

W 2495 **Physiologically-Based Pharmacokinetic/Pharmacodynamic Modeling of Developmental Toxicity.**

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The developing brain is vulnerable to insults and evidence implicates low-level chemical exposures as potential developmental neurotoxins. Organophosphorus (OP) insecticides, like chlorpyrifos (CPF), inhibit cholinesterase (ChE) and are neurotoxic. PBPK/PD models have been exploited to enable cross-species extrapolation of CPF brain dosimetry and ChE inhibition and when linked with a dietary exposure model can predict response across populations. However, recent epidemiology studies suggest that OP neurotoxicity occurs at low-doses, even in the absence of significant brain ChE inhibition. The lack of quantitative cross-species brain dosimetry data associated with epidemiology results hampers any mechanistic based risk assessments. The implication of localized heterogeneous CYP450 brain metabolism has historically not been extensively investigated, but recent research suggests it is of key importance. To address these limitations, we are testing the hypothesis that low-dose exposures of preweaning rats to OP insecticides will result in differential brain region dosimetry, enhanced by localized brain bioactivation, potentially resulting in subtle changes in brain chemistry. Comparative in vitro metabolism studies in rats indicate that the overall brain microsomal metabolism was a fraction (~3%) of the liver. Following in vivo oral administration (1 and 5 mg/kg/day) of CPF to post-natal day-10 pups, CPF and its major metabolite were quantified in the brain with evidence of regional 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. In this regard, these PBPK/PD modeling strategy have significant regulatory implications for assessing developmental neurotoxicity.

W 2496 **Using Epidemiology to Analyze Neurodevelopmental Toxicity across Species.**

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The final presentation of the session will tackle cross-species analysis from a different angle by utilizing epidemiology data. This presentation will explore how epidemiology studies can address toxicity across species by estimating human exposures and/or outcomes with biomarkers and putting these into context with the animal model results. It will focus on how the effect of timing, selection choice, and measurement of biomarkers, as well as the results of toxicology studies, can influence the interpretation of results. Chlorpyrifos and neurodevelopmental effects will be used as a case study. US EPA assessed whether epidemiology data suggest that fetal or early-life chlorpyrifos exposure causes neurodevelopmental effects and, if so, whether they occur at exposures below those causing 10% inhibition of blood acetylcholinesterase (AChE), which is currently considered the most sensitive endpoint. We conducted a hypothesis-based weight-of-evidence analysis and found that a proposed causal association between chlorpyrifos exposure and neurodevelopmental effects in the absence of AChE inhibition does not have a substantial basis in existing animal or in vitro studies, and there is no plausible basis for invoking such effects in humans at their far lower exposure level. The epidemiology studies fail to show consistent patterns; the few associations are likely attributable to alternative explanations. The human data are inappropriate for a dose-response assessment because biomarkers were only measured at one time point, may reflect exposure to other pesticides, and many values are at or below limits of quantification. When considered with pharmacokinetic data, however, these biomarkers provide information on exposure levels relative to those in experimental studies and indicate a margin of exposure of at least 1,000. Because animal data take into account the most sensitive lifestages, the use of AChE inhibition as a regulatory endpoint is protective of adverse effects in sensitive populations.

W 2497 **Cumulative Risk: Toxicity and Interactions of Physical and Chemical Stressors.**

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Recent efforts to update cumulative risk assessment procedures and develop community-based risk assessment methods reflect increased interest in incorporating the totality of variables affecting human health into the risk assessment process. One key roadblock in advancement is uncertainty as to how nonchemical stressors behave in relationship to chemical stressors. An assumption that simplifies incorporation of nonchemical stressors into current risk assessment paradigms is that nonchemical stressors act in the same manner as chemicals. However, evidence is required to support this assumption. The term nonchemical stressors encompasses a

diverse set of variables including physical stressors, such as noise, temperature, disease, and radiation, as well as psychosocial stressors, which involve perception of circumstances. Physical stressors offer a reasonable starting place for measuring the effects of nonchemical stressors and their modulation of chemical effects (and vice versa), as they clearly differ from chemical stressors, present many diverse and highly-relevant stressors, and "doses" of many physical stressors are easily quantifiable. There is a commonly held belief that virtually nothing is known about the impact of nonchemical stressors on chemical-mediated toxicity or the joint impact of coexposure to chemical and nonchemical stressors. While generally true, there are several instances where a substantial body of evidence exists. The objective is to provide expert overviews, for those chemical and physical stressors that have been sufficiently studied to gain at least a limited understanding of their joint impact. In addition to providing the current state of knowledge, data gaps will be identified that should be addressed to facilitate inclusion of nonchemical stressors in risk assessment. (This abstract does not reflect US EPA or NIEHS policy.)

W 2498 **Cumulative Risk: Chemicals and Infectious Disease.**

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At least 4 types of mechanisms underlie potential interactions between toxic chemicals and infectious disease. 1) The best understood is suppression of immune responses, resulting in increased incidence/severity of infectious disease. For example, decreased alveolar macrophage function following exposure to several air pollutants enhances the risk of certain bacterial infections. Research on this model provides both qualitative and quantitative approaches to describe this risk. 2) Certain immune/inflammatory mediators that are activated during infection affect metabolic enzymes and transporters and have the potential to alter chemical toxicity as illustrated by the effects of murine cytomegalovirus on parathion poisoning, sodium pentobarbital induced sleeping time, and cyt P450. Infection and inflammation have also been shown clinically to affect the metabolism, distribution, and elimination of certain drugs. 3) Chemical exposure may enhance inflammation and immune pathology associated with an infection. This is best illustrated by effects of ozone, ultraviolet radiation, and TCDD on influenza infection. In all cases mortality is enhanced in the absence of increased virus titers in the lung or viral dissemination. Deaths appear to be due to increased inflammatory responses. Similarities exist between receptors and subsequently triggered signaling pathways for pathogen associated molecular patterns (PAMPs) and damage associated molecular patterns (DAMPs), which trigger inflammatory responses. A systems approach that examines the integration of these pathways is needed to better describe this phenomenon. 4) Infection enhances chemical induced lesions, e.g., p53 mutations, inflammation, cell proliferation. Such mechanisms might explain the interaction between hepatitis B virus infection and aflatoxin in the induction of liver cancer. These mechanisms are not necessarily comprehensive, distinct, or mutually exclusive and are imperfectly understood. However, they provide a useful framework to further explore the interactions between infectious disease and exposure to toxic chemicals with the ultimate goal of improving our understanding of cumulative risk.

W 2499 **Enhancement of Noise-Induced Hearing Loss by Chemicals.**

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The auditory effects of chemical toxicants have been investigated in the past two decades, in animal and human field and clinical studies. A number of studies demonstrated that some solvents, metals, asphyxiants, pesticides not only affect the sensory organ of the auditory system, as noise does, but also affect central auditory structures. Ototoxicity induces outer hair cell dysfunction in the cochlea (similar to the effects of noise), whereas neurotoxicity induces central auditory dysfunction. Audiological signs of neurotoxicity may or may not include poorer hearing thresholds, in addition to difficulties discriminating sounds such as speech, particularly in adverse listening conditions. The existing evidence prompted the proposal of new guidelines and standards on hearing loss prevention. In the U.S., the National Institute for Occupational Safety and Health has discussed specific research needs regarding the ototoxicity of chemicals used at work. The American Conference of Governmental Industrial Hygienists and the U.S. Army have proposed preliminary practical steps that employers and occupational health professionals can take to improve hearing loss prevention. Australia and New Zealand have developed standards recommending hearing tests for workers exposed to ototoxic agents. In the legislative arena, the European Parliament published a new noise directive (2003/10/EC), that requires employers to give attention to any effects on workers' health and safety resulting from interactions between noise and work-related ototoxic substances, when performing risk assessment of workplaces. Legislation regarding compensation has also changed in Australia and Brazil. In this presentation

the auditory effects of chemical alone or in combination with noise will be reviewed, and the recent guidelines, legislative developments and alternative strategies for the prevention of auditory effects resulting from exposure to chemicals in the workplace will be presented.

W 2500 Exacerbation of Toxicity of Air Pollutants and Pesticides by Thermal Stress.

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Considering the likelihood of global warming in the near future, it is important to understand how heat stress will alter the health effects of toxicants. The toxicity of pesticides and airborne toxicants is generally exacerbated in a warm environment. As air temperature increases, the pulmonary intake of air pollutants and absorption of pesticides applied to the skin is generally accelerated. Cellular toxicity is typically exacerbated when body temperature is elevated. This is primarily a result of a Q10 effect, meaning that the rate of a biochemical reaction doubles with a 10 °C increase in tissue temperature. The generation of reactive oxygen species is also exacerbated with warmer temperatures.

Since warmer temperatures worsen chemical toxicity, a thermoregulatory response to lower body temperature can be protective. Hyperthermia is most likely going to be detrimental in the recovery from toxicant exposure. Rodents and other small mammals have relatively large surface area:volume ratio. Following exposure to pesticides and air pollutants, metabolism is reduced and a rapid reduction in body temperature ensues. If given the opportunity to thermoregulate behaviorally, rodents seek colder temperatures, allowing body temperature to decrease quickly. Thus, the hypothermic response is a regulated response. This is thought to be an adaptive response because the hypothermic response is protective. Large mammals, including humans, have a greater thermal inertia and are unable to mount a hypothermic response as is seen in rodents. A warmer environmental temperature will thus impede the hypothermic response to toxicant exposure in rodents and will also be stressful to large mammals that are unable to undergo a significant cooling response. With the potential impact of global climate change on increased incident of heat stress in urban areas that are also rife with pollution, the topic of the thermoregulatory responses to environmental toxicants is timely. This is an abstract of a proposed presentation and does not reflect US EPA policy.

W 2501 Modulation of X-Ray Mediated Testicular Toxicity by Chemical Exposure.

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High density microarrays and a detailed bioinformatics analytical approach were used to demonstrate that an initial chemical exposure to 2,5-hexanedione (HD) altered the rat testis to ameliorate the response to a subsequent exposure to x-irradiation. Adult male rats were exposed to HD (0.33% or 1%) in the drinking water for 18 days followed by x-ray (2Gy or 5Gy), resulting in a total of 9 treatment groups. Testis samples were collected after 3 hr and gene array analysis was performed. Using a novel bioinformatic approach to summarize the effect of HD across all treatment groups, we focused on the modification of x-ray-induced gene alterations by HD co-exposure. Enrichment analysis was used to identify biological pathways where HD modification of gene expression was the greatest. HD exerted a significant influence on genes involved in Cell Cycle and DNA Replication, Recombination, and Repair. HD also had an antagonistic effect on x-ray-induced alterations of several apoptotic genes (Fas, BBC3, AEN). To further investigate the specific cell populations and stages in which these critical gene alterations occur, laser capture microdissection (LCM) samples were collected from the basal compartment of the seminiferous epithelium, enriching for those germ cells most susceptible to x-ray-induced apoptosis. Quantitative RT-PCR of the LCM samples confirmed the suppression of apoptotic genes by HD co-exposure. The co-exposure attenuation of germ cell apoptosis is the result of an adaptive response to the chemical exposure, causing altered paracrine signalling of the supportive cells in the seminiferous epithelium. These results suggest that toxicity pathway responses determine the outcome of co-exposures, whether chemical or physical in nature, and that complex paracrine interactions between cells modulate the extent of injury.

W 2502 Sunlight Enhancement of the Toxicity of Air Pollutant Mixtures.

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Sunlight can significantly drive photochemical reactions of mixtures of air pollutants commonly observed in the atmosphere, producing many well known toxic compounds such as formaldehyde and other carbonyl containing products. These reactions also contribute to the formation of secondary organic aerosols as well as modifying the composition of existing aerosols or particulate matter (PM). Smog chambers can be used to prepare repeatable, controlled mixtures of simple to increasing complexity and be used to study photochemical atmospheric transformation with natural sunlight or simulated sunlight. Smog chambers can be interfaced with direct exposure to *in vitro* or *in vivo* models for toxicity studies including direct air-liquid-interface *in vitro* or *in vivo* inhalation exposures. Photochemical experiments have been conducted in smog chambers with industrial mixtures and complex mixtures of motor vehicle exhaust in urban atmospheres, often demonstrating enhanced toxicity as measured by markers of inflammation and other biological endpoints such as cytotoxicity. Modifications of experiments, exposure conditions and additional toxicological analyses can provide mechanistic and mode of action understanding. Novel genomic analyses of cells exposed to an urban-like mixtures showed transcriptional changes on a subset of genes, increasing the number of genes with altered expressions from 19 for the un-irradiated mixture, to 709 genes after a one-day sunlight irradiation. The implication is that toxicologists and those dependent on their findings, should consider studies that include mixtures resulting from natural atmospheric photochemical reactivity, transformation of air components, and the resulting enhancement of toxic effects of air pollutants and their products. Not considering such effects, could result in misinterpreting the mode of action and underestimating the potential risk of exposure to air pollution mixtures or its sources.

W 2503 Mechanistic, Occupational, and Clinical Aspects of Lead Exposure.

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The mechanisms of lead toxicity are increasingly being explained by the ubiquitous reactivity of the bivalent lead cation and its ability to substitute for essential cations, notably calcium and zinc. By these means, lead complexes with important functional groups including thiol and carboxyl groups, and damages many fundamental cell processes and structures including enzyme pathways, phospholipid integrity, ion channel specificity and control, and intrinsic protective systems including free radical scavengers and cellular repair mechanisms. Owing to the large sample sizes involved and its nationally-representative nature, NHANES has been the subject of a number of epidemiological analyses relating blood lead concentrations to a range of adverse outcomes such as blood pressure, renal function, auditory thresholds, and a host of other cardiovascular, neurobehavioral, and other developmental or adult outcomes. Some practitioners are now proposing that, as the NHANES data suggest that lead concentrations even less than 5 µg/dL (0.24 µmol/L) can have some health consequences, chelation should be performed at even very low lead concentrations. Is this an appropriate interpretation of these data? There is concern that the occupational intervention concentrations worldwide are not only unsupported scientifically and clinically but also have been set at concentrations that permit unsafe practices to continue. A group of experts has proposed that workers should be removed from occupational exposure if a single blood lead concentration exceeds 30 µg/dL (1.45 µmol/L), or if two successive blood lead concentrations measured over a four-week interval equal or exceed 20 µg/dL (0.97 µmol/L). Will these recommendations prevent clinically significant occupational lead exposure? Due to the paucity of clinical data, there is controversy about the lead concentration at which chelation therapy should be instituted in adults when exposure prevention has failed, the antidote to be used, and the most effective regimen to be employed.

W 2504 Novel Mechanisms of Toxicity.

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The broad spectrum of lead toxicity with adverse manifestations in developmental and functional aspects of many if not all organ systems are increasingly being explained by the ubiquitous reactivity of the bivalent lead cation and its ability to substitute for essential cations, notably calcium and zinc. By these means lead com-

The Toxicologist

Supplement to *Toxicological Sciences*

52nd Annual Meeting and ToxExpo™

March 10–14, 2013 • San Antonio, Texas



OXFORD
UNIVERSITY PRESS

ISSN 1096-6080
Volume 132, Issue 1
March 2013

www.toxsci.oxfordjournals.org

An Official Journal of
the Society of Toxicology

SOT | Society of
Toxicology

Creating a Safer and Healthier World
by Advancing the Science of Toxicology

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