also associated with altered ion channel function, including various forms of epilepsy, neurodegenerative diseases and migraine, among others. Over the last decade, mutations in both the pore-forming and auxiliary subunits of ion channels have been described; they appear to be key contributors to the disease state. Animal models containing these mutant subunits have been constructed or identified from spontaneously-occurring mutations. They can provide excellent models in which to study the influence of environmental contaminants

on these diseases. Electrophysiological techniques permit mechanistic studies of how environmental toxicants may alter the function of these mutant channels. However, to date, there have been few if any investigations of the interactions of environmental toxicants with these channelopathy models, either at the ion channel or the whole animal level. Thus, the relative sensitivity of individuals with channelopathies to effects of environmental toxicants is unknown. This session will present an overview of effects of chemicals on ion channels, background about different channelopathy models, and explore the possibility that individuals with channelopathies may or may not be more sensitive to effects of chemicals. (This abstract does not reflect EPA policy)

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OVERVIEW OF ENVIRONMENTAL TOXICANTS AFFECTING ION CHANNEL FUNCTION. Toshio Narahashi. *Department of Molecular Pharmacology and Biological Chemistry, Northwestern University Feinberg School of Medicine, Chicago, IL 60611, USA*

Channelopathy is a relatively new word, but it has a long history of development over half a century. The modern concepts of ion channel function were well conceived when Hodgkin, Huxley and Katz utilized voltage clamp techniques developed by Cole. Applications of voltage clamp to ion channel pharmacology were greatly accelerated since the discovery of tetrodotoxin as a highly selective blocker of sodium channels in the early 1960s, setting the dawn of physiology, pharmacology and toxicology of ion channels. More recent developments of patch clamp technology greatly widen the applicability of voltage clamp techniques as patch clamp can be used for both voltage-gated and ligand-gated channels. The approach has become more significant as it is used to study pathophysiology of channels thus creating the terminology of “channelopathy”.

Vast numbers of therapeutic drugs have been the subject of investigations for their effects on various ion channels. Among environmental toxicants, insecticides and heavy metals are the most important agents that target ion channels. Whereas heavy metals such as mercury and lead exert multiple actions on various biochemical systems, many insecticides act on neuroreceptors and ion channels as the major target site. In this talk a developmental history of the channel studies of DDT, pyrethroids and fipronil will be briefly introduced. DDT and pyrethroids exhibit the similar mechanism of action on sodium channels despite different chemical structures. Fipronil inhibits GABA receptor-channels and glutamate-gated chloride channels. Although DDT was known to stimulate spontaneous discharge, it was not until the discovery of the increase in depolarizing after-potential that DDT joined a club of channel toxicology in the early 1950s.Voltage clamp and patch clamp studies have clearly demonstrated that DDT and pyrethroids prolong sodium currents by lengthening the open time of single channels. Only about 1% of sodium channels needs to be affected for the development of hyperactivity of animals explaining a high potency of pyrethroids. Another important development occurred when insecticide-resistant insects were discovered to have a reduced sensitivity to the insecticide as a major mechanism of insect resistance to insecticides. This discovery has led to the concept of *kdr* for which a number of mutations in channel amino acids have now been identified as causing resistance to insecticides. The selective toxicity to insects over mammals is another important subject of investigations. Fipronil was found to inhibit, in addition to GABA receptor-channels, glutamate-gated chloride channels very potently, which are present in insects but not in mammals. This factor contributes significantly to the selective toxicity. Channelopathy is now becoming an important concept in biomedical science.

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CALCIUM CHANNELS AS TARGETS OF ENVIRONMENTAL TOXICANTS AND NEUROLOGICAL DISEASE. William Atchison, *Department of Pharmacology and Toxicology, Michigan State University*

Neuronal voltage-gated Ca^{2+} channels play a central role in toxicity induced by some neurotoxins and disease processes. They likely also contribute to the toxicity of certain environmental chemicals. Ca^{2+} channels are multimeric integral membrane proteins consisting of a large pore-forming subunit and several auxiliary subunits, which regulate the functional properties of the channel, as well as its cellular localization and membrane insertion. Ca^{2+} channels regulate secretion of neurotransmitters and hormones, excitability, growth cone elongation and gene expression; thus they are crucial to the neuronal signaling process. They also are key contributors to neuronal plasticity. There are multiple phenotypes of

Ca^{2+} channels; they are divided into three groups (Cav 1-Cav 3) which result from separate gene products and differ in functional properties, chemical sensitivity, and cellular localization. Therapeutic agents, arthropod and mollusk toxins, and environmentally relevant toxic metals and insecticides interact with specific subtypes of Ca^{2+} channels. Inherited trait disorders- "channelopathies", certain forms of seizure and "myasthenic syndromes," also target distinct channel isoforms- especially the P/Q type (Cav 2.1). Conversely, another subtype, Cav 1.3, has been proposed to play a central role in vulnerability of nigrostriatal dopaminergic neurons to cell death in Parkinsons disease. Thus there is potential for environmental neurotoxicants to interact with heritable traits or disease processes leading to a "gene-environment interaction" relative to Ca^{2+} channel function. Lambert-Eaton Myasthenic Syndrome (LEMS) and paraneoplastic cerebellar ataxia are examples of neurological disorders associated with dysfunction of specific subtypes of Ca^{2+} channels. LEMS is an autoimmune disorder characterized by decreased evoked quantal release of acetylcholine (ACh) and disruption of the presynaptic active zones, the sites at which ACh is thought to be released. LEMS antibodies appear to be directed specifically at P/Q-type Ca^{2+} channels (Cav 2.1)located at or near the active zone. This leads to neuromuscular dysfunction and dysautonomia. Several important groups of environmental neurotoxicants also disrupt function of Ca^{2+} channels. These include polyvalent metals, organic solvents, and pyrethroid insecticides. They, too, can exhibit surprising channel subtype specificity. Because of similarities of their chemical structure to Ca^{2+} in terms of hydrated ionic radius, electron orbital configuration, or other chemical properties, polyvalent cations are especially prone to interact with multiple types of voltage-gated Ca^{2+} channels. These metals have been useful in understanding the structure and function of the Ca^{2+} channel, especially its permeability characteristics. Two nonphysiological cations, Pb^{2+} and Hg^{2+}, as well as their organic derivatives, are highly potent Ca^{2+} channel blockers. These metals also apparently gain intracellular access to the neuron in part by permeating through Ca^{2+} channels. Because of the pivotal role which Ca^{2+} channels play in neuronal function, their disruption in disease processes or chemical exposures can have profound effects on neuronal function. Supported by NIH grants R01ES03299 and R01NS051833.

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RYANODINE RECEPTOR CHANNELOPATHIES ARE TRIGGERED BY ENVIRONMENTAL CUES. I. N. Pessah and G. Cherednechenko. *Molecular Biosciences, University of California, Davis 95616, USA*

Ryanodine sensitive Ca^{2+} channels (ryanodine receptors; RyRs) regulate a variety of physiological and pathophysiological processes in a wide variety of tissues including muscle, the central nervous system, and peripheral nervous system. More than 122 mutations within the gene that codes for "skeletal muscle" RyR1 have been associated with malignant hyperthermia (MH) susceptibility. MH is a fulminant pharmacogenetic life-threatening syndrome, which occurs when susceptible individuals are exposed to environmental factors, including halogenated inhalation anesthetics, temperature stress and possibly persistent organic pollutants. Homologous mutations within "cardiac muscle" RyR2 have been associated with catecholaminergic polymorphic ventricular tachycardia (CPVT), a life-threatening stress-induced arrhythmia. Knock-in mice heterozygous (Het) for MH susceptibility mutation R163C-RyR1 are viable and thrive, whereas those homozygous for the mutation are birth lethal. R163C-RyR1 Het mice exhibit fulminant MH episodes after exposure to the halogenated general anesthetic halothane (1.5%) or an ambient temperature of 40°C characterized by rapidly rising core temperature, respiratory rate and inspiratory effort, with significant blood biochemical changes indicating metabolic acidosis, ending in hyper-contracture and death. The homologous mutation, R176Q-RyR2 confers susceptibility to CPVT in KI mice. We have demonstrated that non-coplanar PCBs directly interact with RyR1 and RyR2 to sensitize their physiological activation. We therefore tested if R163C-RyR1 and R176Q-RyR2 were more sensitive using biochemical, electrophysiological, and Ca^{2+} imaging techniques. [^{3}H]ryanodine binding and single channel analyses revealed that R163C-RyR1 and R176Q-RyR2 were ~10-fold more sensitive to activation by PCB 95 compared to their respective WT controls. The amplitude of Ca^{2+} transients in embryonic myotubes elicited by low

frequency electrical pulses was significantly enhanced within 2.5 min after initiating perfusion of PCB 95 in the external medium compared to the solvent control, and PCB 95 prevented recovery of the Ca^{2+} signal to its original baseline. With higher frequency pulse trains, PCB 95 not only amplified Ca^{2+} transient amplitudes but also caused ectopic Ca^{2+} transients immediately after electrical stimuli were suspended, that was not observed in the absence of PCB. Longer (3 hr) exposures to 10 nM PCB 95 caused marked depletion of RyR1 sensitive stores in R163C-RyR1 myotubes, but had minimal effect on WT. Considering that RyR mutations are broadly expressed and can disrupt bidirectional signaling with cell surface channels, R163C-RyR1, R176Q-RyR2, and other RyR mutant mice currently being developed provide relevant experimental models for understanding gene-environment interactions that lead to enhanced susceptibility to environmental and pharmacological agents. Supported by NIEHS and UC Davis SRP.

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ALLETHRIN DIFFERENTIALLY MODULATES VOLTAGE-GATED CALCIUM CHANNEL SUBTYPES IN RAT PC12 CELLS. April P. Neal, Yuan Yukun, and William D. Atchison. *Department of Pharmacology & Toxicology, Michigan State University, East Lansing, MI USA*

(SEE ABSTRACT IN POSTER SESSION ON PAGE 34 #P-73)

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DELTAMETHRIN EXPOSURE RESULTS IN CALPAIN/PROTEASOMAL DEPENDENT DOWN-REGULATION OF VOLTAGE GATED SODIUM CHANNEL EXPRESSION. Jason P. Magby[1,2], Jason R. Richardson[1,2] [1]*Environmental and Occupational Health Sciences Institute and Department of Environmental and Occupational Medicine, Robert Wood Johnson Medical School.* [2]*Joint Graduate Program in Toxicology, Rutgers University, University of Medicine and Dentistry of New Jersey*

(SEE ABSTRACT IN POSTER SESSION ON PAGE 33 #P-72)

Panel Discussion

Moderators:

- Isaac Pessah
- William Atchison

Keynote "Anchor Talks"

SESSION XI: HOT TOPIC KEYNOTES: GENDER DIFFERENCES IN NEUROLOGICAL DISORDERS

Session Chair: **Bernard Weiss**
Co-Chair: **Richard Seegal**

Theme and Rationale: *The IOM report, Does Sex Matter (2001) emphasized the need for examining differences between male and female responses in the research enterprise because of wide gaps between males and females in disease patterns and in response to therapeutic practices and drugs. This platform session is designed to exemplify both current knowledge of sex differences in environmenal chemical vulnerability and to propose insight into the relationship between the environmental exposures, gender, and the development of neurological dysfunction that may lead to a number of neurological diseases or disorders. Platform presentations will include basic, clinical, and translational research aimed at highlighting the integral role of gender in neuronal function and disease disparities and how environmental exposures interact with gender to alter risk of neuronal dysfunction.*

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INTRODUCTION AND OVERVIEW: SEX DIFFERENCES IN NEUROLOGICAL DISORDERS AND RESPONSE TO ENVIRONMENTAL EXPOSURES. D. A. Cory-Slechta, *Department of Environmental Medicine, University of Rochester School of Medicine, Rochester, NY, USA*

Sex differences exist in virtually all aspects of physiological function, differences that no doubt contribute to the corresponding differences between males and females in incidence and prevalence found in a remarkable array of diseases and disorders. Differences in brain structure and ultimately in function are particularly prominent between males and females as shaped by hormones early in the course of development. As would be expected given such differences, prenatal insults like maternal stress and LPS that have been shown to result in lifetime consequences for the central nervous system (CNS) almost invariably manifest differentially by sex. Our studies examining the consequences of maternal or lifetime lead (Pb) exposure ± prenatal stress have demonstrated broad sex differences in outcomes. These inherent sex differences in the CNS result in biochemical functions, such as corticosterone and neurotransmitter levels, that change differentially by sex across time per

se, and these trajectories are then further differentially modified in males vs. females both by behavioral experience and in response to Pb ± stress. As a consequence, both maternal and lifetime Pb ± stress result in behavioral, neurochemical, glucocorticoid and cytokine outcomes that differ, often dramatically, and sometimes in completely opposite directions, in males and females. Such sex differences should assist in furthering the understanding of mechanisms of neurotoxicity. Moreover, such findings indicate that collapsing outcomes across the sexes may obscure actual hazards attendant to such exposures and potentially falsely minimize associated risks. They also underscore the need to determine the validity of uncertainty factor values for sex differences in risk assessment. Supported by ES05017.

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SEXUALLY DIMORPHIC EFFECTS OF PCBS: FROM DEVELOPMENT TO NEURODEGENERATION. RF Seegal, VM Miller, A Dreiem and K Brosch. *Wadsworth Center, New York State Department of Health, Albany, New York, USA.*

Polychlorinated biphenyls (PCBs) are persistent environmental neurotoxicants. There has been, however, little or no examination of whether PCBs differentially affect males and females. We thus examined if PCBs differentially affect neurological disorders where males are affected more than females—attention deficit disorder (ADD) and Parkinson's disease (PD). Rats were developmentally exposed to PCBs and changes in neuroglial development were determined. PCBs increased levels of myelin basic protein (MBP) in the cerebellae of males and decreased MBP levels in females despite similar brain PCB levels. In contrast, levels of glial fibrillary acidic protein decreased in males but increased in females. These findings suggest a mechanism by which PCBs may contribute to greater prevalence of ADD in boys. Epidemiologically, we measured the density of dopamine transporters (DAT) in the basal ganglia of male and female PCB exposed former capacitor workers using β-CIT SPECT imaging. Serum PCB levels were significantly elevated compared to the general population and did not differ between men and women. After controlling for potential confounders we found that women, but not men, showed a significant inverse relationship between serum PCB levels and DAT densities suggesting that postmenopausal women may be at greater risk for PCB-induced reductions in basal ganglia DA function, including PD, than comparably exposed men. In summary, these findings emphasize the need, both experimentally and epidemiologically, to examine the effects of contaminant exposure in males and females. Supported by NIEHS Grants ES014675 and ES015688, and United States Army Grant DAMD17-02-1-0173 to RFS.

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SAME SEX, NO SEX, AND UNAWARE SEX IN NEUROTOXICOLOGY. Bernard Weiss. *Department of Environmental Medicine, University of Rochester School of Medicine and Dentistry, Rochester, New York, USA.*

In 2001, the Institute of Medicine published a report on how sex influences human health. It asked, "Does Sex Matter?" It made a forceful argument that the biology of males and females differs in so many profound ways that to ignore them diminishes the relevance of research that does so. It pointed out that, in essence, "Every cell has a sex," so that, even at cellular and molecular levels, male-female differences have to be taken into account. Most of the literature of neurotoxicology, however, as exemplified by the journal *Neurotoxicology*, seems to assign little importance to sex. Almost without exception, *in vitro* studies disregard the sex of tissues of origin or use media that fail to emulate the sexually dimorphic environment of the brain, even for models of sex-linked disorders such as Parkinson's disease. Studies based on whole animals, if they employ one sex only, favor males over females by a ratio of about 50:1. Only a handful of studies employ both sexes. Some papers, in fact, fail even to report the sex of the animals or simply use some of each for convenience. Yet, in 1993, Congress mandated inclusion of both sexes in research funded by NIH, while, that same year, the FDA required that both sexes be included in clinical trials of new drugs. The public is intensely aware of sex differences, especially in behavior, with dozens of books published on the topic. And, endocrine disruption is now an established marker of environmental exposures. Given such a background, current research in neurotoxicology presents a mystifying discrepancy. (Supported in part by grant RC2 ES018736 from NIEHS).

Panel Discussion

Moderators:

- Bernard Weiss
- Richard Seegal

Discussion

SESSION XII: UNIFYING MECHANISMS OF NEUROLOGICAL DISORDERS: SCIENTIFIC, TRANSLATIONAL, AND CLINICAL IMPLICATIONS: SUMMARY AND RECOMMENDATION

Moderators of the Roundtable:

Conference Co-Chairs:

- Jason R. Richardson
- Kenneth Reuhl

Roundtable Discussants

- Flint Beal
- M. Daniele Fallin
- Benoit Giasson
- Timothy Greenamyre
- Cindy Lawler
- Isaac Pessah
- Timothy Shafer
- Peter Spencer
- Helmut Zarbl

The following papers are presented in
***SESSION V:* POSTER SESSION**
Monday Evening, June 7, 2010
7:00 – 9:30 PM

SESSION VI: POSTER SESSION

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.

MECHANISTIC APPROACHES TO METAL NEUROTOXICITY

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SELENIUM FROM OCEAN FISH PREVENTS MERCURY TOXICITY. CR Ralston and NVC Ralston. *University of North Dakota Energy & Environmental Research Center, 15 North 23rd Street, Grand Forks ND, 58202 USA*

Supplemental selenium (Se) is known to counteract methylmercury (MeHg) toxicity, but the molecular mechanisms responsible for this protective effect have only recently become clear. Since ocean fish are among the richest sources of dietary Se, we hypothesized that their Se contents would protect against the adverse effects otherwise associated with MeHg exposures from seafood consumption. In the current study, 120 weanling male Long Evans rats were fed diets containing either low or high MeHg (0.5 or 50 nmol MeHg/g). These diets were augmented with either sodium selenite (~0.1, 1.0, or 10 nmol Se/g) or Se from delipidated protein isolates from bigeye tuna, swordfish, or mako shark (3.5, 2.3, and 2.1 nmol/g, respectively). Diets were prepared with torula yeast protein or Se from delipidated fish protein isolates added as 10% of the total diet in place of an equivalent amount of torula yeast protein (2 Hg levels × 6 Se diets = 12 dietary treatments, 10 rats per group). Additional MeHg in the diets from tuna, swordfish, and shark protein added to the diets were 1.6, 2.3, and 3.6 nmol MeHg/g. Rats were fed low-Se torula yeast-based diets for 5 weeks to deplete their tissue Se reserves, then switched to assigned dietary treatments for the rest of the study. Rats fed high-MeHg, low-Se diets showed growth inhibition after 4 weeks and hind limb crossing after 9 weeks. MeHg from fish did not increase MeHg toxicity as might have been expected. Instead, the Se from fish protein isolates was effective in preventing MeHg toxicity.

Keywords: Methylmercury, Selenium, Toxicity

P-59

Pre-Doctoral Poster Award Competition

METHYLMERCURY-ASSOCIATED FLUO4 FLUORESCENCE IS REDUCED BY THE GABAA AGONIST MUSCIMOL IN NEONATAL RAT CEREBELLAR SLICES. AB Bradford [1,2] and WD Atchison[2,3]. [1]*Department of Biochemistry/Molecular Biology,* [2]*Center for Integrative Toxicology and* [3]*Department of Pharmacology/Toxicology, Michigan State University, East Lansing, MI, USA.*

Methylmercury (MeHg) causes cerebellar granule cell (CGC) death leading to ataxia and motor dysfunction. Cell death is not immediate upon MeHg exposure, but early onset changes to [Ca2+]i regulation occur. CGCs undergo migration and maturation at the period in development most susceptible to MeHg toxicity. Both depend on Ca2+ signaling and changes to GABAA receptors. We hypothesize a role for GABAA receptors in controlling CGC excitability at this critical point in toxicity. [Ca2+]i was measured during the time of peak migration in acutely isolated live cerebellum slices from 8-12 day old rats. MeHg (10, 20µM) significantly elevated CGC fluo4 fluorescence (1.6-2.0x pretreatment) within 40 min. At 5µM, MeHg significantly elevated fluo4 fluorescence by 40 min but only in dividing CGCs. Pulses of muscimol (100µM), a GABAA receptor agonist, reduced MeHg-associated fluo4 fluorescence increase in dividing (1.5x) and migrating (1.2x) cells and eliminated the change in post-migrational cells. Bicuculline (10µM), a nonspecific antagonist, did not significantly alter the MeHg-induced increase, indicating a possible effect plateau or competition with MeHg. These results support MeHg selectivity for GABAA receptor subunits in developmental CGC susceptibility to toxicity. This work is supported by grants from Johns Hopkins University CAAT and from NIEHS T32ES007255 and R01ES03299-21.

Keywords: GABA-A receptors, MeHg neurotoxicity, cerebellar development

Mentor: William D. Atchison

P-60 (Also presented from Platform in Session IV #21)
Post-Doctoral Poster & Travel Award Competition

ARSENIC INHIBITS NEURITE OUTGROWTH BY INHIBITING LKB1-AMPK SIGNALING PATHWAY.

Xin Wang[1], Dan Meng[2], Qingshan Chang[1], Jingju Pan[1], Zhuo Zhang[1], Gang Chen[3], Zunji Ke[2], Jia Luo[3], and Xianglin Shi[1*]. *[1]Graduate Center for Toxicology, University of Kentucky, Lexington, KY 40536 USA [2]Key Laboratory of Nutrition and Metabolism Institute, Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, 200031, P.R. China [3]Department of Internal Medicine, University of Kentucky, Lexington, KY 40536 USA*

Background: Arsenic is an environmental pollutant inducing numerous pathological effects, including neurodevelopmental disorders. Objectives and Methods: We used Neuro-2a neuroblastoma cells as a model of developing neurons and evaluated the role of LKB1-AMPK pathway in arsenic-induced developmental neurotoxicity. Results: Addition of low concentrations of arsenic (≤5 µM) during differentiation caused an inhibitory effect on the neurite outgrowth in Neuro-2a cells in the absence of cell death. AMPK activation induced by retinoic acid in differentiating cells was blocked by arsenic. Pretreatment with an AMPK specific activator, 5-aminoimidazole-4-carboxamide riboside (AICAR), or overexpression of a constitutively active AMPK-α1 plasmid recovered arsenic-induced neurite retraction. The activation of LKB1, a major AMPK-kinase, was also suppressed by arsenic by inhibiting the translocation of LKB1 from nuclear to cytoplasm. Antioxidants, such as NAC and SOD, but not catalase, protected against arsenic-induced inactivation of LKB1-AMPK pathway, and reversed the inhibitory effect of arsenic on neurite outgrowth. Conclusions: Neurite retraction induced by arsenic results from deficient activation of AMPK as a consequence of LKB1 inactivation. Oxidative stress induced by arsenic, especially excessive superoxide, plays a critical role in the blocking LKB1-AMPK pathway. Our studies provide an insight into the underlying mechanisms of arsenic-induced developmental neurotoxicity and could be important in designing a new strategy for protecting children against this neurotoxic substance.

Keywords: Arsenic, LKB1-AMPK Pathway, Neurodevelopment

Mentor: Xianglin Shi

P-61 (Also presented from Platform in Session IV #20)
Post-Doctoral Poster & Travel Award Competition

THE EFFECT OF ALUMINUM CHLORIDE ON COGNITIVE ABILITY OF APP/PS1 DOUBLE TRANSGENIC MICE AND EXPRESSION OF mGluR1.

ZHANG Qin-Li[1], JIAO Xia[1], JI Xiu-liang[1],Ce Zhang[2] and NIU Qiao[*1]. *Ministry of Education Key Laboratory, [1]Department of Occupational Health, Shanxi Medical University, Taiyuan 030001 P.R. China; Ministry of Education Key Laboratory, [2]Department of Physiology, Shanxi Medical University, Taiyuan 030001 P.R. China*

The aim of present study is to identify the genetype of the APP/PS1 double transgenic mice, and investigate the effects of spacial learning and memory and the expression of mGluR1 in APP/PS1 double transgenic mice exposed to aluminum. The APPswe gene was amplified by PCR from the mice genome DNA ; The APP/PS1 double transgenic mice were divided into 2 groups at random, i.e. normal saline group and Aluminum exposed group (5 mg/kg), 8 for

each group. $AlCl_3$ was administered by intraperitoneal injection for two monthes. ① The Morris water maze test was performed to test the learning and memory abilities of mice. ②The expression of mGluR1 was detected by QRT-PCR and Western blotting. Results were: ① The APP/PS1 double transgenic mice were identified ; ②The latent period of Morris water maze test were significantly (P<0.05) increased in Al-injected mice compared with the control group. ③The Western-blot demonstrated that mGluR1 expression in protein level was increased after Aluminum exposure (P<0.05). The expression of mGluR1 mRNA expressed by QRT-PCR increased after Aluminum exposure (P<0.05). In conclusion,the effects on learning and memory in the APP/PS1 double transgenic mice exposed to aluminum were descended obviously, and aluminum is related with the up- regulation of mGluR1.

Key words: aluminum, learning and memory, mGluR1
Mentor: Qiao Niu
*Corresponding author

P-62

Pre-Doctoral Poster & Travel Award Competition

ARSENITE INHIBITS NEURITE GROWTH THROUGH MODULATION OF THE ACTIN CYTOSKELETON. DJ Swett[1,3] and DA Currie[1,2]. [1]*Maine Center for Toxicology and Environmental Health, and* [2]*Biology Department, University of Southern Maine, Portland, Maine, USA;* [3]*Department of Molecular and Biomedical Sciences, University of Maine, Orono, Maine, USA.*

We previously found that low micromolar concentrations of arsenite inhibited neurite outgrowth in PC12 cells, but the mechanisms leading to this effect were unclear. The proper regulation of actin polymerization and actomyosin contractility is crucial to neurite growth. Growth of the neurite relies on actin polymerization particularly at the growth cone, while promotion of actin-based contraction within the neurite shaft can shorten neurite length. Since previous studies from other laboratories indicate that arsenicals have the potential to modify the actin cytoskeleton in multiple ways, we generated a multiple target hypothesis which predicts that arsenite disrupts neurite growth in PC12s by 1. RhoA activation and the induction of actin-based contractility to shorten neurites 2. by binding to actin monomers in a way that inhibits polymerization and 3. by lowering cellular levels of ATP and therefore reducing actin polymerization. Here we provide evidence that during neuritogenesis arsenite-exposed PC12s have increased levels of active RhoA and that inhibition of this activation partially prevents deficits in neurite outgrowth. Intriguingly, actin polymerization with purified actin appears to be arsenite resistant, suggesting that arsenite does not bind to actin in a manner that would inhibit initial polymer formation. However, we did find that arsenite partially reduces cellular levels of actin and ATP, which has the potential to disrupt actin polymerization in developing neurons.

Key words: Arsenic, Neurite, Actin
Mentor: Douglas A. Currie

P-63

LOW LEVEL LEAD EXPOSURE AND INFLAMMATORY CYTOKINES IN THE BRAINS OF C57BL/6 MICE. M Cervantes, RX Armijos and C Sobin. *Department of Public Health Sciences, the University of Texas at El Paso, El Paso, Texas*

Lead exposure has been a public health concern for many years. Research shows that chronic low level lead exposure may be associated with adverse motor and cognitive dysfunctions. The mechanisms by which low level lead affects brain function are largely unknown. It was hypothesized in the current study that the presence of inflammatory cytokines and an antioxidant enzyme would increase with increasing lead exposure. Twenty-four C57BL/6 mice were observed and grouped in the study: a unexposed group (n=8), a group exposed to 40 ppm lead acetate (n=8), and a group exposed to 230 ppm lead acetate (n=8). Study mice were exposed to lead via the dam's drinking water from PND 1 to PND 40. Gene expression of inflammatory cytokines (Tumor Necrosis Factor-α, Interleukin-6, and Interferon-γ) and an antioxidant enzyme (Heme Oxygenase-1) in mRNA extracted from the brains of mice were analyzed using Quantitative Real Time Polymerase Chain Reaction. Statistically significant increases of Interferon-γ (p=0.002) and Heme Oxygenase-1 (p<0.001) and a decrease in Interleukin-6 (p<0.001) were observed in groups exposed to lead. Differences in cytokine expression were more significant in the group exposed to lowest level lead. Findings suggest that a neuroinflammatory response and oxidative stress may be linked to the motor and behavioral changes observed in mice exposed to chronic low level lead. More studies need to be conducted in this area to clarify this association.

Keywords: Lead, Inflammation, Cytokines

METALS IN HUMAN POPULATIONS

P-64 (Also presented from Platform in Session IX #45)
Pre-Doctoral Poster & Travel Award Competition

RELATIONSHIPS BETWEEN MOOD AND NEUROPSYCHOLOGICAL PERFORMANCE IN ADULTS WITH ENVIRONMENTAL EXPOSURE TO MANGANESE.

Harris, M[1].; Bowler, R[2]. [1]*Alliant International University, San Francisco, CA;* [2]*San Francisco State University*

Background: Manganese (Mn) classified as a neurotoxin affecting brain function (ATSDR, 2000), which can include motor disturbances similar to those found in Parkinson's Disease (Roels et al., 1992), neuro-cognitive effects (Mergler et al., 1994), and mood disturbance (Bowler et al., 2007). Cognitive functioning and motor performance can also be adversely affected by emotional disturbance, particularly anxiety and depression (Lezak, 2004). **Methods:** Adult participants (N=100) were randomly selected from Marietta, OH, which houses the largest Mn-producing plant in the US (EPA, 2004). Participants completed the Symptom Checklist 90-Revised and a battery of neuropsychological tests. Principal components analysis derived seven factors: *Recall Memory, Working Memory, Information Processing/Speed, Trail Making, Grip Strength, Motor Speed*, and *Complex Motor Dexterity*. **Results:** Adjusting for age, gender, and education, Depression was negatively related to Trail Making (β= -.218, *p*=0.030), Working Memory (β = -.197, *p* = .035), Information Processing/Speed (β= -.260, *p*=0.008), Motor Speed (β= -.249, *p*=0.019), and Complex Motor Dexterity (β = -.216, *p* = .039), while Anxiety was negatively related to Trail Making (β = -.310, *p* = .002), Information Processing/Speed (β= -.274, *p*=0.006), Motor Speed (β= -.288, *p*=0.007) and Complex Motor Dexterity (β= -.224, *p*= 0.034). Neither mood variable was related to Recall Memory or Grip Strength. Living proximity to the Mn-producing plant was negatively related to Information Processing/Speed and Trail Making, and positively related to Anxiety. Interaction effects between plant proximity and mood were found for Trail Making. **Conclusion:** Mood has a moderate negative impact on neuropsychological functioning in several domains, especially cognitive flexibility, motor speed, and information processing speed. This relationship may be stronger with higher exposure to Mn, and should be considered when examining the neuropsychological effects of exposure to neurotoxins.

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Key words: Manganese, Neurocognitive, Mood
Mentor: Rosemarie Bowler

P-65

OCCUPATIONAL DIFFERENCE IN MEMORY PERFORMANCE*

Hage, D.; Leary B.; Bowler, R. San Francisco State University

Background: Occupational demand has shown to be positively associated with cognitive function (Bosma et al., 2003). This study seeks to investigate whether different occupational groups exhibit different scores in verbal and visual short term memory (STM). **Methods:** Work history data was collected from participants (*N*=190) from a larger study by Bowler et al. (2010) investigating Manganese (Mn) exposure conducted in two towns in Ohio, Marietta and Mt. Vernon. 23 participants' occupational data was missing, resulting in a sample of 167 participants from both towns. Verbal and visual subtests, adjusted by years of education (*E*), age, and gender, from the Neuropsychological Assessment Battery (NAB) Memory Module were administered to participants (N=88) in Marietta and participants (N=79) in Mt. Vernon: List Learning Immediate, Short and Long Delayed, Story Learning Phrase Unit Immediate and Delayed, and Daily Living Memory Immediate and Delayed. Visual subtests were Shape Learning Immediate and Delayed Recognition. Participants' occupations were categorized into the Standard Occupational Classification System (U.S. Bureau of Labor Statistics, 2010) then into 5 different categories: 1) professional (*N*=28, *E*=18.6), 2) administrative (*N*=45, *E*=14.8), 3) technical (*N*=51, *E*=15.2), 4) service (*N*=23, *E*=13.4), and 5) labor (*N*=20, *E*=12.5). **Results**: Significant differences between occupational categories were found for the NAB Story Learning Phrase Unit Immediate Recall, $F_{4,162} = 3.08$, $p = .018$. Service occupations ($M = 49.65$, $SD = 10.969$) and professional occupations ($M = 48.96$, $SD = 13.911$) scored significantly higher than labor occupations ($M = 39.45$, $SD = 11.194$). The remaining NAB memory subtests did not differ significantly by occupation. **Conclusion:** Service, one of the lowest educational groups (*E*=13.4), had the highest score on verbal short term memory.

Acknowledgment: *This study was funded by the U.S. EPA under Co-op. Agreement P8341600*
*Presented at the Twenty-Sixth International Neurotoxicology Conference *Unifying Mechanisms of Neurological Disorders: Scientific, Translational, and Clinical Implications*. Portland, Oregon. June 7, 2010.
Keywords: Short Term Memory, Occupation, NAB

P-66

THE INFLUENCE OF DIETARY CONSUMPTION AND IRON STORES ON MANGANESE IN BLOOD. R Rasalan and R Bowler. *San Francisco State University, San Francisco, CA, USA*

Background: Diet is known to be one of the primary forms of manganese (Mn) exposure (ATSDR, 2008). The typical average daily intake for adults consuming a western diet is between 0.7mg and 10.9mg, with an adequate daily intake of 1.8mg/day and 2.3mg/day for females and males respectively (ATSDR 2008). Small effects of dietary Mn on levels of Mn in blood may be attributed to iron stores preventing the over absorption of this element that may otherwise increase likelihood of toxicity. **Method:** A total of one hundred ninety adults were randomly selected from Mt. Vernon and Marietta, OH, where one of the largest plants in ferroalloy production using Mn is still in operation (EPA, 2004). A blood sample and information about recent dietary habits were collected. In diet, participants were compared by gender. Analyses between participants above or below the 'safe' levels of blood Mn using the Centers for Disease Control's cut-off of 12.1 μg/L were also conducted. **Results:** Comparing males and females against adequate dietary Mn reveals that males showed an average daily intake of 2.62mg compared to an adequate intake of 2.3mg/day while females showed an average daily intake of 2.85mg compared to an adequate intake of 1.8mg/day. As expected, blood Mn did not correlate with diet Mn intake (r=0.02, p=0.78), but a significant relationship is present with serum ferritin (r=-0.22, p=0.003). In addition, comparison of participants above and below the cut-off for 'safe' levels of Mn in blood show no differences in diet Mn intake (t=-0.07, p=0.94), but significantly differ in levels of serum ferritin (U=1,467.00, p<.001) and Mn in blood (U=0.00, p=<.001). **Conclusions:** Participants have within normal levels of average daily intake of dietary Mn with the exception of higher average daily intake in females. Parallel with previous findings, dietary Mn intake was not a significant predictor of Mn in blood, but the significant relationship that exists with serum ferritin reinforces the consideration of iron stores when examining elevated levels of Mn in blood.

Acknowledgment: *This study was funded by the U.S. EPA under Co-op. Agreement P8341600*

Keywords: Manganese, Diet, Gender

P-67

MERCURY IN FISH: WHAT'S A SAFE LEVEL? SG Gilbert. *Institute of Neurotoxicology & Neurological Disorders, Seattle, WA, USA*

There is no doubt that methylmercury (MeHg) is neurotoxic and an undesirable contaminate of fish. The potential toxicity of mercury exposure was known from ancient times but the more modern awareness of the tragic consequence of MeHg exposure occurred in the 1950's at Minamata, Japan. Dumping industrial mercury waste into Minamata Bay demonstrated that indeed the "solution to pollution was not dilution". This incident and others lead to the realization the inorganic mercury was readily biomethylated by bacteria to MeHg which biomagnified and bioaccumulated up the food chain. Humans and other wild life were exposed to MeHg from high order fish such as shark, tuna, and sword fish which accumulate MeHg in their muscle. In 1969, the US Food and Drug Administration (FDA) set a 0.5 ppm action level for total mercury in fish. The FDA raised the acceptable mercury fish level to 1 ppm in 1979 and in 1984 switched the standard from total mercury to 1 ppm methylmercury in fish. In 2001 the US Environmental Protection Agency set an oral RfD for MeHg at 0.1 ug/kg and later advised a MeHg fish tissue level of 0.3 ppm for human health based upon water quality standards. In 2000, 41 states have issued 2,242 fish advisories for mercury and the FDA advised that certain fish not be consumed. While much of the concern about the hazards of excessive MeHg exposure has focused on the sensitivity of the developing organism there is also ample documentation of adult MeHg toxicity from consumption of fish with elevated MeHg levels. Vulnerable populations include subsistence fishers that depend on fish as a significant source of food. There are also people that consume high levels of fish and suffer from MeHg toxicity such as Richard Gelfond, as recently documented in the Wall Street Journal. Canada established a guideline of 0.5 ppm mercury for retail fish and seafood for people who infrequently eat commercially-produced fish and seafood. Setting fish advisories is clearly challenging given the well documented benefits of fish consumption. The sources of mercury release into the environment such as coal fired plants, cement kilns, and boilers are well documented and the EPA is currently proposing a new federal rule that would reduce mercury emissions more than 50% by requiring changes

at companies with industrial boilers, heaters and incinerators. Is it time for the US FDA to lower the acceptable level of MeHg in commercial fish to 0.5 where the EPA has set the level for sport fish? Or even to 0.3 ppm based upon human health concerns? Lowering the acceptable level of mercury in fish would send a clear message that we all need to work together to prevent mercury contamination of fish, a very important source of nutrition for many.

Keywords: Mercury, FDA, Fish

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RELATIONSHIPS BETWEEN OBESITY, MANGANESE, AND HEALTH IN ADULTS WITH ENFIRONMENTAL EXPOSURE TO MANGANESE.

Wilson, K[1].; Gocheva, V[2].; Bowler, R[2]. [1]Alliant International University, [2]San Francisco State University

Background: Obesity has been linked with various chronic health problems (Malnick & Knobler, 2006). There is evidence that obesity may exacerbate neurotoxic effects in the dopaminergic system of animals (Sriram, Benkovic, Miller, & O'Callaghan, 2002). Manganese (Mn), an essential element, is required in small amounts for normal bodily functions. At higher doses, Mn has neurotoxic effects associated with parkinsonian-like symptoms, such as tremor, rigidity, and bradykinesia (ATSDR, 2000), as well as mood disturbances, e.g. depression and anxiety (Bowler et al., 2006). The potential role of obesity, as measured by Body Mass Index, in the effects of Mn neurotoxicity is examined here to determine if obese individuals comprise a vulnerable population. **Methods:** One hundred adults were randomly selected from Marietta, OH, where the largest Mn-producing plan in the US is located (EPA, 2004). Eight participants were excluded due to missing height data. Participants provided a blood sample, their height and weight, completed a brief neuropsychological battery and an extensive questionnaire on illnesses, symptoms, and diet. Participants were categorized into obese and non-obese based on a body-mass index cut-off of 30 kg/m^2 and differences between the two groups were examined. **Results:** Obese individuals had significantly more Mn in blood (MnB) (t = -2.525, p =.013) but did not differ from non-obese individuals on serum ferritin, dietary Mn intake, or total food consumption. Obese individuals reported more somatization (U = 710.5, p = .014) and higher number of anxiety symptoms (U = 779, p = .039). For symptom categories, 68% of obese vs. 44% of non obese participants reported experiencing musculoskeletal symptoms (χ^2 (1) =5.089, p =.024) and 62% of obese vs. 38% of non-obese participants reported experiencing neurological symptoms (χ^2 (1) =5.098, p = .024). **Conclusions:** These findings suggest that despite similar dietary Mn intake, obese individuals have greater MnB than non-obese individuals. Obese individuals reported more somatization, anxiety symptoms, and musculoskeletal and neurological problems. The relationship between MnB and obesity should be considered when examining the effects of environmental exposure to neurotoxins.

Acknowledgment: *This study was funded by the U.S. EPA under Co-op. Agreement P8341600*

Keywords: Manganese, Obesity, Health

P-69

RELATIONSHIP BETWEEN LOW-LEVEL LEAD EXPOSURE AND PHYSICAL GROWTH IN CHILDREN OF EL PASO TEXAS.

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Blood lead levels in children have declined over the past 30 years following to removal of lead in paint and gasoline. Despite this advance, low-level lead exposure continues to threaten to an unknown number of children. Previous research has indicated that blood lead levels are negatively correlated with physical growth in children. The lowest levels at which exposure to lead may cause growth differences are not known. Few studies have examined the effects of lead on growth in children with very low level lead exposure. Blood lead levels and anthropometric measures were collected from 223 children of 5 to 12 years of age. Anthropometric measures were compared for children with and without lead exposure using three different exposure criteria cut points (2.0, 2.5 and 3.0 µg/dL). Significant differences in head circumferences, Body Mass Index (BMI) and weight were found for children with blood lead levels as low as 2.0 µg/dL. Height differed only among girls with blood lead levels of 2.5 µg/dL or higher. This evidence suggests that lead can affect children's physical development at the lowest-level of lead exposure.

Keywords: Lead Exposure, Children, Physical Growth

P-70

THE POSSIBLE PROTECTIVE EFFECTS OF ACTIVE BILINGUALISM IN CHILDREN EXPOSED TO LOW LEVEL LEAD, AS MEASURED BY THE SIMON TASK. Marisela Gutierrez and Christina Sobin. *Department of Psychology, University of Texas at El Paso, El Paso, Texas, US*.

Many children are at a high risk of chronic low-level lead exposure, particularly minority children living in low socioeconomic conditions. Past research has suggested that chronic low level lead exposure (< 10 µg/dL) is associated with diminished attention and executive function in children. It has also been suggested that bilingualism has a positive impact on the development of several neurocognitive functions including executive problem-solving, attention, and inhibitory control. Specifically inhibitory control processes have been shown to be enhanced in bilingual children during a visual problem-solving task requiring inhibitory control (Simon Task). The present study examined bilingualism as a possible protective mechanism in children with low blood lead levels. Active bilingualism (daily use of two languages), blood lead level, and performance on the Simon task were assessed in 267 children (mean age = 8.21 years). Consistent with past studies, children with subclinical blood lead levels were slower to respond. As compared with exposed monolingual children, exposed bilingual children had faster response times for both congruent and incongruent trials. Bilingual children without subclinical blood lead levels were the fastest responders. These data may suggest that active bilingualism protects the brain from the negative effects of exposure to low-level environmental lead. Active bilingualism might be considered a natural protective mechanism against low level lead exposure.

Keywords: Low-level Lead, Bilingualism, Inhibitory Control

INSECTICIDE NEUROTOXICITY: MECHANISMS AND POPULATIONS

P-71

ORGANOPHOSPHATE (OP) PESTICIDES INDUCE OXIDATIVE STRESS AND OXIDATIVE DNA DAMAGE IN HUMAN SH-SY5Y NEUROBLASTOMA CELLS. Juan F. Muniz[1], Linda A. McCauley[2], and Glen E. Kisby[3] [1]*University of Pennsylvania-School of Nursing, Philadelphia PA 19104-6096, USA;* [2]*Emory University, Atlanta, GA;* [3]*Center of Research on Occupational & Environmental Toxicology (CROET), Oregon Health & Science University, Portland, OR 97239, USA*

Epidemiological studies indicate that pesticide exposure is a significant risk factor for neurodegenerative disease (e.g., Parkinson's). Our previous studies indicate that oxidative stress and DNA damage are significantly increased in agricultural workers who used organophosphate (OP) pesticides (Muniz *et al*., Toxicol Appl Pharmacol 227:97-107, 2008). Thus, oxidative stress and DNA damage may be important mechanisms that link chronic exposure to pesticides with neurodegenerative disease. To determine if OP pesticides induce their effects on human neurons through similar mechanisms, dopaminergic human SH-SY5Y neuroblastoma cells were treated with AZM (10 µM to 350 µM) for 24-48h and examined for cell viability, oxidative stress and DNA damage [double-strand breaks (DSB), single-strand breaks (SSB) and 8-oxodeoxyguanosine DNA lesions]. Cell viability and glutathione (GSH) levels were significantly reduced ($p<0.0001$) by AZM in a dose-dependent manner and this correlated with increase levels of reactive oxygen species. Pre-treatment with a GSH inhibitor (i.e., BSO) significantly reduced the viability ($p<0.0001$) of AZM treated SH-SY5Y cells whereas pre-treatment with *N*-acetylcysteine, vitamin E or alpha-phenyl-tert-butylnitrone (PBN) primarily preserved cell viability *($p<0.0001$).* These studies demonstrate that AZM induces neuronal cell death through an oxidative stress-mediated mechanism. DNA damage studies (Neutral Comet and Alkaline Comet assay with and without *fpg*) showed a dose-dependent increase in oxidative DNA damage ($p<0.0001$**),** but not DSB in AZM treated SH-SY5Y cells. These findings indicate that oxidative stress and oxidative DNA damage are consistent features in both agricultural workers who used OPs and human neural cells treated with an OP pesticide. Thus, OP pesticides have the potential to induce oxidative stress and DNA damage and these mechanisms may explain their role in neurodegenerative disease.

Keywords: OP pesticides, DNA Damage, Oxidative Stress

P-72 (Also presented from Platform in Session IV #54)
Pre-Doctoral Poster & Travel Award Competition

DELTAMETHRIN EXPOSURE RESULTS IN CALPAIN/PROTEASOMAL DEPENDENT DOWN-REGULATION OF VOLTAGE GATED SODIUM CHANNEL EXPRESSION. Jason P. Magby[1,2,] Jason R. Richardson[1,2]

[1]*Environmental and Occupational Health Sciences Institute and Department of Environmental and Occupational Medicine, Robert Wood Johnson Medical School.* [2]*Joint Graduate Program in Toxicology, Rutgers University, University of Medicine and Dentistry of New Jersey*

The voltage gated sodium channel is essential for action potential generation in neurons. *In vitro* studies have shown that prolonged exposure to sodium channel agonists can down-regulate sodium channel expression and function. However, it is unknown if these effects occur *in vivo*. The pesticide deltamethrin is thought to exert toxicity by acting as an agonist of voltage gated sodium channels leading to persistent depolarization. Here, we sought to determine whether developmental exposure to deltamethrin causes persistent changes in sodium channel expression *in vivo*. Pregnant mice were exposed throughout gestation and lactation to either 0 or 3 mg/kg deltamethrin every three days. The offspring were sacrificed at 10-11 months of age for determination of isoform-specific sodium channel ($Na_{(V)}$) mRNA expression by QPCR. In the male offspring following developmental exposure to deltamethrin, $Na_{(V)}$ 1.3 expression was decreased by 27% in the frontal cortex, with no other significant effects on α subunit isoforms in this region. In the striatum of exposed males, $Na_{(V)}$ 1.1, $Na_{(V)}$ 1.2, $Na_{(V)}$ 1.3, and $Na_{(V)}$ 1.6 were decreased by about 25%. To gain insight into the mechanism of this down-regulation we used an *in vitro* culture system. Exposure of SK-N-AS neuroblastoma cells to 100 nM deltamethrin for 24 hr resulted in a 20% decrease of $Na_{(V)}$ 1.2 and $Na_{(V)}$ 1.3. Similar results were observed with the voltage gated sodium channel agonist veratridine and these effects were blockedy by the $Na_{(V)}$ antagonist tetrodotoxin (TTX). Interestingly we were also able to prevent the down-regulation of $Na_{(V)}$ mRNA with the calpain inhibitor PD 150606, the proteasomal inhibitor lactacystin, and the intracellular calcium chelator BAPTA-AM. Taken together, these data suggest that developmental deltamethrin exposure results in long-term alterations of isoform-specific sodium channel subunit mRNA expression. Furthermore, the in vitro data suggest that sodium influx and calcium signaling, culminating in activation of protein degradation pathways lead to down-regulation of Na(v) expression following deltamethrin exposure.

Keywords: Sodium channels, Pyrethroid, Calpain

Mentor: Jason R. Richardson

P-73 (Also presented from Platform in Session X #53)

Post-Doctoral Poster & Travel Award Competition

ALLETHRIN DIFFERENTIALLY MODULATES VOLTAGE-GATED CALCIUM CHANNEL SUBTYPES IN RAT PC12 CELLS. April P. Neal, Yuan Yukun, and William D. Atchison. *Department of Pharmacology & Toxicology, Michigan State University, East Lansing, MI USA*

Pyrethroid insecticides are one of the most widely-used classes of insecticides. Previous studies revealed that pyrethroids potently affect the insect voltage-gated sodium channel (VGSC), resulting in prolonged channel open time. However, recent findings have suggested that pyrethroids may affect targets other than the VGSC. In particular, several studies have shown that pyrethroids can modulate the activity of voltage-gated calcium (Ca^{2+}) channels (VGCCs). However, these studies often reported conflicting results; some studies observed stimulatory while others inhibitory effects of pyrethroids on VGCCs. The present study investigated whether allethrin (AL), a well-characterized type I pyrethroid, altered VGCC characteristics measured by whole-cell recording in rat pheochromocytoma cells (PC12) differentiated with nerve growth factor (NGF). AL (5 μM) increased peak, end, and tail composite VGCC current independent of its effects on VGSCs. After blocking VGCC subtype-specific current with ω-conotoxin GVIA (GVIA, N-type) or nimodipine (NIM, L-type), our data further suggest that AL differentially affects VGCC subtypes. Thus AL apparently stimulated GVIA-insensitive current while inhibiting NIM-insensitive current. AL shifted peak and end currents towards more hyperpolarized potentials through interaction with the L-type VGCC, and shifted the inactivation of L-type VGCCs to more hyperpolarized potentials. AL appears to differentially modulate specific VGCC subtypes, which may explain some of the conflicting observations of other studies.

Keywords: Pyrethroid Neurotoxicology, Voltage-gated Calcium Channel, Electrophysiology

Mentor: William Atchison

P-74

Post-Doctoral Poster & Travel Award Competition

N-ISOPROPYLACRYLAMIDE INCREASES ACETYLCHOLINE RELEASE IN RAT STRIATAL SLICES. LKM Wright, J Liu and CN Pope. *Department of Physiological Sciences, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, OK, USA*

N-isopropylacrylamide (NIPA) is a monomer used in the preparation of thermoresponsive nanomaterials that are being evaluated in a variety of applications including controlled drug delivery and tissue engineering. Although the toxicological effects of NIPA have not been extensively evaluated, subchronic dosing reportedly led to altered rotarod performance and morphological changes in peripheral nerves, similar to acrylamide (ACR). Axonal damage in peripheral nerves following ACR exposure has been reported for decades, but more recent studies suggest that ACR neurotoxicity may be mediated pre-synaptically by adduction of cysteine sulfhydryls on proteins important in neurotransmitter uptake, vesicle packaging and/or release. In the present study, the *in vitro* effects of NIPA, ACR and the non-neurotoxic analog propioniamide (PRO) on acetylcholine (ACh) release were evaluated in rat striatal slices. Slices (400 µm) from adult, male Sprague Dawley rats were pre-loaded with [3H]choline (30 nM, 40 min, 37°C), superfused with physiological buffer containing hemicholinium-3 (10 µM, to block reuptake of choline), and then depolarized twice (S1, S2) with high potassium (20 mM). NIPA, ACR and PRO were added 10 min before S2, and the radioactivity peak ratio of S2/S1 was used to evaluate effects on ACh release. NIPA (0.1-100 mM) increased striatal ACh release in a concentration-dependent manner (maximal increase, 62%), with an EC_{50} of ~30 mM. ACR (100 mM) increased ACh release by ~30%, while PRO had no effect. In contrast to previous studies reporting concentration-dependent reduction in synaptosomal dopamine uptake and release by *in vitro* or *in vivo* ACR exposure, both NIPA and ACR appear to increase ACh release in striatal slices, possibly mediated through changes in dopaminergic transmission. While high *in vitro* NIPA concentrations were needed to increase ACh release, neurotoxicity and adduct formation following exposure to ACR and its analogs are cumulative, thus biologically relevant concentrations/dosages could lead to altered CNS cholinergic signaling. Knowledge of the potential neurotoxicity of NIPA may be important in the future development and application of NIPA-based nanomaterials. (Supported by OSU Board of Regents).

Keywords: Acetylcholine release, N-isopropylacrylamide, Nanomaterials

Mentor: Carey Pope

P-75

Post-Doctoral Poster & Travel Award Competition

MECHANISM OF PYRETHROID PESTICIDE-INDUCED APOPTOSIS: ROLE OF THE ER STRESS PATHWAY AND CALPAIN ACTIVATION.

M. M. Hossain and J. R. Richardson. *Department of Environmental and Occupational Medicine, Environmental and Occupational Health Sciences Institute, Robert Wood Johnson Medical School, Piscataway, NJ 08854, USA.*

Exposure to the pyrethroid pesticide deltamethrin has been demonstrated to cause apoptosis both in vitro and in vivo (Wu and Liu, 2000; Elwan et al., 2006). However, the molecular pathways leading to deltamethrin-induced apoptosis have not been established. Here, we sought to identify the molecular pathways by which deltamethrin causes apoptosis in the dopaminergic-like SK-N-AS cell line. Following 24-48 hr exposure to deltamethrin (100 nM-5 µM), we observed a time and dose-dependent increase (21-121%) in DNA fragmentation, an indicator of apoptosis. DNA fragmentation was accompanied by increased caspase-9 and 3 activities and was abolished by specific caspase-9 and 3 inhibitors. However, deltamethrin did not increase cytosolic cytochrome *c* levels, indicating that the mitochondrial pathway was likely not involved. Indeed, the initiation of DNA fragmentation resulted from interaction of deltamethrin with Na+ channels and subsequent calcium influx, as tetrodotoxin and the intracellular Ca^{2+} chelator BAPTA-AM completely prevented apoptosis. Additional studies demonstrated that deltamethrin exposure activated caspase-12 activity as early as 12 hr after exposure, suggesting a role for the ER stress pathway in deltamethrin-induced DNA fragmentation. The role of the ER stress pathway was confirmed by the finding that the calpain and eIF2α inhibitors completely prevented the deltamethrin-induced DNA fragmentation. Furthermore, siRNA knock down of calpain-1 abolished deltamethrin-induced DNA fragmentation. Together, these findings demonstrate that deltamethrin activates the ER stress pathway through its interaction with Na+ channels and subsequent calcium overload and leads to initiation of the apoptotic cascade through calpain-1 activation of caspase-12. Because ER stress and the subsequent unfolded protein response have been observed in a number of neurodegenerative diseases, these data provide mechanistic information supporting the link between pesticide exposure and neurodegeneration. Supported by NIH R01ES015991

Keywords: Pyrethroid, ER Stress, Apoptosis

Mentor: Jason Richardson

P-76

CHLORPYRIFOS DISRUPTS NEUROLIGIN-MEDIATED SYNAPSE FORMATION. Dongren Yang and Pamela Lein. *Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, CA, 95616, USA*

The causes of autism spectrum disorder (ASD) are poorly understood. While genetic factors are important in determining susceptibility, there is evidence that environmental factors also influence ASD risk and severity. Because environmental factors are more readily controlled than genetic factors, there are compelling reasons to identify specific environmental factors that contribute to the adverse neurodevelopmental changes observed in ASD. In this study, we are examining the possibility that organophosphorus pesticides (OPs) interact with neuroligin to interfere with synapse formation in the developing brain. This hypothesis is derived from observations that 1) mutations in the gene that encodes neuroligin have been linked to ASD; 2) increasing evidence suggests that the patterns of synaptic connections between neurons are altered in the brains of autistic children; and 3) the OP chlorpyrifos and neuroligins have both been shown to independently influence the formation of synaptic connections. Additional reasons to suspect that OPs may contribute to determining the risk for ASD include documented widespread exposure of pregnant women and children to OPs in both agricultural and residential settings as well as findings from recent human studies suggesting that perinatal exposure to OPs may be linked to increased risk for ASD. Using embryonic hippocampal neurons co-cultured with COS-7 cells transfected with neuroligin, we show that chlorpyrifos disrupts neuroligin-mediated synapse formation in vitro in the absence of effects on cell viability and neuroligin expression. These data support our hypothesis and provide a biological mechanism to support the epidemiological data linking OP exposure to increased risk for ASD. This work supported by the UC Davis M.I.N.D. Institute.

P-77

CHANGES IN THE ACTIVITY OF SEROTONERGIC NEURONS FOLLOWING ACUTE EXPOSURE TO ORGANOPHOSPHATE PESTICIDES. R Dodds, PG Blain & SJ Judge. *The Medical Toxicology Centre, Wolfson Unit, Newcastle University, Newcastle upon Tyne, UK*.

Organophosphate pesticides (OPs) can cause hypercholinergic symptoms as a result of cholinesterase inhibition. OP exposure can also increase the risk of depression and anxiety, suggesting that the 5-HT (serotonin) system may also be affected. Indeed, it has been reported that OP exposure in animals can affect 5-HT forebrain levels and 5-HT receptor and transporter expression[1,2]. Forebrain 5-HT release, and consequently 5-HT receptor and transporter expression, is dependent on the firing activity of 5-HT neurons in the dorsal raphe nucleus (DRN). We used extracellular electrophysiology in adult rat brain slices to examine the effect of acute OP exposure on the activity of DRN 5-HT neurons. Ten minute exposure to 50 μM chlorpyrifos (n = 7) or diazinon (n = 8) had no significant effect on the basal firing rate of the 5-HT neurons or on their inhibitory response to 5-HT. However, exposure to the metabolites of DZN and CPF, diazinon oxon (n = 9) and chlorpyrifos oxon (n = 7) (50μM; 5 mins) caused a persistent increase in the basal firing rate of the 5-HT neurons and a decrease in firing regularity. These data indicate that OP metabolites can rapidly alter the firing activity of 5-HT neurons, supporting the evidence that the 5-HT system is affected by OP exposure. This OP effect may contribute to the development of psychiatric disorders.

1. Slotkin *et al*., Environ Health Perspect. (2006) 114:1542-6.
2. Judge *et al*., Neurotoxicology (2009) 30:865.

Keywords: Organophosphate, 5-HT, Electrophysiology

P-78

COMPOUNDS-SPECIFIC EFFECTS OF MUTATIONS AT V787 IN DII-S6 OF NAV1.4 SODIUM CHANNELS ON INHIBITION BY SODIUM CHANNEL BLOCKER INSECTICIDES (SCBIS). R.T. von Stein and D.M. Soderlund. *Department of Entomology*, *Cornell University, Geneva, NY, USA.*

Sodium channel blocker insecticides (SCBIs) inhibit Na^+ conductance in both insect and mammalian neurons by stabilizing voltage-gated Na^+ channels in a non-conducting slow-inactivated conformation. The mode of action for SCBIs is similar to local anesthetic (LA) drugs that bind to the LA receptor putatively formed by residues in DI-S6, DIII-S6, and DIV-S6. Site-directed mutagenesis has demonstrated that LA receptor residues in DIV-S6 are important modulators of SCBI sensitivity, indicating that insecticidal and therapeutic Na^+ channel blockers share common inner-pore binding determinants. Inner-pore residues are

also important determinants of slow inactivation gating. In $rNa_v1.4$ channels, slow inactivation is enhanced by substituting lysine for the native valine at position 787 in DII-S6, whereas cysteine or alanine substitutions at V787 inhibits slow inactivation to variable extents. To investigate the state-dependence of SCBI block, we substituted amino acid residues at V787 in $rNa_v1.4$ and characterized the effects on SCBI sensitivity using the *Xenopus* oocyte expression system and two-electrode voltage-clamp technique. Mutations at V787 modulated SCBI sensitivity in a manner independent from mutation-induced changes in slow inactivation gating. Furthermore, the effects of mutations at V787 on block of channels by SCBIs were compound-specific. In particular, $Na_v1.4$-V787K channels were completely resistant to block by 10 mM metaflumizone, despite being more sensitive to block by indoxacarb. Taken together, these results suggest that V787 is either part of or in close proximity to the binding domain for SCBIs. Moreover, it is likely that the precise molecular determinants for the binding of SCBIs to Na^+ channels are not identical for all members of this insecticide class. Supported by NIEHS Grant No. R01-ES014591

Keywords: Sodium Channels, Slow Inactivation, Insecticides

P-79

NEUROBEHAVIORAL EVALUATION OF CHILDREN LIVING IN AN AGRICULTURAL COMMUNITY. DS Rohlman, E Hohn, T Moomey, A Kirk, S Huszar. *Center for Research on Occupational and Environmental Toxicology, Oregon Health & Science University, Portland, OR 97239.*

There is increasing concern that the use of pesticides in agriculture may be affecting farm worker communities, including children. Organophosphate (OP) pesticides are among those of greatest concern, due to their persistence once in the home and their established neurotoxic effects. Many factors have been associated with increased levels of pesticide metabolites in children and adults and pesticide residues found in homes. Neurobehavioral tests have identified deficits in adult populations exposed to and poisoned by OP pesticides on farms. However, little research has examined OP pesticide exposure in children. While the neurotoxic effects of acute exposure to OP pesticides are well established, chronic low-level exposures are not well studied in adults and very few studies provide evidence of neurobehavioral deficits in farm worker children compared to controls. Children of farm workers are presumed to be exposed to pesticides throughout development, and this exposure may produce subtle health effects that would not be detected by clinical examinations nor recognized by parents. Preliminary data from an ongoing study to examine health effects of children living in an agricultural community will be presented. The goal of the current study is to examine health effects from chronic exposure to organophosphate pesticides in children and determine if they are associated with current home pesticide exposure. We have developed methods to assess neurobehavioral functioning in school-age children and a measure of lifetime exposure to pesticides. Children's exposure to pesticides from the parent's work or residence in an agricultural community was measured through dust samples collected from the home.

Keywords: Pesticides, Children, Exposure

P-80 (Also presented from Platform in Session VII #33)
Post-Doctoral Poster & Travel Award Competition

BIOMARKERS OF ORGANOPHOSPHORUS (OP) EXPOSURES IN HUMANS. J Marsillach[1,2], RJ Richter[1,2], JH Kim[3], RC Stevens[1,2], MJ MacCoss[2], D Tomazela[2], SM Suzuki[1,2], LM Schopfer[4], O Lockridge[4], CE Furlong[1,2]. *Univ. of Washington, Departments of [1]Medicine (Div. Medical Genetics), [2]Genome Sciences, [3]Anesthesiology, Seattle, WA, [4] University of Nebraska Medical Center, Omaha, NE*

There have been a number of recent events where aircraft engine bleed air has become contaminated with engine lubricating oil that contains tricresyl phosphate (TCP), the molecule that caused thousands of cases of paralysis during prohibition when it was used to adulterate ginger extracts. The bleed air enters the aircraft cabins unfiltered. It has been known since 1954 that the ortho-isomers of TCP are metabolized into neurotoxic metabolites. It is now known that the bioactivation is carried out primarily by liver cytochromes P450. Since these enzymes vary greatly among individuals in response to diet and medications as well as from genetic differences, some individuals are much more susceptible to neurological damage from TCP exposure. Mass spectrometric protocols are being developed to determine the percentage of "biomarker proteins" that are modified by such exposures. Both plasma butyrylcholinesterase (BChE) and red cell acylpeptide hydrolase (APH) are readily inhibited by cresyl saligenin cyclic phosphate (CBDP) or phenyl saligenin cyclic phosphate (PSP) and have the potential

to provide information about both the level of exposure and the P450 status of an exposed individual. We have characterized the active site serine adducts and have developed immunomagnetic bead-based single-step purification protocols for both BChE and APH. Sensitive individuals have experienced tremors for a number of years following an exposure. Supported by NIH Grants ES009883, ES04696 and funding from pilot and crew unions, the Royal Australian Air Force, and the Norwegian Union of Energy Workers.

Keywords: Biomarkers, Cabin air, Neurotoxicity

Mentor: Clement E. Furlong

P-81 (Also presented from Platform in Session VIII #39)

Pre-Doctoral Poster & Travel Award Competition

ENVIRONMENTAL NEUROTOXIC PESTICIDE INDUCED HISTONE ACETYLATION AND ITS ROLE IN EPIGENETIC MECHANISMS OF NEURODEGENERATION. Song C, Kanthasamy A, Anantharam V, Sun F, Kanthasamy AG. *Iowa Center for Advanced Neurotoxicology, Department of Biomedical Sciences, Iowa State University, Ames, IA 50011, USA.*

Pesticide exposure has been implicated in the etiopathogenesis of Parkinson's disease (PD); in particular, the organochlorine insecticide dieldrin is believed to be associated with PD. Emerging evidence indicates that histone modifications play a critical role in cell death. In this study, we examined the effects of dieldrin treatment on histone acetylation and its role in dieldrin-induced apoptotic cell death in dopaminergic neuronal cells. In mesencephalic dopaminergic neuronal cells, dieldrin induced a time-dependent increase in the acetylation of core histones H3 and H4. Histone acetylation occurred within 10 min of dieldrin exposure indicating that acetylation is an early event in dieldrin neurotoxicity. The hyperacetylation was attributed to dieldrin-induced proteasomal dysfunction, resulting in accumulation of a key histone acetyltransferase (HAT), cAMP response element-binding protein. The novel HAT inhibitor anacardic acid significantly attenuated dieldrin-induced histone acetylation, Protein kinase C delta proteolytic activation and DNA fragmentation in dopaminergic cells protected against dopaminergic neuronal degeneration in primary mesencephalic neuronal cultures. Furthermore, 30-day exposure of dieldrin in mouse models induced histone hyperacetylation in the striatum and substantia nigra. For the first time, our results collectively demonstrate that exposure to the neurotoxic pesticide dieldrin induces acetylation of core histones because of proteasomal dysfunction and that hyperacetylation plays a key role in dopaminergic neuronal degeneration after exposure of dieldrin.

Mentor: Anumantha Kanthasamy

MODELING NEUROLOGICAL DISORDERS

P-82

Pre-Doctoral Poster Award Competition

MECHANISM OF PRODUCTION OF SPECIFIC ALPHA-SYNUCLEIN C-TERMINAL TRUNCATIONS AND IMPLICATIONS TO DISEASE. Caroline M. Ritchie[1], Karen A. Lewis[1], Yang Su[2,3], Olina Jou[2,3], Charles L. White III[2,3], Kimmo J. Hatanpaa[2,3], George N. DeMartino[1], Philip J. Thomas[1]. *Departments of Physiology[1], Pathology[2], and the Alzheimer's Disease Center[3], University of Texas Southwestern School of Medicine, Dallas, TX, USA*

It is believed that the formation of C-terminally truncated alpha-synuclein fragments may accelerate the formation of Lewy bodies in brains of patients with Lewy body dementia and Parkinson disease. However, the mechanism by which these truncations are produced is unknown. We have identified the 20S proteasome as a putative protease responsible for the formation of these C-terminal truncation products. We have developed polyclonal antibodies specific to several C-terminal truncation products of alpha-synuclein (α-syn119 and α-syn110) that are produced by the 20S proteasome *in vitro* and found that these truncated species are present in the brains of patients with Lewy body disease at much higher levels than in age-matched controls. This data also suggests that aggregated full-length alpha-synuclein predominantly colocalizes with aggregated α-syn119, but not α-syn110. We are currently investigating whether the presence of these C-terminal alpha-synuclein truncations in cerebrospinal fluid can be used as a biomarker for Lewy body disease and Parkinson disease. We are also studying the mechanism by which alpha-synuclein is recognized and degraded by the 20S proteasome. We have shown that not all natively-disordered proteins are good substrates for 20S proteasomal degradation. Furthermore, our data suggests that the position of the cleavage site within the substrate influences its susceptibility to degradation. Future studies aim to elucidate the properties of a protein that make it a good substrate for degradation by the 20S proteasome.

Keywords: alpha-synuclein, proteasome, aggregation

Mentor: Phillip J. Thomas

P-83 (Also presented from Platform in Session IV #15)
Pre-Doctoral Poster & Travel Award Competition

HIGH-THROUGHPUT ASSAY TO ASSESS MANGANESE TRANSPORT KINETICS IN A STRIATAL CELL MODEL OF HUNTINGTON'S DISEASE. GF Kwakye, D Li, AB Bowman. *Dept. of Neurology, Vanderbilt Kennedy Center. Vanderbilt University Medical Center. Nashville, TN, USA.*

Expansion of a polyglutamine-tract in Huntingtin (Htt) leads to the degeneration of medium spiny neurons in Huntington's disease (HD). Recently, we discovered that expression of mutant Htt is associated with impaired manganese (Mn) uptake upon over-exposure in a striatal cell line and mouse model of HD. To evaluate the mechanism by which expression of mutant HTT alters cellular Mn accumulation, we have developed a fluorescence-quenching based assay (Fura-2 Mn assay) to measure Mn transport kinetics (e.g. Vmax and Km) and Mn storage properties (e.g. Bmax and Kd). The assay utilizes changes in Fura-2 fluorescence as an indirect measure of total extractable cellular Mn content. Here, we report the development and validation of the assay by graphite furnace atomic absorption spectrometry (GFAAS). The Fura-2 Mn assay is able to accurately measure extracted Mn levels over a range of at least 0.1 – 1 µM. We have applied this assay to assess Mn transport and storage in wild-type ST*Hdh*$^{Q7/Q7}$ and mutant ST*Hdh*$^{Q111/Q111}$ striatal cell line model of HD. We find that mutant ST*Hdh*$^{Q111/Q111}$ have decreased Mn uptake in a time and concentration-dependent manner relative to wild-type ST*Hdh*$^{Q7/Q7}$ cells as early as one hour after exposure with 200 uM $MnCl_2$. We observe a substantial decrease in uptake capacity (Bmax) in the mutant cells, with only moderate differences in Mn uptake efficiency (Kd). Finally, we have utilized small molecule inhibitors of divalent metal transporter 1 (DMT1) in the context of Fura-2 Mn assay. We find that while DMT1 inhibitors impaired Mn uptake, it did so equally between wild-type and mutant cell lines. To conclude, we have developed a novel assay, which provides a rapid means of assessing the dynamics of Mn transport kinetics in disease and cellular toxicity models.

Keywords: Manganese, High- throughout assay, Huntington's Disease
Mentor: Aaron Bowman

P-84

EFFECT OF CHRONIC MPTP ADMINISTRATION ON GLYCOLYSIS AND TCA CYCLE PATHWAYS IN MOUSE MODEL OF PARKINSON'S DISEASE. Bakary J Sonko[1], Tom Schmitt[1], Laszlo Boros[3], Sumit Sakar[2], Ali Syed[2] and Richard Beger[1]. *[1]Division of Systems Biology, NCTR, FDA, Jefferson, AR USA; [2]Division of Neurotoxicology, NCTR, FDA, Jefferson, AR USA; [3]SIDMAP LLC. 2990 S. Sepulveda Blvd, Los Angeles, CA, USA.*

Introduction: Parkinson's disease (PD) affects nearly 1.5 million Americans. It is poorly understood, poorly diagnosed and has no cure yet. PD is characterized by slow mobility, resting tremor, rigidity and gait disturbance. The underlying molecular mechanisms of PD are not well understood. A neurotoxin, 1-methyl-4-phenyl 1, 2, 3, 6-tetrahydropyridine (MPTP) has been used to investigate the condition. Deranged ATP production is implicated in 1-methyl-4-phenylperidinium ion (MPP^+), induced PD- like symptoms. In the current study, we used SIDMAP flux analysis techniques to examine: a) chronic effects of MPTP on glycolytic and TCA cycle pathways in a mouse model of PD and, b) whether by-products of these metabolic pathways could provide biomarkers for better characterization of PD.

Methods: Four groups of C57BL/6 mice (8/group) obtained from the NCTR colony were given either saline (Control), 5mg/kg, 10mg/kg or 20mg/kg MPTP by ip injection for five consecutive days after which, they lived for 21 days and were then given (i.p.) U-^{13}C-glucose (0.5mg/10ml/kg). Blood was collected at 30 and 90min. Animals were sacrificed at 120min and terminal blood and brain were collected. Samples were analyzed for ^{13}C mass isotopomer distribution using Gas Chromatography/Mass Spectrometry.

Results: MPTP increased pyruvate kinase flux and pushes glucose to replenish TCA cycle intermediates through anaploresis. Increased M1 compared M2 extracellular glutamate at 20mg dose level suggests more glutamate being pushed out of the tissues into the circulation, while more $^{13}CO_2$ production reflects the cells ATP needs to cope with these demands. Inability to meet these energy requirements could lead to pathway collapse and cell death. The increase in glutamate levels could be used as a marker for patients with neurological diseases or those taking drugs that cause nerve damage.

Keywords: MPTP, Parkinson, Glutamate

P-85

RESVERATROL PROTECTS AGAINST MPP+ AND METHAMPHETAMINE NEUROTOXICITY BY MODULATING THE PKCδ APOPTOTIC SIGNALING PATHWAY AND MICROGLIAL ACTIVATION. K. Kanthasamy*, R. Gordon, C. Hogan, V. Anantharam, A.G. Kanthasamy, and A. Kanthasamy, *Parkinson's Disorder Research Laboratory, Dept. of Biomedical Sciences, Iowa State University, Ames, IA 50011-1250*

Resveratrol (3, 4', 5-trihydroxy stilbene) is a phytoalexin that has been shown to produce beneficial effects in various disease models. Generally recognized as a potent antioxidant, resveratrol has also been shown to modulate various signaling molecules including SIRT-1. We hypothesized that resveratrol could have a dual neuroprotective function in models of dopaminergic neurotoxicity by protecting against neuronal apoptosis as well as by suppressing microglial mediated neurotoxicity. Specifically, we examined whether i) resveratrol protects against dopaminergic neurodegeneration induced by the Parkinsonian toxicants MPP+ and methamphetamine (Meth), ii) resveratrol attenuates microglial activation, and iii) resveratrol modulates the PKCδ dependent proapoptotic signaling in dopaminergic neuronal cells. Resveratrol treatment in the N27 mesencephalic dopaminergic neuronal cell model significantly attenuated MPP+ and Meth -induced cell death in a dose-dependent manner. This protective effect was accompanied by attenuation of neurotoxicant-induced caspase-3 enzyme activity as well as proteolytic cleavage of proapoptotic kinase PKCδ. Interestingly, resveratrol effectively blocked the expression of PKCδ in a dose-dependent manner. Furthermore, we confirmed the neuroprotective effect of resveratrol in mouse primary mesencephalic cultures by determining the number of TH positive neurons. Resveratrol also attenuated LPS-induced nitric oxide production and proinflammatory cytokines in BV-2 microglial cells in a dose-dependent manner, indicating that resveratrol can also suppress microglial activation and the subsequent neuroinflammatory response. Collectively, our results demonstrate that resveratrol protects against the degenerative process in dopaminergic neurons by modulating the PKC-delta dependent apoptotic pathway as well as by suppressing microglial mediated neurotoxicity (NS 38644 and NS65167).

Keywords: Neurotoxicity, Neuroinflammation, Neuroprotection, Parkinson's Disease

P-86

USING A BIOMARKER OF OXIDATIVE STRESS TO DETERMNE SYNERGISTIC INTERATIONS BETWEEN IRON AND PARAQUAT. T. Brown, F. Cardozo-Pelaez. *Center for Environmental Health Sciences. Department of Biomedical and Pharmaceutical Sciences, The University of Montana, Missoula, Montana, USA*

It has been shown that iron and paraquat can act together causing a synergistic reaction in which they cause more damage to cells when combined than when they are applied separately. These ROS are able to react with DNA to generate a number of possible DNA lesions. Two-electron oxidation of guanine results in the formation of 8-hydroxy-2'-deoxyguanosine (oxo^8dG), which is the major oxidation product of guanine in DNA, and it has attracted considerable interest as a biomarker of oxidative stress. Guanosine 5'-triphosphate (GTP) can also be modified by ROS. GTP concentrations in the cytoplasm are hundreds of times larger than dGTP. This suggests that under conditions of high ROS levels, significantly more oxidized GTP (oxo^8GTP) than oxo^8dGTP could be produced in the cell. Previously we have described a method in which the level of oxo^8GTP and GTP in the cytoplasm of cells can be quantified using high performance liquid chromatography with electrochemical detection (HPLC-EC). In this study the same method was implemented to determine if ShSy-5y cells, a line of human neuroblastoma, underwent oxidative stress when exposed to PQ (500 µM), iron (500 µM), or PQ and iron (500 µM each) in a 4 hr exposure. The ratio of oxo^8G to G was determined and it was found that iron and PQ exposure alone did not increase it compared to the negative control. However, when the cells were exposed to both PQ and iron the ratio of oxo^8G to G did increase compared to control or each toxin alone. This data supports the idea of PQ and iron working synergistically to create ROS in cells. It also confirms the use of HPLC-EC in determining oxo^8GTP and GTP levels in cells as a biomarker for oxidative stress.

Keywords: Paraquat, Biomarkers, Oxidative Stress

DEVELOPMENTAL NEUROTOXICITY

P-87 (Also presented from Platform in Session IV #17)
Pre-Doctoral Poster & Travel Award Competition

SINGLE POSTNATAL METHYLMERCURY (MEHG) EXPOSURE INITIATES ACUTE STEM CELL DEATH IN HIPPOCAMPUS LEADING TO SUBSEQUENT NEURONAL DEFICITS AND LEARNING IMPAIRMENT. K. Sokolowski[1,2] Kelsey Robinson[1] and E. DiCicco-Bloom[1,2]

Joint Graduate Program in Toxicology, Rutgers[1]; Neurosci & Cell Biol, RWJMS, Piscataway, NJ[2]

MeHg, an environmentally persistent teratogen, induces a spectrum of harmful effects on brain structure and function. Our recent studies (Falluel-Morel et al. 2007) indicated that a single sc injection of 5µg/gbw MeHg in 7 day old (P7) rat pups acutely activates caspase-3, an apoptotic protease, leading to cell cycle arrest and cell death specifically in hippocampus and not cerebellum. Caspase-3 immunoreactivity was therefore used to define lower exposures of MeHg that induce significant increases in hippocampal cell death. We found a five-fold increase in caspase-3 positive cells 24h after 0.6µg/gbw MeHg, a ten-fold lower exposure. Also, BrdU labeled mitotic cells were decreased by ~30% of the control in proliferative regions of hippocampus (32% hilus (H), 27% granule cell layer (GCL)). Double labeling revealed that ~80% of caspase-3-positive cells were colabeled with nestin and ~35% with Sox2, both neuronal stem cell markers. In contrast, GFAP staining in hilus was unchanged with 0.6µg/g MeHg compared to controls, although an increase was detected at 5µg/gbw MeHg. The apparent selective sensitivity of neuronal stem cells in hippocampus at this low exposure correlated with a 22% and 27% decrease in total cells in H and GCL two weeks after MeHg (P21) defined by stereology. In addition, there was a subtle learning deficit coupled with a significant increase in escape latency in the 2-day memory trial assessed by Morris water maze (2-day memory: Con: 18±3sec, MeHg: 28±4sec, p=0.03). Together, these results illustrate that a single injection of 0.6µg/g MeHg at P7 selectively targets neuronal stem cells for apoptosis that may lead to decreases in neurons in the dentate gyrus two weeks later at P21 and memory deficits during adolescence at P35. NIEHS: NS06259, ES07148, ES05022, ES11256; USEPA R829391; FRM SPE2006.

Keywords: Methylmercury, Hippocampus , Neural Precursors

Mentor: Manny DiCicco-Bloom

P-88

Post-Doctoral Poster & Travel Award Competition

MICROMANAGING BRAIN DEVELOPMENT AND FUNCTION: ETHANOL-INDUCED NEUROBEHAVIORAL TOXICITY IN ZEBRAFISH. TL Tal, JA Franzosa, KS Saili, and RL Tanguay. *Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, Oregon, USA*

Prenatal alcohol exposure produces a range of outcomes in offspring, collectively referred to as fetal alcohol syndrome disorders (FASD). Epidemiological studies suggest that the majority of children with FASD present with deficits in neurobehavioral function in the absence of clinically discernable physical abnormalities. MicroRNAs (miRNAs) are non-coding RNAs that direct post-transcriptional repression of protein coding genes. While miRNA biogenesis is critical to neural development, the neurobehavioral function of miRNAs is unknown. To explore the role of miRNAs in ethanol-induced neurobehavioral toxicity, zebrafish larval neurobehavioral assessments were obtained by quantifying distance moved during alternating periods of light and dark at 120 hours post fertilization (hpf). Transient developmental exposure (6-24 hpf) to ethanol (20-300 mM) results in concentration-dependent reductions in locomotor activity relative to unexposed controls. To identify miRNAs that are misexpressed following developmental ethanol exposure, miRNA microarray analyses were conducted. At developmental stages consistent with ethanol-mediated neurobehavioral abnormalities, expression of multiple CNS-enriched miRNAs were significantly reduced relative to time-matched controls. miRNA target predictions were employed to identify putative mRNA targets and subsequent computational analyses revealed that ethanol disrupts expression of miRNAs that direct neurogenesis. Antisense oligonucleotide morpholinos were used to transiently knockdown miRNA expression during development. Knockdown of multiple CNS-enriched miRNAs phenocopied ethanol-induced behavioral hypoactivity. Taken together, these data indicate that ethanol exposure misregulates the expression of miRNAs that may collectively choreograph neural development and function and suggest that miRNA signaling pathways are targets of developmental neurotoxic agents. This research was supported by P30ES00210 and T32ES07060.

Keywords: microRNA, behavior, zebrafish

Mentor: Robert L. Tanguay

P-89

EARLY POSTNATAL ANESTHESIA AND LONG LASTING COGNITIVE DEFICITS IN RHESUS MONKEYS. M. G. Paule[1], M. Li[1], X. Zou[1], C. Hotchkiss[2], J.P. Hanig[3], T.A. Patterson, W. Slikker, Jr.[1] and C. Wang[1]. *[1]Division of Neurotoxicology, National Center for Toxicological Research, FDA, [2]Bionetics Corporation, Jefferson, AR,*

and [3]Center for Drug Evaluation and Research, FDA, Rockville, MD

Previously, our laboratory has shown that ketamine exposure (24 hrs of clinically-relevant anesthesia) causes significant increases in neuronal cell death. Sensitivity to this ketamine-induced neurotoxicity in monkeys was observed on gestational days 122-124 (*in utero* exposure via maternal anesthesia) and on postnatal days (PNDs) 5-6, but not on PND 35. In the present study, six monkeys were exposed on PND 5 or 6 to intravenous ketamine anesthesia (light surgical plane) for 24 hrs; six control animals were unexposed. At 7 months of age all animals were weaned and began training to perform a series of cognitive function tasks as part of the National Center for Toxicological Research (NCTR) Operant Test Battery (OTB). The OTB tasks used here included those for assessing aspects of learning, motivation, color discrimination, and short-term memory. Subjects responded for banana-flavored food pellets by pressing response levers and press-plates during daily (M-F) test sessions (50 min), and were assigned training scores based upon their individual task performance. As reported earlier, beginning around 10 months of age control animals outperformed (had higher training scores) ketamine-exposed animals for approximately the next 30 months. In this study, for animals now over three and one-half years of age, the cognitive impairments continue to manifest in the ketamine-exposed group as poorer performance in the OTB learning and color and position discrimination tasks, primarily as deficits in accuracy of task performance, but also in response speed. There are also apparent differences in the motivation of these animals, which may be impacting OTB performance. These observations provide further evidence that a single 24-hr episode of ketamine anesthesia, occurring during a sensitive period of brain development, results in very long-lasting deficits in brain function in primates that are not just a result of developmental delay. Supported by NICHD, CDER/FDA and NCTR/FDA.

Keywords: Pediatric Anesthesia, Cognitive Function, Developmental Neurotoxicity

P-90

AGE-DEPENDENT CHANGES IN THE NIGROSTRIATAL SYSTEM AND MOTOR BEHAVIOR IN MICE DEFICIENT IN THE DNA REPAIR ENZYME

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Parkinson disease (PD) is a neurodegenerative disease that affects predominantly motor functions and presents with resting tremor, muscular rigidity, bradykinesia, and postural instability. Loss of dopaminergic neurons in the substantia nigra (SN) and the presence of Lewy bodies are pathologic hallmarks of the disease. Although the cause remains unknown, oxidative stress appears to play an important role in the pathogenesis and progression of PD. Several lines of evidence indicate increased oxidative damage to macromolecules in the brain of PD patients. Particularly, levels of damage to DNA (as determined by level of oxidized guanine, oxo^{8}dG) are increased in PD. To test whether increased oxo^{8}dG is a mere epiphenomena of neurodegeneration or whether it plays a role in the process, we evaluated the nigrostrial and motor behavior of mice deficient in the enzyme 8-hydroxyglycosylase (Ogg1, responsible for the removal of oxo^{8}dG). Ogg1knockout mice accumulate Oxo^{8}dG at a faster rate than wild-type mice and show age-dependent loss of nigrostriatal neurons, loss of striatal dopamine and development of ubiquitin-positive inclusions in surviving SN neurons. Additionally, Ogg1 knockout mice exhibit an increased susceptibility to the dopaminergic neurotoxin MPTP and this susceptibility is augmented during aging. The changes in nigrostriatal system are paralleled by changes in spontaneous motor activity. Aged (18-20 month old) ogg1 knockout exhibited decreased line crossings, speed, and number of rearings while time immobile was longer compared to wild-type mice. The differences found in the aging group were not found in 3 months old mice. These studies indicate that levels of oxo^{8}dG can modulate neuronal and behavioral changes that mimic the pathology seen in PD.

Keywords: Parkinson's disease, DNA Repair, DNA damage

P-91

IN RATS, REPROGRAMMING OF EARLY DIET MAY REDUCE THE THRESHOLD OF REACTIVITY TO TOXICITY.

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Studies in man and animals have shown that certain developmental factors, e.g chemical exposure or (maternal) nutrition, may reprogram (neuro)physiological set points and have lasting consequences for health later in life. The aim of present research was to study effects of early diet focussing especially on 1) body weight (BW), 2) glucose tolerance and 3) vulnerability to early methylmercury (MeHg) exposure.

Three groups of female Wistar rats (n=24) were kept on a semi-synthetic control diet (n=24), a high-caloric diet (15% butter oil, n=48) or a caloric restricted (deficient) diet (75% of control diet, n=38) during 6 weeks premating, mating (1 week) and gestation (3 weeks) period. Pups were cross-fostered at birth to obtain 5 diet groups, i.e. (gestation/LACTATION): cont/CONT; high/HIGH; def/DEF; def/HIGH and high/DEF. A selection of male (n=100) and female (n=100) pups was additionally dosed sc. with saline (control) or MeHg (3 mg/kg BW) from PN2 to 21. BW and food intake were measured, behavior tested and neuropathology survey carried out, as well as glucose tolerance and fat tests and measurement of some (satiety) hormones.

The results showed that specifically the reprogrammed def/HIGH group showed 'catch-up growth' during lactation, persistently increased body weight at adulthood, and increased response in the glucose tolerance test, suggesting an increased prevalence for obesity and diabetes. Moreover, an increased vulnerability to MeHg was observed in the def/HIGH group exposed to MeHg, demonstrated by neural impairment in auditory startle response which was not observed by diet or MeHg exposure alone.

Speculating on the mechanisms underlying the findings in the def/HIGH group it is hypothesized that early dietary reprogramming affects the Hypothalamus Pituitary Adrenal (HPA)-axis, leading to an increase in adipose tissue and increased levels of glucocorticoids. The latter, in turn might have caused a reduction in the threshold of reactivity to toxicity.

Keywords: Maternal Diet/Crossfostering, Postnatal MeHg Exposure, Pre-imprinting Obesity/Diabetis

P-92

PRENATAL METHYLMERCURY EXPOSURE INDUCES IN RATS STRUCTURAL BRAIN DEFICITS AT WEANING AND FUNCTIONAL IMPAIRMENT AT ADULT AGE. D. de Groot[1], M. Radonjic[1], M. Bogaard[1], N. Cappaert[3], R. Dierckx[2], F. Kuper[1], R. Nederlof[1], M. Verwei[1], B. Voet[1], E. de Vries[2], A. van Waarde[2], W. Wadman[3], A. Wolterbeek[1], R. Stierum[1]. *[1]TNO Quality of Life, Zeist, The Netherlands; [2]University Medical Center Groningen, PET Center Groningen, The Netherlands; [3]University of Amsterdam, the Netherlands*

(SEE ABSTRACT IN PLATFORM SESSION IV ON PAGE 9 #16)

ENDOCRINE SYSTEMS AND RECEPTOR ACTIVATION

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Pre-Doctoral Poster & Travel Award Competition

NEUROPROTECTIVE EFFECTS OF ESTRADIOL ON ALTERED AGE RELATED NEURONAL MARKERS IN AGING FEMALE RATS. Pardeep Kumar, R.K. Kale, S.M.Cowsik and N. Z. Baquer. *School of Life Sciences, Jawaharlal Nehru University, New Delhi-110067, India*

Biological aging is a fundamental process observed in almost all living beings. During aging the brain experiences structural, molecular, and functional alterations. Aging in females and males is considered as the end of natural protection against age related diseases like osteoporosis, coronary heart disease, diabetes, Alzheimer's disease and Parkinson's disease. These changes increase during menopausal condition in females when the level of estradiol is decreased. The objective of this study was to observe the changes in activities of Na^+K^+ ATPase, Ca^+ATPase, membrane fluidity, DNA degradation, glucose transporter 4 (GLUT4) and neurolipofuscin accumulation occurring in brains of female rats of 3 months (young), 12 months (adult) and 24 months (old) age groups, and to see whether these changes are restored to normal levels after exogenous administration of estradiol (0.1 µg/gm body weight for one month). The results obtained in the present work revealed that normal aging was associated with significant decrease in the activity of Na^+K^+ATPase, Ca^+ATPase, GLUT4 levels and membrane fluidity, in the brains of aging female rats, and an increase in DNA degradation and neurolipofuscin accumulation. The present study showed that estradiol treatment significantly decreased DNA degradation, and neurolipofuscin accumulation in brain of aging rats, and a reversal of Na^+K^+ATPase, Ca^+ATPase, membrane fluidity and GLUT4 levels was achieved. It can therefore be concluded that estradiol's beneficial

effects seemed to arise from its antilipofuscin, antioxidant, antilipidperoxidative effects, implying an overall anti-aging action. The results of this study will be useful for pharmacological modification of the aging process and applying new strategies for control of age related disorders.

Keywords: Brain aging, Estradiol, Glucose transporter

Mentor: Prof N.Z. Baquer and Prof R.K. Kale

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Post-Doctoral Poster & Travel Award Competition

***PARA*-SUBSTITUTION IS A KEY DETERMINANT OF BROMINATED DIPHENYL ETHER ACTIVITY TOWARD RYANODINE RECEPTORS**

KH Kim, DD Bose, J Riehl, IT Padilla, CD Barnhart, PJ Lein, and IN Pessah. *Department of Molecular Biosciences, The School of Veterinary Medicine, University of California, Davis. USA*

Polybrominated diphenyl ethers (PBDEs) are widely used flame retardants that bioaccumulate in human and animal tissues. Structurally similar non-coplanar polychlorinated biphenyls (PCBs) sensitize ryanodine receptors (RyRs) that are broadly expressed in excitable and non-excitable cells. [^{3}H]Ryanodine ([^{3}H]Ry) binding analysis and Ca^{2+} efflux measurement indicate that *para-ortho*-bromo substitution(s) of specific BDE congeners critically influence activity toward RyR1 and RyR2. BDE-4 (2,2'-di-BDE) enhanced RyR1 activity in a dose-dependent manner, whereas diphenyl ether and BDE-15 (4,4'-di-BDE) lack activity. Disruption of the RyR1-FKBP12 complex abrogates BDE-4 activity. Environmentally relevant BDE-17 (2,2',4-tri-bromo) and BDE-49 (2,2',4,5'-tetra-bromo) significantly increased [^{3}H]Ry binding to RyR1 (~450 %) and RyR2 (~300%). Addition of a methoxy (MeO) group at the free *para*-position of BDE-49 reduced activity relative to the corresponding parent congener in both binding and microsomal Ca^{2+} efflux assays. BDE-49 triggered Ca^{2+} efflux from microsomal vesicles is inhibited by ruthenium red, indicating these effects are mediated through RyR activation. BDE-47 (2,2',4,4'-tetrabromo) has low efficacy toward activating RyR1 and RyR2 (<150% of control). Interestingly, 6-OH-BDE-47 is a potent inhibitor, whereas 4'-OH-BDE-49 shows dose-dependent biphasic activity toward RyR1. HEK 293 cells expressing either RyR1 or RyR2 pretreated (16 hr) with BDE-49 (250 nM) showed significantly sensitized responses to acute application of RyR agonist caffeine or 4-CMC compared to vehicle or BDE-47 pretreatment. Pretreatment of mouse primary cortical neurons in culture with BDE-49 (0.1-10 μM; for 48 hr between 6~8 DIV) significantly decreased viability, whereas exposure to BDE-47 (< 10 μM) had no influence on neuronal viability. These results provide insight into the structure-activity relationship of PBDEs toward RyRs and identify specific congeners that may be potent neurotoxicants due to their activities on RyR mediated Ca^{2+} signaling.

Keywords: PBDE, Ryanodine Receptors, BDE-49

Mentor: Isaac N. Pessah

P-95

EFFECTS OF DIBP ON BEHAVIOR OF LEARNING AND MEMORY IN THE MALE MOUSE.

Ma Ning, Student, and Xu Hai-bin, Advisor. *National Institute of Nutrition and Food Safety, China CDC, Beijing ,china*

The neurobehavioral changes and ultrastructural alterations in the brain hippocampus region were investigated in adult mice (KunMing strain) following 8-weeks exposure to diisobutyl phthalate (DiBP). Briefly, 120 adult mice were divided randomly into five groups by their body weights (control, 50mg/kg, 250mg/kg, 500mg/kg and 1000mg/kg). Animals were dosed with DiBP dissolved in corn oil via gavage for 8 weeks. Control animals received an equal volume of corn oil. Doses were adjusted daily to account for changes in body weight. The effects of DiBP on locomotor activity, passive avoidance response and Morris water maze were examined in this study. After 8 weeks exposure, six mice from each group were randomly selected for morphometric analysis of the structural alterations in hippocampus by electron microscopy. Our study demonstrated that total locomotor activity in open field test was not affected following exposure, but the time spent in center was longer in 1000mg/kg group compared with controls ($p<0.05$); In the Morris water maze test, latency to find a hidden platform was significantly increased in the 500mg/kg and 1000mg/kg groups ($p<0.01$). In the passive avoidance test, learning was also significantly impaired in 1000mg/kg group compared with control ($p<0.05$). Morphometric analysis by transmission electron microscopy revealed that neuronal ultrastructural alterations, such as damage of nuclear membrane, mitochondria, rough surfaced endoplasmic reticulum and polyribosome were found in DiBP-exposed hippocampal neurons. In addition, the total number of synapses shows decreased in the 500mg/kg and 1000mg/kg group ($p<0.01$). All together, these results indicated that 8 weeks of DiBP exposure could cause significant neurobehavioral and morphological changes. However, further research is

still needed to address whether these changes are reversible in nature after a long period of washout.

Keywords: Diisobutylphthalate (DiBP), Neurobehavioral, Neuronal Ultrastructural

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Post-Doctoral Poster & Travel Award Competition

NANOMOLAR NON-COPLANAR PCB 95 INCREASES SPONTANEOUS SYNCHRONIZED Ca^{2+} OSCILLATIONS AND DENDRITIC GROWTH IN PRIMARY HIPPOCAMPAL NEURONS. DD Bose, D Yang, ES Kaplan, PJ Lein and IN Pessah. *Department of Veterinary Molecular Biosciences, University of California, Davis, CA, USA.*

Polychlorinated biphenyls (PCBs) are a group of structurally related chemicals with widespread distribution in the environment. The neurotoxicity of non-coplanar PCBs may be mediated, at least in part, by their potent activity towards ryanodine receptors (RyRs), which are broadly expressed and contribute to Ca^{2+} dependent signaling events. Primary mouse hippocampal cultures dissociated from P1 mice were plated at high density and exposed to PCBs at 7 days *in vitro* (DIV). Spontaneous oscillations of Ca^{2+} in hippocampal neurons exposed to solvent alone, PCB 95 (2,2'3,5'6-pentachlorobiphenyl) or PCB 66 (2,3, 4,4'-tetrachlorobiphenyl), were monitored using the Ca^{2+} sensitive dye Fluo-4 at 9 DIV (48hr exposure). Spontaneous Ca^{2+} oscillations in neurons exposed to PCB 95 increased in a dose dependent manner (0.03 to 1 µM), compared to DMSO controls. Neuronal cultures treated with 0.1, 0.3 and 1 µM PCB 95 exhibited significantly increased frequency of spontaneous Ca^{2+} oscillations in the dendrites and the cell soma (3.5 and 15 fold increase compared to control for 0.1, 0.3 and 1µM PCB 95 respectively). High frequency oscillations were also observed within an individual Ca^{2+} transient in the neurons exposed to 0.3 and 1 µM PCB 95. Treatment with mono-ortho PCB 66 (0.1µM) treated hippocampal neurons did not alter the frequency of Ca^{2+} transients compared to solvent controls, nor did it elicit high frequency oscillations. Treatment of rat hippocampal neurons with PCB 95 (48 hr, 20nM) enhanced dendritic growth. PCB 95 mediated changes in dendritic morphology in mouse hippocampal neurons are being investigated. These results indicate that exposure to non-coplanar PCB 95 that lacks dioxin-like activity alters the fidelity of spontaneous intracellular Ca^{2+} events that contribute to the developing hippocampal neuron. Supported by NIEHS R01 ES014901.

Keywords: PCBs, Ryanodine Receptors, Calcium

Mentor: Isaac N. Pessah

RISK IDENTIFICATION

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THE WORLD LIBRARY OF TOXICOLOGY (WLT): A GLOBAL COMMUNITY OF TOXICOLOGISTS SERVING PUBLIC HEALTH. SG Gilbert[1], P Wexler[2], EM Faustman[3], and N Thorp[1]. [1]*Institute for Neurotoxicology and Neurological Disorders, Seattle, WA, USA.* [2]*National Library of Medicine, Bethesda, MD, USA.* [3]*University of Washington, Seattle, WA, USA.*

The science of toxicology, and its associated policy and societal issues, is of paramount global concern. With the use of toxic chemicals increasing in all corners of the world, particularly in developing countries, toxicology is stepping into the forefront as a major science that can help in our understanding of environmental problems and provide solutions for protecting public health. Developing countries tend to be at particular risk for human health and environmental effects. To understand and manage the risk associated with chemical exposures and improve our understand of the effects of their use on global health it is essential that there be a medium for trained toxicologists and public health professionals in all countries to exchange toxicological research, ideas, and experiences in the field. The multi-lateral, multi-lingual, Web-based, World Library of Toxicology (WLT as www.wltox.org) was developed to facilitate this exchange by decreasing the barriers to sharing information among countries and building capacity within countries. Through the enlistment of volunteer Country Correspondents to share their country-specific knowledge of toxicology and public health, the WLT is creating a global portal to resources from countries on all seven continents. The Correspondents build their respective country pages by contributing links to resources, such as relevant governmental and non-governmental organizations, university or other training programs, poison control centers, professional associations, databases, books, journals, general country information and other topics. The WLT uses a modified Wiki based technology that provides the Country Correspondents with control of their individual country content, allowing for rapid and continually updated information. There are currently some 50 countries represented and we actively recruit new

country correspondents as part of our efforts to expand the program. The underlying support for this technology is provided by Toxipedia (www.toxipedia.org). The WLT is a joint project the International Union of Toxicology (IUTOX), the National Library of Medicine (NLM), the Institute of Neurotoxicology & Neurological Disorders (INND), and Toxipedia (www.toxipedia.org). The WLT web site was formally launched in South Africa at the 7th IUTOX Congress for Toxicology in Developing Countries (CTDC) in September 2009.and has had over 2,500 unique visitors from around the world during the month following its release. The WLT offers a unique way to share information and build global capacity in toxicology research, application, and training for a healthy and sustainable world. Minimizing duplication of effort and stimulating collaboration are additional ethically responsible goals of the WLT in its quest to create a virtual global village, populated by toxicologists and public health professionals from around the world.

Keywords: Global, Toxicology, Resources

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EXAMINING THE RELATIONSHIP OF SELF-REPORTED DIAGNOSED ILLNESSES AND MEDICATION USE.

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It has been contended that the use of prescribed medications, compared to the use of self-reported symptoms, is a more reliable means for studying a populations' health (Dayal *et al*, 1994). Bowler *et al*.2002, extended these findings to include the use of diagnosed illnesses and medications to validate symptoms. This paper explores the relationship between self-reported medication use and diagnosed illnesses. Data was collected to examine the heath effects of long-term, low-dose environmental manganese exposure. One hundred and ninety participants were randomly recruited from water and tax records and screened for serious pre-existing conditions. No significant mean differences were found in blood Manganese levels between the exposed and control group. Participants were asked to submit a list of their current medications and to fill out a Health Questionnaire to assess their symptoms, illnesses and daily living habits. Medications and illnesses were categorized into ICD9 categories. Of the participants (44.68%) taking medication(s) for a circulatory illness, only 42.8% of them reported having been diagnosed as having a circulatory illness (χ^2=.12, p=.82, OR=1.17). Thus, taking a circulatory medication was not significantly related to being diagnosed with a circulatory illness. Of the participants (19.58%) who reported taking medication(s) for an endocrine illness, only 21.6% reported having an endocrine disorder (χ^2=.37, p=.55, OR=1.20). Similarly, of the participants (20.21%) who reported taking medication(s) for a psychiatric illness, only 18.4% reported a having a psychiatric diagnosis (χ^2=2.52, p=.146, OR=1.19). Seventy one percent of participants who reported taking medication(s) for a respiratory illness reported having a respiratory illness (χ^2=.181, p=.431, OR=1.23). Of the participants who reported taking medication(s) for a musculoskeletal illness, only 50% reported a diagnosed musculoskeletal illness (χ^2=1.182, p=.285, OR=1.48). There is no significant relationship between self-reported medication use and diagnosed illnesses for any of the five categories listed above. Participants may be unaware that they have a diagnosed medical illness, which points to issues with patient-physician communication and informed consent regarding diagnosis and prescription of medications. Illnesses may well be significantly under reported by health study participants, perhaps due to a misunderstanding regarding their own health, Adding medication use in health studies may give further information on unreported medical diagnoses.

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HYPOXIA HAZARDS FROM SULFUR DIOXIDE DERIVED FROM SHRIMP DIP.

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Shrimping is a major industry in the Gulf of Mexico. Harvested shrimp are placed on the shrimp boat deck and de-headed before being placed into a tank of shrimp dip (formulated as a 1.25% (w/v) solution of sodium metabisulfite (SMB) in salt water). The dip is used to prevent the formation of an undesirable post-catch condition, "Black Spot". "Black Spot" (melanosis), is a non-toxic esthetic condition that reduces consumer acceptance, increases wastage and decreases shrimp value. After dipping, shrimp are transferred into the storage holds of the shrimp boat and cooled or frozen. The holds are enclosed spaces with little to no

ventilation. Shrimp boat personnel work in the hold to move, arrange, and stack containers of shrimp. Injuries and deaths have occurred while crew worked inside shrimp boat holds. Medical evaluation has shown that some of these deaths and injuries were due to hypoxia resulting from inhalation of toxic atmospheres. One specific cause of the hypoxia has been identified as inhalation of sulfur dioxide (SO_2) gas. Conditions responsible for the presence of SO_2 include: release from improperly stored SMB, release from SMB placed directly on wet ice used to cool shrimp and release from freshly prepared SMB shrimp dip. Analysis of work flow and thru-put was conducted modeling different rates of shrimp harvest. Laboratory testing, using a scaled model of a shrimp boat hold, was performed to determine the influence of several variables on SO_2 concentrations in hold air. Variables included: temperature of the shrimp dipped, temperature of the shrimp dip, hold temperature, hold humidity, refrigeration frost removal, and quantity of shrimp dip transferred to the hold. SO_2 concentration increased with temperatures of the shrimp, the shrimp dip and the hold. SO_2 concentrations correlated positively with quantity of shrimp dip, the rate of desiccation of frozen shrimp dip and refrigeration frost removal. Results support the conclusion that under foreseeable conditions of use, SMB shrimp dip can produce, Immediately Dangerous to Life and Health (IDLH = 100 ppm.) levels of SO_2 in the refrigerated holds of shrimp boats. This work suggests that an evaluation of shrimp boat hold atmospheres, while at sea and under normal working conditions, is justified.

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