Proceedings

Human Health Symposium—A STAR Progress Review Workshop

October 28-29, 2004
Philadelphia, PA

Introduction

The U.S. Environmental Protection Agency's (EPA) mission is to protect human health and to safeguard the natural environment upon which life depends. Science supports this mission by providing EPA with the knowledge needed to make informed decisions about risks to human health and the environment, and with opportunities to prevent or mitigate these risks. Within EPA, the Office of Research and Development (ORD) provides leadership in science and engineering and conducts most of the Agency's research and development within its own laboratories and through an extramural grants program. ORD has identified research to improve human health risk assessment and management as a high priority, emphasizing emerging concerns like aggregate and cumulative exposure and susceptible human populations such as children.

As part of its research program, ORD's National Center for Environmental Research (NCER), through its Science To Achieve Results (STAR) grants program, supports research to improve the ability to assess the health risks posed by exposure to a variety of toxic chemicals in the environment. NCER's emphasis is on:

- Understanding the impact of environmental exposures from multiple routes (air, water, soil, food) and multiple pathways (breathing, eating, skin contact);
- Characterizing the unique environmental threats faced by sensitive sub-populations such as children;
- Developing new tools, such as biomarkers, to better quantify exposure, dose, and effects; and
- Investigating new risk assessment and management approaches that incorporate mechanistic information by applying cutting-edge techniques such as molecular biology and genomics.

Through a variety of Request for Applications, NCER has accumulated a diverse portfolio of extramural research in the area of human health risk assessment, including grants dealing with exposure assessment, susceptible subpopulations, biomarkers to assess exposure and toxicity in humans, and the impact of exposure to chemical mixtures.

This Human Health Symposium brought together EPA's extramural scientists as well as scientists and policymakers from government, academic, and non-government organizations to hear about the latest research being conducted to address these priority issues in environmental health. EPA uses meetings like these to allow EPA and other federal and non-federal scientists to discuss research progress on topics of major scientific interest to the Agency. The research reported here is of critical importance to EPA, as it has the potential to strengthen the scientific basis for both assessing the risk from exposure to chemicals in the environment and developing appropriate risk-management practices to mitigate any adverse effects.

The research described in this report has not been subjected to the Agency's required peer review and policy review, and does not necessarily reflect the views of the Agency. Therefore, no official endorsement should be inferred. Any opinions, findings, conclusions, or recommendations expressed in this report are those of the investigators who participated in the research or others who participated in the Human Health Symposium, and not necessarily those of EPA or the other federal agencies supporting the research.

For more information on EPA's STAR program on human health research, please contact either Chris Saint at 202-564-6909 (saint.chris@epa.gov); Nigel Fields at 202-564-6936 (fields.nigel@epa.gov); or Kacee Deener at 202-564-8289 (deener.kathleen@epa.gov).

U.S. EPA 2004 Science To Achieve Results (STAR) Progress Review Workshop—Human Health Symposium

Loews Philadelphia Hotel
1200 Market Street
Philadelphia, PA 19107

October 28–29, 2004

OVERVIEW

The U.S. Environmental Protection Agency (EPA) 2004 Science to Achieve Results (STAR) Progress Review Workshop—Human Health Symposium convened EPA-funded scientists, public sector scientists, and policy-makers to share research findings on cutting-edge issues in environmental health. This research is critically important to EPA because it strengthens the scientific basis for assessing risk from chemical exposures and for developing appropriate risk management practices to mitigate adverse effects. Through a variety of grant solicitations, EPA's National Center for Environmental Research (NCER) has developed a diverse portfolio of extramural research in the area of human health risk assessment. Research presented at the 2-day Human Health Symposium covered several topics presented either to the general forum or in a poster session. Symposium topics included biomarkers, exposure assessment, susceptibility and vulnerability, and chemical mixtures. Approximately 70 individuals attended the workshop.

Welcome and Introductory Remarks
Kacee Deener and Becki Clark, U.S. EPA

Kacee Deener, Health Scientist at NCER, welcomed participants to the STAR Human Health Symposium and thanked EPA Region 3 colleagues, particularly Ronald Landy, who helped plan the Symposium. Becki Clark, Division Director for NCER's Environmental Sciences Research Division, welcomed participants and introduced several colleagues in the Office of Research and Development (ORD) including Pauline Mendola of the National Health and Environmental Effects Research Laboratory, Jim Quackenboss of the National Exposure Research Laboratory (NERL), Richard Hertzberg of the National Center for Environmental Assessment, and Brenda Foos of EPA's Office of Children's Health Protection. She also introduced members of the Human Health Team at NCER including Kacee Deener, Chris Saint, Nigel Fields, Susan Laessig, and David Mustra. Two Association of Schools of Public Health (ASPH) Fellows, Allan Davis and Richard Callan, also attended the meeting. Barbara Levinson (NCER's liaison with EPA Regions), Deborah Segal (NCER's Peer Review Division), and Roger Cortesi (NCER Senior Scientist) also were acknowledged.

STAR Human Health Research Overview
Chris Saint, U.S. EPA

EPA's ORD consists of approximately 1,950 employees in 13 laboratories or research facilities located across the country, with a budget of $700 million. The STAR Grants program has a budget of approximately $100 million and is an extramural research program whose aim is to provide technical support and research results to inform environmental decisions made by EPA. The STAR program is a competitive grants program that funds investigator-initiated research in response to targeted Requests for Applications (RFAs). ORD's human health research priorities include: (1) aggregate exposure assessments; (2) toxic mechanisms of action and incorporation of these mechanisms into risk assessment; (3) cumulative risk assessment (for mixtures and multiple chemical exposures); (4) understanding the specific risks faced by vulnerable populations (i.e., children); and (5) public health outcomes including the development of indices and methods to evaluate the effectiveness of environmental decisions and programs from a public health standpoint.

The role of the STAR program is to encourage the development of a modern, molecular-based method of risk assessment. The basic risk assessment paradigm moves from exposure to dose to effects to disease. Exposure leads to dose, which is impacted by pharmacokinetics. Dose leads to effects, such as cellular changes and disease, and is impacted by pharmacodynamics. A unifying goal of ORD is to encourage the development of biomarkers to help understand these processes. Biomarkers can target different points and processes along the risk paradigm, leading to the development of methods to measure and understand exposure and effects. Eventually, this will lead to the development of cheaper and less invasive methods to assess exposure and risk directly in humans rather than in rodent models. Data gathered from these efforts can be used to strengthen risk assessment and reduce uncertainties associated with traditional risk assessments, such as those that result from the use of animal models and indirect exposure measurements. EPA then will be able to better evaluate the effectiveness of assessments and the risk management process by measuring public health outcomes to determine if EPA is making a difference in the improvement of public health. Current and future areas of research include: (1) biomarkers for risk assessment; (2) small grants to fund statistical methods and analyses of existing data sets; (3) children's health research; and (4) aggregate exposure assessment, with an emphasis on new methods to identify and characterize human activity patterns that lead to exposure.

Region 3 Health Science Issues
Michael Kulik, U.S. EPA

This presentation focused on increasing awareness of the impact of various environmental exposures on children's health and on development of measures to accurately assess these effects. October is Children's Health Month, and the theme of this year's program is Protect Children Where They Live and Learn. Sponsored events include online postings of several events and presentations on topics including: mercury; asthma; indoor air quality; environmental influences related to mental retardation, autism, attention deficit disorder, Parkinson's disease, and bioterrorism; and successful community intervention programs (i.e., asthma and lead poisoning prevention). Letters were sent to health care providers to inform them of the availability of this information. Grants also have been provided to programs to help children suffering from asthma and to the Retired Senior Volunteers Program, whose participants help communicate children's health and environmental messages to the community.

Because children experience different environmental exposures than adults and experience them in different ways, they may respond differently to exposures. Studies aimed at estimating risks for adults need to be augmented and amplified with information on the specific vulnerabilities of children. Research presented at this Symposium included studies aimed at developing a better understanding of human sensitivity to environmental agents at different stages of life and how this affects risk, and it will contribute to the development of effective risk-reduction programs with the ultimate goal of improving children's health.

SESSION 1: BIOMARKERS

Session Overview
Pauline Mendola, U.S. EPA

The mission of EPA is to protect human health and the environment by conducting and sponsoring research designed to examine the relationship between environmental contaminants and health. To promote this mission, biomarkers can be used to assess the mechanisms and mode of action of contaminants, look for subclinical health effects, identify susceptible subgroups, and describe pharmacokinetics and pharmacodynamics. The results of this research will be used to strengthen exposure assessments and risk estimates to improve public health. The overriding goal of biomarker development is to improve risk assessment for more effective public health protection. EPA does not regulate individual behavior or exposure; rather, it regulates contaminants in the environment and strives to do this in an effective way that will protect human health and the environment. Biomarkers will help to define the relationship between environmental levels of compounds of concern and human health risks.

Biomarkers of exposure help clarify the relationship between levels of environmental contaminants of concern and the presence of the contaminant in a person, and whether this makes a difference in health (i.e., the biologically effective dose). Biomarkers of effect help researchers examine the impact of exposure on targeted biologic systems at the molecular and cellular levels, as well as systemic effects. Ideally, these biomarkers could be assessed before clinical symptoms are apparent. Biomarkers of susceptibility can help to define population subgroups that may have different risk profiles, including not only biomarkers that indicate increased susceptibility, but also those that may indicate increased resiliency such as genetic polymorphisms or antioxidant levels. Biomarkers also can help to determine the utility and relevance of surrogate tissue analysis and will help to improve the extrapolation of results from animal models to humans through a better understanding of underlying physiological mechanisms. Epidemiological evidence of an effect associated with environmental exposure can be linked to biological mechanisms discovered in the laboratory.

Biomarkers of Prenatal Exposure to Non-Persistent Pesticides
Robin Whyatt, Columbia University

Residential insecticide use accounts for substantial levels of indoor exposure, and pesticides are easily transferred from mother to fetus. There also appears to be a link between insecticides and adverse fetal growth and neurocognitive development. The goal of this study is to determine the extent to which pesticide levels in biologic samples collected during pregnancy and at delivery reflect residential exposures during the third trimester. A cohort of 100 African American mothers and newborns from New York City was established; prior research showed widespread pesticide exposure during pregnancy among this cohort. Of 79 women in the cohort, 61 percent used some form of pest control in the last 2 months; 20 percent was applied by an exterminator, whereas 41 percent was applied by "others." Sticky traps accounted for 51 percent of pest control measures and the use of unregulated pesticides accounted for 8 percent. Two-week integrated air samples to detect nonpersistent pesticides (NPP) in the mothers' residences were taken in the last 2 months of pregnancy and provided the gold standard for biomarker validation. NPP levels were assessed in biweekly spot maternal urine samples collected over the last 2 months of the pregnancy, in maternal and umbilical cord blood samples, and in postpartum meconium.

The organophosphates chlorpyrifos and diazinon, and the carbamate propoxur, were detected in 99-100 percent of air samples. Levels of these insecticides were correlated highly with maternal personal air levels. A weak but significant correlation between average indoor air levels of chlorpyrifos and diazinon and levels of their respective chemical-specific metabolites (3,5,6-trichloro-2-pyridinol [TCPY] and 2-isopropyl-4-methyl-6-hydroxypyrimidinol) in biweekly maternal urine samples was observed. Detection of chlorpyrifos and diazinon in maternal blood was correlated highly with detection in newborn blood samples. No association was observed between these blood levels and levels in indoor air samples. Diethylphosphate (another organophosphate metabolite) was detected in 61 percent of meconium samples and TCPY was detected in 69 percent of samples, although these levels were not associated with corresponding levels of insecticide in indoor air. The presence of chlorpyrifos and diazinon in umbilical cord plasma was associated with low birth weight. Preliminary data suggest an inverse association between high chlorpyrifos levels and mental and motor development at 3 years of age.

Discussion

A participant asked if a quantitative assessment of drug or pesticide metabolite levels in meconium could be made. Dr. Whyatt responded that at present, she is using dry weight, but would like to have a measure similar to creatine equivalents to use for meconium. Although use of the first meconium would be optimal, the nursing staff generally is too busy to collect the first meconium, so often the sample is obtained the day after delivery. A participant asked if Dr. Whyatt had controlled for socioeconomic status because low birthweight often is associated with low socioeconomic status. Socioeconomic status, housing quality, availability of basic necessities, and maternal education levels were included in the analysis and did not affect the association between birthweight and exposure. Dr. Whyatt was asked to clarify whether diet could contribute to TCPY levels. She replied that she had no data on this, but that some TCPY could come from dietary sources. A participant asked

why there was no correlation between indoor air and urine and blood levels. Dr. Whyatt speculated that exposure could come from sources besides indoor air (e.g., dietary sources). Intestinal metabolism also could account for the difference because this varies greatly between individuals. Dr. Whyatt was asked whether she had measured breakdown products in meconium or whole compounds. Dr. Whyatt responded that she had looked mainly at breakdown metabolites and generally did not find the parent compound in the meconium.

Analysis of Genotoxic Biomarkers in Children Associated With a Pediatric Cancer Cluster and Exposure to Two Superfund Sites

Barry Finette, University of Vermont

This project evaluates the utility of specific biomarkers of effect and susceptibility to cancer risk using a pediatric population exposed to environmental genotoxins. The study population consists of children residing in Tom's River, New Jersey, the site of a pediatric cancer cluster. Children residing here were exposed to toxins from two different Superfund sites and had a 70 percent higher rate (compared to statewide levels) of acute lymphocytic leukemia in females under 5 years of age and brain and other central nervous system tumors. Toxicologic and hydrogeologic studies show that over a 50-year period, residents were exposed to a wide variety of chemicals including aniline and benzene-based dyes, radionucleotides, and over 150 other chemicals. These conditions offer the ideal setting in which to conduct a biomarker study because there is strong exposure data and a documented increase in cancer risk in the population. Three biomarkers will be assessed in this study: (1) hypoxanthine guanine phosphoribosyltransferase (HPRT) mutation frequencies; (2) chromosome aberrations; and (3) a genotype comparison of 19 polymorphisms in 11 genes. Children with cancer will be compared to their cancer-free siblings (exposed group) and to an unexposed control group.

Analysis of HPRT mutation frequency has been completed in the exposed siblings of children who developed cancer and in age- and gender-matched unexposed children. No difference in HPRT mutation frequency was observed between these two groups. Analysis of the HPRT mutational spectrum may show differences because the types of mutation may be different in exposed versus unexposed children. Cryopreserved peripheral blood, rather than fresh blood, was used to make metaphase spreads of 92 subjects; as of this meeting, 10 spreads have been analyzed. Mutation spectra and frequency will be analyzed for 11 genes, such as those encoding glutathione s-transferases, cytochrome P450 enzymes, and N-acetyltransferases. These data will be used to detect links between exposure and genetic effects and to assess pediatric cancer risk.

Discussion

In response to a question, Dr. Finette confirmed that the polymorphisms studied in this project did affect enzyme activity and would therefore be more likely to reflect exposure. To clarify the HPRT assay, Dr. Finette explained that the mutations themselves are not significant but instead are used to represent genome-wide mutation events. The participant also asked if the type of mutation observed in HPRT (mutation spectrum) correlated with mutations in genes responsible for cancer risk. Dr. Finette responded that the HPRT assay is a classic reporter assay for somatic mutations that can be extrapolated to the entire genome, and that certain types of mutations (e.g., CpG mutations), could be associated with certain tumors. A participant asked whether there was any explanation for the increased rate of cancer in females in this population. Dr. Finette commented that during fetal development, differences in the types and frequencies of mutations could depend on gender. Males and females also express different kinds and levels of detoxification enzymes during fetal growth and early childhood; additionally, differences in absorption, particularly in fat, as a result of the influence of estrogens, could play a role. A participant asked whether measurements of environmental toxins would be made at schools because children spend a great deal of time there. Dr. Finette confirmed that measurements at schools would be made as part of new epidemiological studies.

Development of a Physiologically Based Pharmacokinetic and Pharmacodynamic (PBPK/PD) Model To Quantitate Biomarkers of Exposure to Organophosphorus Insecticides

Charles Timchalk, Battelle Memorial Institute

The development of a physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model to quantitate biomarkers of exposure to organophosphorus insecticides (chlorpyrifos) will be used to assess exposure and biological response during development. Coupling kinetic models with dynamic models allows the assessment of both target tissue dose and target interaction with a pharmacodynamic response. For this model system, cholinesterase inhibition is used as the marker for biological response, and the parent compound or its metabolites serve as the dosimetry marker. Both children and young animals (neonatal rats) are more sensitive to insecticides than are adults. The increased sensitivity may be related to age-dependent differences in the metabolism of the insecticides. The ontogeny of developing enzyme systems are similar in rats and humans; therefore, neonatal rats were used to develop this model. A model system previously developed for chlorpyrifos will be modified using different types of metabolism scaling and enzyme systems to incorporate age-dependent changes into the dynamics. The modeling strategy focuses on quantitating the chlorpyrifos metabolites oxon and trichloropyridinol (TCP); chlorpyrifos is metabolized by cytochrome P450 to the reactive oxon, which inhibits acetylcholinesterase.

A series of experiments in rats involved exposing neonatal rats to two doses (1 mg/kg or 10 mg/kg) of chlorpyrifos, determining kinetics and dynamics in these rats, and fitting experimental data points to the model. Levels of chlorpyrifos in blood at postnatal days 5, 12, and 17 are proportional; a higher concentration was observed in neonates than in adults, which indicated lower rates of chlorpyrifos metabolism in the neonates. Dynamic data show a clear dose response between 1 and 10 mg/kg of chlorpyrifos, with the 1 mg dose resulting in greater inhibition in neonates than in adults. Greater inhibition of cholinesterase is observed in younger animals. The model reasonably simulates age-dependent responses in the rat, although many questions remain. Sensitivity analysis is needed to determine parameters that estimate age-related sensitivity more accurately.

Another goal of this project is to develop noninvasive biomonitoring strategies to measure levels of cholinesterase in saliva. Using classical pharmacological approaches, cholinesterase activity in the presence of inhibitors was measured in the rat brain, plasma, and saliva. Inhibition was observed in the blood and plasma but not in saliva. Further studies suggest that in rat saliva, cholinesterase is mostly butyrlcholinesterase, consistent with what has been reported in humans. Comparing blood to saliva shows a good parallel response, although the magnitude of cholinesterase levels in saliva is significantly less than in blood. This suggests that saliva could be used to measure dosimetry and cholinesterase response; however, quantitation limits may be an issue.

Discussion

A participant asked about plans to develop a fetal rat PBPK/PD model. Dr. Timchalk plans to pursue this and also is working on developing a monkey model system. A participant asked about plans to scale the PBPK/PD model for human fetal development, particularly correlating the brain development in rats with humans. Dr. Timchalk responded that these plans are underway, that a scaling approach would be needed, and that the monkey model might be more relevant and could help with extrapolation.

Biomarkers of Human Exposure to Pesticides Utilizing a New PBPK/PD Model and Kinetic Data on Pesticide Metabolism in Humans

James Olson, The State University of New York at Buffalo

This project aims to develop age- and gender-specific kinetic parameters for the metabolism of model pesticides (parathion and chlorpyrifos) in the liver of humans in various age groups. These parameters will be used in a PBPK/PD model (the Exposure Related Dose Estimating Model [ERDEM]) to estimate individual susceptibilities using biomarkers of susceptibility (paraoxonase and cytochrome P450 genotypes), exposure (urinary

metabolites), and effects (blood acetylcholinesterase). This model incorporates multiple routes of exposure to multiple chemicals, multiple metabolic pathways and distribution to target tissue, and receptor inhibitions.

Kinetics for the metabolism of parathion were characterized in human liver microsomal specimens from donors ages 0 to 60 years and from recombinant human cytochrome P450s. The cytochrome P450 enzyme CYP3A4 was observed to have the highest V_{max} for paraoxon and p-nitrophenol formation, although the high K_m indicates that it may play a more limited role in low-level human exposures. The parameters derived from this experiment were used to determine CYP-specific content in liver microsomes and CYP-specific kinetic parameters and apply them to the PBPK/PD model. Values for the pooled liver microsomes compared favorably to that derived for the recombinant CYPs. Age-dependent differences in hepatic CYP content will be used along with these data in the PBPK/PD and ERDEM models to improve risk assessment for these pesticides.

Discussion

A participant commented that the human pooled microsomes had lower V_{max} for dearylation and desulfation. Dr. Olson responded that this was true, but that the pool incorporated liver microsomes from five different livers. A better quantitation of CYP content and activity is needed for human liver microsomes to develop a representative range of values. A participant commented that CYP3A4 is the most prominent cytochrome P450, but in mixes, it appears to play a smaller role. CYP 3A4 is the only P450 in small intestine and could affect compound availability. Dr. Olson responded that this could affect risk assessment based on a liver model because many children are exposed to pesticides through the oral route of exposure.

Species-Specific Xenobiotic Metabolism Mediated by the Steroid and Xenobiotic Receptor, SXR

Bruce Blumberg, University of California–Irvine

This project is aimed toward providing a molecular basis for understanding similarities and differences in the ways humans and model animals respond to chemical exposure. The steroid and xenobiotic receptor (SXR) is a member of the nuclear hormone receptor superfamily, but behaves as a sensor rather than a classic endocrine receptor. Compounds that activate SXR increase expression of genes involved in xenobiotic metabolism, such as the cytochrome P450s and Phase I and II enzymes. Unlike many of the steroid receptors, the pharmacology of SXR is different in mice than in humans; compounds that activate the receptor in humans fail to activate it in mice. These differences are relevant for determining the risk posed by natural and synthetic endocrine disruptor compounds.

In rodents, polychlorinated biphenyls (PCBs) are SXR agonists, inducing activation of the receptor and expression of target genes. In contrast, certain highly chlorinated PCBs that are strong activators of rodent SXR antagonize human SXR. Competitive binding assays show that PCBs bind SXR in vitro, which correlates with the ability of PCBs to antagonize SXR activity. In humans, PCBs are the least metabolized, most persistent compounds and are suspected of having adverse effects in humans and in wildlife. Given the difference between the actions of PCBs in a rodent model compared to humans with respect to SXR activity, rodents may not be a suitable model system for assessment of the risk these compounds pose to human health.

Recent research has described mutually inhibitory crosstalk between SXR and the NF B signaling pathway. Rifampicin, a strong activator of SXR, also has anti-inflammatory and immune suppression effects and inhibits NF B in the presence of SXR. SXR ligands inhibit NF B target gene expression in human primary hepatocytes through a p65-dependent mechanism. Induction of NF B activity has been observed to block CYP3A4 expression and also inhibits SXR-dependent reporter gene expression. A mouse model in which SXR is knocked out shows upregulation of NF B targets, including inflammatory cytokines such as interleukin (IL)6, IL2, TNF- , and Cox-2; inhibition of NF B target gene expression does not occur in these knockout mice. Conversely, inhibition of NF B results in enhancement of SXR-dependent reporter activity. The SXR and NF B signaling pathways are mutually antagonistic through competition for a common cofactor, the protein P300, and inhibition of one path results in upregulation of the other. This antagonism provides a mechanism for the anti-inflammatory effects of rifampicin modulated through its effects on SXR. This also may provide an explana-

tion for the decreased expression of some cytochrome P450 genes observed under conditions of infection and inflammation. Disruption of the xenobiotic response by chronic inflammation may result in increased chemical sensitivity, whereas inhibition of the immune response by xenobiotics may occur through SXR antagonism of NF B signaling.

Discussion

A participant asked whether glucocorticoid-mediated stress could affect chemical toxicity, or if toxicity could affect stress. Dr. Blumberg replied that in humans, SXR responds to certain glucocorticoids, so this type of interaction is possible. A participant asked whether inflammatory molecular events had been connected to cellular activities such as proliferation, growth regulation or regulation of apoptosis, and to outcomes such as neurodevelopmental delays, immune response problems, or cancer. Dr. Blumberg replied that studies on immune function are ongoing and that he hoped to extend these studies to outcomes.

A participant asked whether Dr. Blumberg had examined the effects of polybrominated biphenyls such as flame-retardants. Dr. Blumberg expected that these would behave similarly to PCBs. A participant asked for clarification of the ability of SXR to activate PCBs such as PCB 209. Dr. Blumberg responded that nuclear receptor antagonists can act as slight agonists at high concentrations. Environmental levels of these antagonists are low and probably behave as antagonists.

A participant asked about the kinetics of NF B and SXR response to acute xenobiotic stress. Dr. Blumberg answered that experiments examining acute exposure showed that the observed mutual antagonism is independent of protein synthesis. A participant asked about SXR expression and activity in fetal versus adult life. Dr. Blumberg answered that SXR is expressed at approximately day 12 or 13, and that expression peaks between 20 and 50 years of age.

Toxic Metal Ion-Synthetic Chelating Agent Interactions in Aqueous Media
Alan Stone, The Johns Hopkins University

This presentation focused specifically on interactions of industrial chemicals with metals such as chromium (Cr) and manganese (Mn) present in sediment in places such as Baltimore Harbor. Most Cr present in sediment is precipitated and not harmful, but release of industrial chelating agents found in substances such as hospital detergents can solubilize and release the Cr. Chelating agents adsorb onto particulates in the sediment and extract the Cr, releasing it as soluble Cr^{III}, which can be oxidized to Cr^{VI}. This process can be affected by the presence of other contaminants (e.g., acetate, which has a synergistic effect on Cr solubilization). Solubilization of Cr by chelating agents is also pH dependent. Monitoring the solubilization process using capillary electrophoresis showed that adsorption occurs and that several different reactants, intermediates, and products could be detected. This work suggests that although efforts are underway to ban PCBs and reduce the use of pesticides such as chlorpyrifos and diazinon, other hydrophobic chemicals such as neutral ligand-metal complexes also should be assessed for their ability to harm human health.

Naturally occurring Mn also interacts with synthetic chelating agents. Mn is present in the environment as Mn^{3+}or Mn^{4+}, usually in a precipitated state. Dissolved Mn^{3+} is capable of interacting with industrial chemicals such as pyrophosphate to form Mn^{3+}-pyrophosphate, which is a strong and potentially dangerous oxidant. Other industrial chemicals and pharmaceutical compounds such as the antiviral triamcinolone acetonide also can solubilize Mn^{3+}. Another potential source of solubilized Mn^{3+} arises from the use of ozone, rather than chlorines, in water treatment plants. At present, there is little concern about Mn in drinking water, although a Parkinson's-like disease is seen in people exposed to inhaled Mn through certain kinds of welding. Understanding the chemistry of and interactions between metals such as Cr and Mn and industrial chemicals present in water and sediment will help to develop predictive tests to determine which of these chemicals may pose a risk to human health.

Discussion

Dr. Stone was asked whether any characteristics of the sediments themselves might influence the release of Cr in real-world situations. Dr. Stone answered that factors such as water salinity and the presence and nature of organic matter in the sediment would affect Cr release.

Session Wrap-Up
Pauline Mendola, U.S. EPA

This session stressed the importance of relating laboratory results to the environment and the ways it impacts human health. Age and gender differences affect sensitivity, and it is important to understand crucial windows of exposure. Early exposure may have long-term effects because it provides enough time for the accumulation of deleterious genetic lesions that could have serious health consequences. It is necessary to expand our knowledge of the ways different species respond to and metabolize chemicals, particularly when extrapolating data from a model organism to humans; species-specific differences in terms of receptor activity, metabolism, and sources of exposure also must be considered.

SESSION II: EXPOSURE ASSESSMENT

Session Overview
Jim Quackenboss, U.S. EPA

This session overview described the goals of exposure assessment including the determination of aggregate and cumulative risk, protection of sensitive subpopulations (i.e., children), and an evaluation of public health outcomes. Intervention can occur at the source of exposure, whereas assessment of the effects of intervention involves evaluation of exposure. The goals for NERL's Children's Exposure Research Study include: (1) development and evaluation of approaches, methods, and models for assessing children's aggregate and cumulative exposure; (2) identification and characterization of key determinants of exposure; (3) quantifying and understanding the relative importance of each route and path of exposure; and (4) collection of population data on exposure to better assess risk.

EPA-sponsored studies on risk assessment include the National Human Exposure Assessment Survey, Particulate Matter Panel Studies, Minnesota Children's Pesticide Exposure Study, Children's Exposure Program, and Agricultural Health Survey. These and other ongoing studies use different approaches to understand exposure and develop exposure models. The studies are aimed toward understanding the mechanisms of exposure and include longitudinal measurements of exposure from early childhood onward. The National Children's Studies, involving collaboration between the National Institute of Child Health and Human Development, EPA, and the Centers for Disease Control and Prevention, are designed as longitudinal studies to follow 100,000 children from before birth to adulthood. Multiple types of exposure will be assessed and related to any developmental issues that arise.

Estimating Human Health Risk From Dermal Exposure to Contaminated Soils
Annette Bunge, Colorado School of Mines

The paradigm for assessing risk from dermal exposure to contaminants in soil must incorporate exposure, absorbed dose, and the probability of adverse health effects. Dermal exposure differs from inhalation exposure as a result of absorption differences; a chemical may be extremely toxic, but poses little danger to health from dermal exposure if it cannot cross the skin barrier. Estimation of absorbed dose from dermal exposure must include the effects of soil loading and soil solubility limits.

The fraction absorbed approach calculates absorbed dose per contacted area by measuring the amount of chemical present in the soil and the amount of soil on the skin surface, and incorporating the fraction absorbed value (F_{ABS}), which is specific to each chemical compound. Most F_{ABS} values have been determined by the

same laboratory using the same soil with the same organic carbon content, and using only large particles and high soil loading. This is different from real-life situations, in which the soil composition is more complex, usually includes smaller particles, and soil loading is lower. F_{ABS} accuracy, therefore, may be affected by the amount of soil that is on the skin; contaminants present in upper layers of the soil may not have contact with the skin and may not contribute significantly to the dose. Absorption from multiple layers of soil was determined using 2,4-dichlorophenoxyacetic acid (2,4-D) and lindane. The F_{ABS} increases in a linear fashion until a plateau is reached; this plateau occurs when coverage of the skin with a soil monolayer is complete. These experiments demonstrate that only layers of soil in direct contact with the skin contribute to exposure. Rates of absorption decrease as the first layer of soil is depleted of contaminants. This work demonstrates that F_{ABS} values are not independent of the amount of soil in contact with the skin surface; therefore, these values should be adjusted for soil loading.

Discussion

A participant asked whether highly soluble compounds can diffuse through hydrated soil and contact the skin, even if they are present in the outer layers of soil. Dr. Bunge replied that her experiments used dry soils without pore water, but that the presence of pore water could facilitate transport to the skin's surface and increase exposure. A participant asked how the kinetics of diffusion and transfer, plus the water content of the soil, could be incorporated into a calculation of absorbed dose. Dr. Bunge replied that most real-life exposure involves a fine layer of dry soil (unless the exposure is to mud). Data indicate that water has little effect on absorption of hydrophobic compounds, although sweat may help transfer compounds from the soil to the skin's surface. A participant asked whether particle size affected adherence of soil to the skin. Dr. Bunge answered that there is an effect, and that particles less than 63 µm adhere. The effect of particle size on absorption has not yet been examined.

A Longitudinal Approach of Assessing Aggregate Exposure to Organophosphate Pesticides in Children

Chensheng (Alex) Lu, Emory University

The goals of this longitudinal study to determine pesticide exposure in children are to: (1) determine exposure to organophosphates and pyrethroids in school-age children over the course of 1 year; (2) assess the contribution of dietary exposure to the total exposure; and (3) investigate aggregate exposures. The variability of total organophosphate pesticide exposures was characterized with respect to residential pesticide use and dietary intake. The effects of dietary intervention (organic diet) on pesticide levels also were examined. Organophosphate and pyrethroid metabolites were assessed in urine before, during, and after dietary intervention. Children ate a conventional diet, and then conventional food items were replaced with organic versions. Analysis showed that most chlorpyrifos exposure came from dietary sources, and that levels of the chlorpyrifos metabolite TCPY could be "washed out" during the organic diet phase. The malathion metabolite, malathion dicarboxylic acid, also fell to undetectable levels during the organic diet, and levels of 2,4-D (dichlorophenoxyacetic acid) also decreased. Organophosphate and pyrethroid levels also were assessed in food items. Levels of chlorpyrifos were highest in apples, soybean grain, wheat, and peaches, whereas pyrethroid levels were highest in wheat, soybean grains, strawberries, and cherries. For this cohort, wheat-based food items were the most commonly consumed foods, followed by strawberries and grapes.

Organic dietary interventions resulted in decreased levels of several pesticide metabolites in this cohort of children and showed that children are exposed to organophosphates primarily through food consumption. Exposure to pyrethroids appears to be primarily through residential exposure. Interventions of this sort will allow identification of the source of exposure, assessment of the human pharmacokinetics for these pesticides, and calculation of the risk of dietary pesticide exposures and the benefit of organic diets.

Discussion

A participant asked whether compliance with the organic diet was good because recovery of pesticide residues before the end of the organic diet was sometimes observed. Dr. Lu responded that lack of compliance was a possibility, and that the food journals the children kept could be used to help confirm this. A participant asked whether washing food would affect the results because many compounds are found mainly on the surface of the food. Dr. Lu answered that participants were not asked to wash the food or treat organic or conventional foods differently. Because the same diet composition was maintained for both the organic and conventional phases, the inability to obtain certain organic food items led to use of some conventional food items during the organic phase, which may have affected the results. A participant asked if it was possible to calculate cumulative risk based on guidelines for organophosphates. Dr. Lu answered that they have archived replicate urine samples and can use these for cumulative risk assessment in the future.

Longitudinal Study of Children's Exposure to Pyrethroids
Ye Hu, RTI International

This longitudinal study to assess children's exposure to permethrin involves collection of multimedia samples to assess exposure to environmental pesticides in a cohort of stay-at-home children under 3 years of age. The research plans include: (1) developing a time course of the redistribution of pyrethroid pesticides following residential application; (2) determining the functional relationship between the concentrations of pesticides found in different media; and (3) analyzing the differences between adults and children in the time course of pyrethroid metabolism. Sampling took place after pesticide application (the type of pesticide and method of application were noted) and environmental, personal, biologic, and food samples were collected. Samples included air, surface wipe, toy wipe, hand wipe, pajama, and urine from both the child and his or her caregiver. Followup was planned for 12 months. Videotape recordings also were made to understand eating and hand-to-mouth activity of the children to assess nondietary ingestion.

Urine samples were collected from diapers to analyze pyrethroid metabolites. The polyacrylate granules contained in the diapers posed a challenge for urine extraction from diapers. These granules have a high capacity for water, and pyrethroid metabolites are water soluble, leading to dilution of the urinary metabolites if water is used for the method of extraction. Magnesium and calcium salts were tested; these salts collapse the hydrated polyacrylate granules and release the water and urine from the diaper. Calcium salts at concentrations of 150 grams per liter were found to yield the highest percent recoveries of pyrethroid metabolites. The stability of the metabolites in urine stored at 37 °C overnight (to mimic urine remaining in a diaper overnight) also was tested, as was storage stability at 1 °C. Recovery of metabolites under these conditions was satisfactory.

Discussion

A participant asked how much urine could be recovered from the diapers. Dr. Hu answered that approximately 75 mL, at least 70 percent of which is urine, was recovered. A participant asked if data on pesticide application rates and methods were gathered. Dr. Hu answered that the identity of the pesticide, as well as the time and method of application, were recorded.

Assessing Levels of Intermittent Exposures of Children to Flea Control Insecticides From the Fur of Dogs
Janice Chambers, Mississippi State University

This project assesses children's levels of exposure to organophosphates present in dog flea collars. Fleas on pets are a large problem in the South, Southwest, and West, and the use of flea control agents can lead to significant levels of exposure for children through dermal exposure and, in young children, oral exposure. Organophosphorus insecticides have a long, well-documented history of widespread use. Organophosphates inhibit acetylcholinesterase, resulting in the accumulation of acetylcholine in the synapse and cholinergic hyperstimulation. These compounds are metabolized and detoxified by several routes involving cytochrome P450 enzymes and esterases. The nervous system of children is more susceptible to organophosphate toxicity be-

cause they have lower levels of P450 enzymes, carboxylesterases, acetylcholinesterase, and body fat, which serve to sequester these compounds.

The objective of this study is to determine the levels of chlorpyrifos or tetrachlorvinphos residues transferable from the fur of dogs wearing flea collars and the levels of exposure in people in contact with the dogs, particularly children. Healthy dogs at least 4 months old of diverse breeds were selected for the study. Children between the ages of 3 and 12 years were assessed for exposure from contact with the dogs. Dogs first were bathed to remove any residues and then petted with a white cotton glove (treated to remove possible contaminants) to obtain a baseline level of organophosphate residues. After applying the flea collar, dogs were petted with gloves on the back and on the neck above the collar at 14 and 20 days after placement of the collar. Transfer of residues to cotton T-shirts worn by the children and levels of metabolites in the urine of the children and adults also were analyzed. Chlorpyrifos residues were not detected on the back of the dog but were detected on the dog's neck. These residues transferred to and were detectable on gloves and T-shirts. Evidence of the chlorpyrifos metabolite TCP was observed in urine from the adults and children, but no statistically significant increase between pre- and post-flea collar placement was observed.

Discussion

A participant asked how flea collars could be effective if insecticide residue is not detected on the back of the animal, away from the flea collar. Dr. Chambers responded that the insecticide allegedly is absorbed and becomes systemic, but that most veterinarians do not consider flea collars to be effective for killing fleas. A participant asked whether increasing the moisture of the glove to mimic skin could have an effect on residue transfer. Dr. Chambers answered that they did not check this, but that it could have an effect. A participant asked whether younger children showed higher levels of exposure or ingestion. Dr. Chambers answered that this has not been assessed because they do not have enough children in different age groups to get statistically significant results. She added that no correlation was observed between exposure and the type of dog or fur, nor the age of the dog.

Ingestion of Pesticides by Children in an Agricultural Community on the U.S./Mexico Border—Variations in Behaviors and Metabolite Levels

Natalie Freeman, University of Florida

This objectives of this project are to: (1) investigate the sources of childhood exposure to pesticides in a rural community; (2) determine the influence of children's activities on exposure; and (3) develop a model to assess how source and activity affect exposure. The study took place over 2 years and included four rounds of sampling during the summer and winter. Sixty children between the ages of 6 and 60 months were involved. Pesticides present in the environment (soil) and inside the house were measured, and exposure was sampled by taking wipes of the children's hands and by collecting first-morning voids and analyzing for organophosphate pesticide metabolites. Children were videotaped around the home for 4-hour sessions during two of the four rounds of sampling, and the videotape was transcribed to measure activity and behaviors. Urine was collected using 100 percent cotton diaper inserts.

Age-related differences in children's behaviors were noted, and these differences were analyzed to determine if the behaviors influenced exposure. Infants tended to show more mouthing behavior than older children, but their exposure to pesticides was limited by their limited mobility. Soil consumption was reported most frequently for children between 12 and 23 months old, and time spent outdoors led to increased contact with potentially contaminated soil and plants. Hygiene habits improved, however, which may have reduced exposure. Levels of urine metabolites varied over the four seasons as a result of changes in pesticide use at nearby agricultural areas. Higher urine metabolite levels also were observed in younger children who tended to eat food that had been in contact with the floor, ate with their fingers, and slept with a toy or blanket, which resulted in increased inhalation exposure. Analysis is ongoing to relate exposure to time spent outdoors or in the house. Future plans include observing the children's behaviors at school and assessing any neurobehavioral or genetic changes that may occur.

Discussion

A participant asked if the higher levels of metabolites in urine associated with hand-to-mouth behaviors in younger children could be related to a higher intake of food. Dr. Freeman answered that she does not have dietary data adequate to answer this question.

Vulnerability of Young Children to Organophosphate Pesticides Through Intermittent Exposures in Yuma County, Arizona

Mary Kay O'Rourke, University of Arizona

Young children in Yuma County, Arizona, are exposed to agricultural pesticides and have urinary biomarkers indicative of exposure to organophosphate pesticides. Previous work determined that about one-third of exposure was caused by ingestion of contaminated food and beverages, whereas the majority of exposure (more than 68 percent) was a result of secondary ingestion (hand-to-mouth transfer) of house dust. For this study, 210 children between the ages of 16 and 48 months were recruited. Exposure was assessed using videotaped observations of behavior, measurement of pesticides in the children's homes and environments, hand wipes, and analysis of urinary metabolites.

Urine levels of metabolites indicating exposure to chlorpyrifos and malathion were higher in children who had more contact with food outdoors. Hand wipe samples showed increased levels of diazinon, cis-permethrin, and trans-permethrin on the hands of children who had more contact with the entry floor or rug of their homes. Analysis of multiple hand wipes showed a loading limit; no increase in pesticide levels were seen for multiple wipes, indicating that wiping may remove all contamination and subsequent reloading does not go above a maximum loading level. Some pesticide contamination of the hands was associated with specific locations, including the entry to the home, laundry areas, and the outdoors. Outdoor food consumption also was associated with pesticides on the hands.

Discussion

A participant asked if acetate, which affects organophosphate solubility, could affect pesticide uptake. Dr. O'Rourke answered that RTI may have some data on this for chlorpyrifos, pyrethrin, and hepatochlor. When asked whether she had plans to develop the distributions of contact factors to use for modeling, Dr. O'Rourke answered that her data were collected only during a short time period and may not be useful. Initially, some data appeared to indicate a relationship between creatine loading and activity, but this association disappeared when more data points were included in the analysis. A participant commented that these data and those of Dr. Freeman showed higher measurements of exposure than those from the National Health and Nutrition Examination Survey (NHANES), and asked whether there had been attempts to calibrate both sets of data. Dr. O'Rourke answered that this had been done, but that there are differences between her data and the NHANES data because this study involved younger children living in an agricultural community. The Centers for Disease Control and Prevention may plan on assessing exposure in younger children.

Session Wrap-Up

Jim Quackenboss, U.S. EPA

Dr. Quackenboss commented on commonalities between the biomarkers and exposure assessment sessions and defined pathways and activities in terms of how they influence exposure. Dr. Bunge discussed experimental studies to define soil loading and how it influences exposure; these data will need to be extrapolated to the "real world" to generate more accurate risk assessment measures. Drs. Freeman and O'Rourke documented the complexities of behaviors influencing exposure in real life. Determining contact rates with contaminants involves considering these activities and relating them to the transfer of materials to the skin and to the mouth. Activities such as chewing on nonfood items and the handling of food, and the location where the food is eaten, all affect dietary exposure. Exposure to toxins in food is assumed to be primarily through ingestion, but these studies have shown that children's food-handling behaviors also are significant contributors to dietary

exposure. Dr. Chambers showed that pet care products are another way in which children are exposed to pesticides. Dr. Lu demonstrated that diet contributes to pesticide exposure but that substituting organic foods can decrease the levels of these pesticides in children. Overall, this session demonstrated the complexity of real-life behaviors and their contribution to exposure, and subsequent research is required to try to identify trends in behavior that may significantly increase exposure.

SESSION III: SUSCEPTIBILITY AND VULNERABILITY

Session Overview
Brenda Foos, U.S. EPA

The Office of Children's Health Protection coordinates, integrates, and promotes children's health concerns. EPA's science inventory contains more than 300 projects for children's health, including high profile intramural studies such as the Children's Environmental Exposure Research Study and the Children's Health Study. EPA's intramural program is interested in vulnerability and susceptibility studies, because EPA's mandate promotes the protection of human health and the environment, including assessment of vulnerable populations and life stages and consideration of the risks to children. The agency-wide policy states that EPA should consider risks to infants and children as part of risk assessment during the policy setting process; therefore, it is necessary to understand the vulnerable populations to set policy. Research on childhood lead poisoning, for example, led to the removal of lead from gasoline and to clean water and air policies. Additionally, lead abatement programs and parent education programs arose from these efforts. EPA and the Food and Drug Administration have a joint fish advisory to limit the exposure to methyl mercury in pregnant women, women of reproductive age, and young children. EPA's smoke-free home pledge specifically is a result of children's vulnerability to secondhand smoke. Overall, the goal of this office is to strengthen the scientific tradition of the agency's efforts, particularly with respect to children's health.

Bioaccumulative Toxics in Native American Shellfish
Felix Basabe, Swinomish Tribal Community

Members of the Swinomish Indian Tribal Community are exposed to low level, chronic bioaccumulative toxics because of their substantial dietary intake of marine animals, which are the main source of protein for most of the Swinomish people. Tribal members gather shellfish from reservation land located 70 miles north of Seattle, an area adjacent to polluting industries such as oil refineries, chemical plants, aluminum smelters, and power generators. The goals of this project are to: (1) determine whether consumption of subsistence-harvested shellfish exposes the Swinomish people to toxics at a level that could cause chronic and acute health risks; (2) communicate any identified health risks to tribal community members; (3) develop mitigation strategies; and (4) confirm whether health problems on the reservation are linked to eating contaminated shellfish. In the summer of 2002, butter clams, little neck clams, and crabs were gathered and tested for contamination. Analysis of both clam species showed significant levels of mercury, arsenic, cadmium, dioxin, and several species of polychlorinated biphenyls. Cancer risk and hazard index analysis show that the harvested butter clam and steamer clams exceed the standards for health hazards. Although ingestion rates have not been calculated, they are expected to be near 1 to 2 pounds of shellfish per day, which is 27 to 30 times more shellfish than nontribe members eat. Strategies to reduce risk must take into account the cultural identity of the Swinomish people, which includes consumption of marine species as a substantial part of the traditional Swinomish diet. Community outreach and education efforts include programs in schools and day care centers to educate children about the risks and benefits of consuming shellfish and show them where levels of contamination preclude fishing.

Discussion

A participant asked if risk assessment for cancer was biased for PCBs and dioxins. Dr. Basabe answered that risk assessment was mainly dioxin based. A participant commented that risk based on a normal U.S. diet is not considerably different from that for the tribes, and that perhaps toxin levels in beef and pork may be similar to the levels detected in shellfish. Substitution of other protein sources for shellfish may, therefore, not have a

large health benefit. A participant commented that the New Bedford, Massachussetts, harbor has high levels of PCBs in sediment and that another group obtained serum samples from those at high risk for PCB exposure from this harbor. Those aged 70 years and older had higher levels of serum PCBs that randomly associated with some diseases, but no strong associations were observed. A participant asked whether leukemia had been observed in any of the mollusks harvested from contaminated areas. Dr. Basabe answered that they had not looked for leukemia, but had detected some anomalies and a slight decline in population. They did not have the data to link this to toxin levels.

The Effects of the World Trade Center Disaster on Pregnant Women and Their Infants
Trudy Berkowitz, Mount Sinai School of Medicine

The destruction of the World Trade Center resulted in the release of soot, benzene, polycyclic aromatic hydrocarbons, heavy metals, pulverized glass and cement, and thousands of tons of alkaline particulates into the atmosphere. This study involved 187 women who were pregnant on or about September 11, 2001, and were present in one of five exposure zones near the World Trade Center that day or within the succeeding weeks. Given the special vulnerability of fetuses and children, this study sought to determine whether exposure of pregnant women to these environmental pollutants had an effect on perinatal outcome and early infant growth and development. Plasma organochlorines, brominated biphenyl ethers, and blood and urinary biomarkers for metals were analyzed in the women, as well as levels of stress and anxiety, which were documented through the use of questionnaires and salivary cortisol levels. Birth weight, size for gestational age, and presence of pre- and neonatal complications were determined. Infants were assessed at 9 months, 2 years, and 3 years to evaluate growth and cognitive, psychomotor, and behavioral development.

Women who were exposed to environmental toxins arising from the World Trade Center collapse had high levels of lead, similar to those seen in firefighters working at the site and higher than those detected in the National Health and Nutrition Examination Survey. In general, these women also had higher levels of organochlorines and brominated biphenyl ethers, particularly PCBs and octachlorodibenzodioxins (OCDD). No significant differences were observed between the World Trade Center cohort and a control group for the frequency of preterm births or low birthweight. Women in the World Trade Center cohort did have a twofold increase in risk for small-for-gestational age infants and preliminary data suggest that infants of mothers who were closest to the World Trade Center on September 11, 2001, scored more poorly on some tests of early cognitive development.

Discussion

A participant asked how exposure zones were established. Dr. Berkowitz answered that the zones were based on EPA monitoring data, data from other environmental health centers, and on the travel of the pollutant plume eastward during the first few days after September 11, 2001. A participant asked if the plume data had been overlaid on Dr. Berkowitz's zones of exposure. She answered that plume data had validated the exposure zones, but added that monitoring data were not available for September 11, 2001. She commented that they also have data on the women's perceptions of air quality, and that these were highly correlated with the exposure index.

Genetic Basis of the Increased Susceptibility of Children to Inhaled Pollutants
Terry Gordon, New York University

Most diseases have a nongenetic trigger, but genetics can influence susceptibility and response to environmental contaminants. The objectives of this study are to: (1) quantify the contribution of genetic versus environmental factors; (2) identify candidate genes critical to the increased susceptibility of the neonatal lung to inhaled pollutants; and (3) compare these genes to those involved in adult lung toxicity. A mouse model was used, in which eight inbred strains of mice ages 12 to 18 days were exposed to ozone for 5 hours; lung lavage was performed 24 hours after exposure to detect lung damage. Inflammatory effects of ozone exposure were not observed in 12-day-old pups, but 15-day-old pups showed strain-related differences in the amount of lung

damage; Balb/C and SJL mice showed the highest amount of damage. Young mice also were more sensitive to ozone-induced lung injury than were adult mice.

To determine how these differences in sensitivity arise, dosimetry experiments to detect ozone absorption were performed on neonatal and adult mice from sensitive and resistant strains. These experiments showed that the increased sensitivity of neonatal lung to ozone-induced injury is not caused by differences in ozone absorption. Differences between strains also were not a result of differences in absorption, indicating that the observed strain differences were most likely caused by genetic factors that contribute to the effects of pollutants in the lung. Future studies will seek to identify these factors using microarray experiments and quantitative linkage analysis.

Discussion

Dr. Gordon was asked if he had performed ozone dosimetry experiments on 12-day-old mice to determine why these mice do not show ozone-induced damage. Dr. Gordon responded that he thought mice this age had not developed the chemotactic pathways or inflammatory and immune system components necessary for the ozone-induced response. Dr. Gordon was asked whether crosses between mice of resistant and sensitive strains resulted in a graded response to ozone. Dr. Gordon answered that the offspring of these mice show damage closer to that seen in resistant mice, but that the results are variable. A participant commented that recently another group has shown that Balb/C and Black 6 strains are very resistant to lung cancer, most likely as a result of differences in the inflammatory response. Dr. Gordon was asked if he had examined sensitivity following multiple exposures across the placenta or shortly after birth; he has not yet performed these experiments.

Impact of Phthalates on the Male: Frog and Rabbit Models

Rao Veeramachaneni, Colorado State University

This project seeks to determine the effects of exposure to dibutyl phthalate (DBP) on the reproductive system using the South African clawed frog (*Xenopus laevis*) and rabbits as model animals. Rabbits are useful models for studies of the effects of DBP on the reproductive system because their pre-reproductive years are comparable to those of humans as a percentage of lifespan. In this set of experiments, rabbits were exposed to DBP in utero and during puberty, and reproductive development and function were assessed after puberty (25 weeks of age). Rabbits exposed in utero had increased numbers of abnormal sperm and a reduction in numbers of ejaculated sperm. Most abnormal sperm had defects associated with the acrosome (acrosomal dysgenesis) and plasma membrane. One of 17 DBP-treated males rabbits had hypospadias, defects in the prostate and bulbourethral gland, and cryptorchid testes with carcinoma in situ-like cells. A rabbit exposed during adolescence had unilateral cryptorchidism. The rabbits displayed normal sexual behavior and serum testosterone profiles were only slightly changed.

The frog also was used as a model animal to observe the effects of DBP on morphogenic changes. *Xenopus* embryos were exposed to DBP at 8 hours postfertilization for 12 weeks at levels ranging from 1 to 15 ppm. Approximately 35 to 40 percent of day 1 to 4 embryos were killed by exposure to 1 ppm. Surviving embryos showed lags in development; embryos exposed to the highest levels of DBP underwent metamorphosis at an extremely slowed rate and remained abnormally small. Secondary sex characteristics of exposed animals were altered; laryngeal growth and mating calls were affected. Treated frogs were unable to perform proper mating calls and also showed abnormal pelvic amplexus. Changes in mating calls and behavior were observed upon exposure to 5 ppm or less DBP. Spermatogenesis of exposed frogs also was abnormal, with a high incidence of spermatid death. Some males exposed to DBP during sexual differentiation had oviducts and missing testes. Further characterization of these defects is under way.

Discussion

In response to the question if changes such as those he reported are observed in the wild, Dr. Veeramachaneni responded that similar defects have been reported for other teratogens, but solid data were not available for phthalates.

Mutations in Steroid 5-Alpha Reductase Type 2 and the Severity of Hypospadias
Jeanne Manson, Children's Hospital of Philadelphia

Allelic variants in genes affecting androgen action and metabolism may be associated with the risk and severity of hypospadias, and parental exposure to environmental contaminants may increase risk to fetuses with susceptible genotypes. A large population study has been initiated to investigate gene-environment interactions that may influence the risk for this defect. The database contains infants diagnosed with hypospadias, infants with other urogenital abnormalities, and unaffected controls. Parents were interviewed to determine types and sources of exposure indicating a relationship between exposure and hypospadias. For the approximately 350 families currently in the study, no differences between parents of infants affected with hypospadias and unaffected infants were observed for occupational exposure to substances such as pesticides, heavy metals, paints, stains, fuels, or solvents. Parents of cases of hypospadias reported higher home exposures (i.e., fathers reported higher exposure to insecticides and herbicides). Paternal pesticide exposure was the only independent risk factor for hypospadias identified in this study.

Several candidate genes for hypospadias were analyzed. Analysis of the steroid 5-alpha reductase gene SRD5A2 showed that there was a highly significant association between a V89L missense mutation in exon 1 and severity of hypospadias. No differences in frequency of this mutation were observed between cases and controls or between Caucasians and African Americans. Trinucleotide repeat lengths in the androgen receptor (increasing numbers of repeats decrease receptor activity) also were not independent risk factors for hypospadias.

Discussion

A participant asked if the control regions of SRD5A2 had been analyzed to determine polymorphisms in these regions and if they affect the risk or severity of hypospadias. Dr. Manson responded that these regions of the gene had not yet been analyzed.

Prenatal Exposures of Children to Polybrominated Diphenyl Ethers: The Collection of Animal and Human Data Along with the Development and Validation of a PBPK Model
Ed Garner, RTI International

A PBPK rat model is being developed and validated to assess the effects of in utero exposure to environmental chemicals such as the polybrominated diphenyl ethers (PBDEs) on fetal development. PBDEs may contribute to neurological and endocrine abnormalities and are found widely in the environment. The objectives of this project include an analytical assessment of PBDEs in human blood and meconium, and determining if these are the appropriate media for measurement of cumulative exposure in infants. Analysis of 12 mothers and their infants found BDE-47 to be the major PBDE in cord and maternal blood; BDE-99 was present at approximately one-third the levels of BDE-47. Levels of PBDEs in maternal blood correlated with levels in infant blood, indicating that measurements of PBDE levels in maternal blood could be used to assess fetal cumulative exposure.

Measurements of PBDEs in rats will be used to develop a PBPK model that can be scaled to humans. Synthesis of 99.5 percent pure BDE-47 and 65 percent pure BDE-92 to use in dosing studies has been completed. Rats will be dosed with PBDEs for 10 days, and various tissues (maternal tissues, placenta, amniotic fluid, and pooled fetuses) will be analyzed. A solvent extraction and fractionation method also has been developed to analyze PBDE levels in tissues. A gas chromatography/electron capture detection chromatogram of liver ho-

mogenate extract showed good recovery and sensitivity of detection. Future work includes validating this method for rat tissues, determining partition coefficients, completing animal studies, and continuing model development and its scaling to humans for application to human samples.

Discussion

Dr. Garner was asked if he had identified a target population for these studies with known exposure to PBDEs. Dr. Garner responded that they had not yet identified such a population, and that would make human studies more difficult. A participant suggested that North Carolina's furniture industry may provide access to a population with exposure to flame retardants, many of which contain PBDEs, which are used in furniture manufacturing.

Strain-Dependent Susceptibility to Transplacentally Induced Murine Lung Tumors
Mark Miller, Wake Forest University

Exposure to environmental toxins may contribute to cancer risk, particularly when exposure occurs in utero. A mouse model was used to assess strain-dependent susceptibility to lung tumors induced in utero by chemicals to discover gene-environment interactions that increase the risk of developing cancer as a result of exposure during fetal development. Levels of activity of Phase I and II enzymes and their effects on the metabolism of 3-methylcholantrene (MC) were measured. Additionally, the levels of DNA adducts and rate of DNA repair were measured to examine whether differences in lung tumor incidence could be attributed to differences in the amount of DNA damage arising from transplacental exposure to MC.

Strain-related differences in lung tumor incidence were observed. C57BL/6 mice were relatively resistant to induction of lung tumors by transplacental exposure to MC, whereas Balb/c mice and crosses between these two strains had a 100 percent tumor incidence. Mutations in Ki-ras were analyzed, and tumors in Balb/C mice had a prevalence of G-C transversions in this gene versus the G-T mutations in tumors in resistant mice. Experiments examining gene and protein expression patterns demonstrated that these differences in susceptibility to lung tumors were not a result of differences in induction of metabolic enzymes involved in MC metabolism and detoxification, such as cytochrome P450s 1A1 and 1B1 and glutathione S-transferases. This work points to a novel genetic polymorphism that influences the risk of developing cancer after in utero exposure to environmental chemicals.

Discussion

A participant asked if Dr. Miller had examined the location of the glutathione S-transferases; past studies have shown that nuclear glutathione S-transferase leads to a different gene expression response than cytosolic glutathione S-transferase. Dr. Miller answered that they had examined supernatants and therefore had not analyzed nuclei. A participant asked at what stage C57BL/6 mice block tumor formation. Dr. Miller answered that hyperplasia is not observed in these mice, so inhibition of tumor formation likely occurs early in the carcinogenic process. Dr. Miller was asked if his research had examined rare Ki-ras mutations in the genome. He answered that they have performed preliminary experiments to assess this, which were promising, although the error rate was still very high. Dr. Miller was asked if he had examined specific DNA adduct formation. He responded that he had measured total adduct formation and also had examined and tracked each individual adduct.

Session Wrap-Up
Brenda Foos, U.S. EPA

Research sponsored by EPA will continue to increase an understanding of susceptibility and vulnerability to develop more accurate risk assessments. Presentations at this meeting brought together many research topics such as unique exposure, postnatal development, birth defects, genetics, amphibian and mammalian toxicity, and kinetic modeling, which will contribute to a better understanding of susceptibility and vulnerability. This

meeting also provided an opportunity to bring together scientists and experts from a wide variety of disciplines including the medical sciences, epidemiology, and toxicology to present research on the impact of susceptibility and vulnerability on human health and provide a stronger scientific foundation for future work.

Upcoming EPA Research Initiatives
Paul Gilman, U.S. EPA

EPA has developed a children's health research program over the past 5-7 years. EPA recently announced an additional RFA for more than $3 million focused on creating tools useful to the implementation of the National Children's Study, which is designed to explore and understand the impact of environmental stressors on children. The RFA will help to support development of biological and nonbiological tools such as biomarkers, survey and collection techniques, and analytical computing resources. EPA is cooperating with the National Institutes of Health to re-orient a computing center to serve as a backup in anticipation of large amounts of incoming data.

Research on children's health is a high priority for government-funded research, particularly for EPA; however, it is an area in which diplomatic approaches cognizant of ethical considerations are needed. As an example, EPA recently announced the initiation of an effort to assess comprehensively household pesticide exposure in children 0 to 3 years of age in Duval County, Florida. The American Chemistry Council (ACC) offered to contribute funds to the study, which would allow examination of exposures to additional chemicals such as phthalates and flame retardants. Questions arose, however, concerning the appropriateness of accepting funding from the chemical industry for work of this sort. NCER was careful to "insulate" EPA from undue influence by the ACC, creating a solid firewall between funding and scientific entities associated with the study. There also were ethical concerns related to possible unnecessary exposure of children to pesticides over the course of this study. Nonetheless, comprehensive, substantive knowledge of the extent of children's exposure to household chemicals and their effects will provide an important basis for EPA regulatory decisionmaking efforts.

Discussion

Dr. Manson commented that she had served as a member of the external advisory board for the ACC and participated in attempts to develop a joint RFA between the National Institute of Environmental Health Sciences and the ACC for the fetal basis of adult disease. She believes that the ACC is a good collaborator, and despite the concerns of the environmental activist community, if researchers retain solid rights to publish their data, there is no conflict of interest. Further interactions with industry will become more and more essential, given the funding situation at the National Institutes of Health and EPA. Better studies can be performed if more funding is available, and institutional review boards will serve to protect participants, particularly children, in these studies.

Dr. Gordon commented that a firewall between public and private agencies is important, but that communication rules and standards must be clear and consistent. In the past, Dr. Gordon received a grant from Philip Morris to study lung cancer; although there were strict rules, there also was confusion about implementing them.

Dr. Freeman discussed the reaction of the Children's Health Advisory Panel on the Florida study described by Dr. Gilman. Part of the negative reaction to the study could be traced to a brochure describing the study. Two issues were of particular concern: (1) Families would receive $900 over a 2-year period. The brochure did not clarify that the $900 was commensurate with the intensity of participation and activity required of participants, considering the number and variety of samples they would be required to gather. These included hand wipes, dust samples, interviews, questionnaires, and videotapes, and these activities would continue for 2 years. The $900 was actually trivial given the demands placed on participating families, and the brochure did not explain this well. (2) Regarding intentional exposure of children to pesticides that EPA recommends parents avoid using in their homes, exposure to household pesticides in the southern United States is higher than in the North because of the greater levels of pests in the South. The risk of deleterious health effects from exposure to pests

may outweigh the risk from exposure to pesticides. Better communication could have resolved both of these issues; the study is an important one and should proceed.

Dr. Knaak commented that in his field of pesticide exposure caused by percutaneous absorption, there is little available data on compounds and a dearth of information for developing pesticide PBPK models for exposure. What is needed especially is transfer data for children such as information on the transfer of pesticides from hand to mouth, through the skin, and measurements of what is excreted.

Dr. Bruckner commented that he served as a member of the National Research Council (NRC) community that examined the scientific and ethical questions of an intentional dosing study. This group decided that these studies could be performed in adults if the studies were deemed worthwhile. For children, studies on incidental exposure were approved. Dr. Bruckner asked how EPA intends to use recommendations from these groups and data from exposure studies. Dr. Gilman answered that decisionmaking takes place at Scientific Policy Council meetings, and at the next meeting (November 15), the Council will be signing off on an interim policy acknowledging NRC recommendations and also recommendations concerning how to proceed on a longer term policy, which also will concern oversight of human experiments.

Dr. Gilman was asked if there were any upcoming initiatives for pollution prevention and/or transport. Dr. Gilman answered that for pollution prevention, there were significant fluctuations within the research office recently. The Office of Management and Budget evaluates these programs, and a previous evaluation found that the pollution prevention programs were not achieving results. The intramural programs also are being reinvented. A group at NCER has been asked how better scientific and technical support can be provided to achieve sustainability. Efforts are underway to try to better organize efforts in pollution prevention, green chemistry, and other topics in the category of science and technology for sustainability to better define and achieve agency goals. RFAs have been written for research efforts to tackle large problems (transportation or water related, etc.) and EPA wants to provide funding that focuses on the use of science and technology to identify goals, measure progress, and disseminate practices that come out of these efforts. These programs seek to establish a connection between real-world problems and the development of tools and approaches to the science. This is not strictly an applied research program, but it does try to encourage investigators to focus their research efforts more closely on real-world problems.

Dr. Blumberg commented that this meeting was very valuable for obtaining the perspective of researchers with a different focus and has helped bring new and different insights. Meeting EPA scientists also helps researchers at universities to understand what EPA wants and needs.

Dr. Gilman commented that currently, there is much discussion within the Federal Government, including the National Institutes of Health, concerning how well the government promotes interdisciplinary research. He asked whether the work EPA encourages becomes somewhat more interdisciplinary than usual when it is driven by applied needs. A participant responded that this does not change actual research, but encourages thinking about new directions. Interdisciplinary research arises from a meeting like this one because it allows researchers with access to different resources (i.e., clinical samples) to share resources; these meetings provide not only intellectual stimulation but also practical access.

SESSION IV: CHEMICAL MIXTURES

Session Overview
Richard Hertzberg, U.S. EPA

The most recent laws arising from research on chemical mixtures include the Food Quality Protection Act and Safe Drinking Water Act amendments, which specifically mention chemical mixtures. Initial laws of this sort included the Comprehensive Environmental Response, Compensation, and Liability Act in1980, which provided for the federal Superfund program and was among the first laws to address both mixtures of single chemicals coming together at a Superfund site and intentional commercial mixtures. The Toxic Substances

Control Act of 1976 gave EPA the ability to track and screen industrial chemicals and allows EPA to require reporting or testing of chemicals that may pose an unreasonable risk to the environment or human health. These laws, however, did not define unreasonable risk, chemical mixture, or exposed population.

More guidance came in 1986 during EPA's first foray into areas of mixtures risk assessment, which led to the development of Guidelines for Health Risk Assessment of Chemical Mixtures. A mixture was defined as a group of chemicals that, regardless of spatial or temporal proximity, jointly contribute to health risk. Exposure to multiple chemicals does not need to occur at the same time, which is important for consideration of the initiator/promoter concept for cancer risk.

Besides the STAR Grants program, EPA works with the U.S. Forest Service and the Department of Energy (DOE) on chemical mixtures research. DOE sites often involve large exposures to complex mixtures. Together with these agencies, EPA researchers have examined dose response combinations and developed mathematical models, and developed artificial processes to model degradation of chemicals in soil. Legal issues concerning responsibility for cleanup and issues impacting risk assessment arise when toxic components at a site are different than the parent compounds. Because it is impossible to test all possible chemical mixtures, researchers must understand the basic science pertaining to mixtures, including key processes and mechanisms of mixtures and the magnitude of changes in response. More information also is needed on the transformation, transport, and toxicity of mixtures to allow for more precise risk assessment.

PAH/Metal Mixtures—Human In Vitro Mutagenicity Studies
Laurence Kaminsky, New York State Department of Health

Polycyclic aromatic hydrocarbons (PAH) and heavy metals often are found together in the environment, and heavy metals may affect PAH carcinogenicity. The heavy metal arsenite affects bioactivation of the PAH benzo[k]fluoranthene (BKF). Bioactivation of BKF, essential for its carcinogenic activity, requires the cytochrome P450 enzyme, CYP1A1, which catalyzes the metabolic activation of PAH to mutagenic and carcinogenic derivatives. BKF also induces CYP1A1, thus enhancing its own metabolism and production of bioactivated forms. The human liver cancer cell line, HepG2, was used to determine if bioactivation of BKF by CYP1A1 was affected by arsenite. Induction of CYP1A1 mRNA by BKF was sustained for between 40 and 50 hours and was decreased by arsenite along with the levels of CYP1A1 protein.

To determine the mechanism by which arsenite inhibits BKF-induced transcription of CYP1A1, promoter assays were performed to identify transcriptional control elements. The CYP1A1 promoter has xenobiotic response elements wherein CYP1A1-inducing agents bind through the xenobiotic receptor. Removal of the xenobiotic response elements resulted in a construct within a 1.7-fold higher responsiveness than the intact promoter, suggesting a negative response element had been removed. Arsenite still decreased expression of this reporter construct, indicating that arsenite does not affect BKF induction directly through these xenobiotic response element sites. Arsenite also did not affect the stability of CYP1A1 mRNA. A synthetic construct containing only the xenobiotic response element was tested and responded to BKF; arsenite, however, had no effect on this reporter construct, indicating that arsenite inhibition of BKF induction does not involve the xenobiotic response element. Heme oxygenase also is induced by arsenite in HepG2 cells and increased heme oxygenase levels could be responsible for degradation of CYPA1 via the CYP1A1 heme moiety. RNA interference was used to knock down heme oxygenase levels, which resulted in the decreased ability of arsenite to reduce CYP1A1 levels. This work indicates that heme oxygenase has a role in arsenite-mediated inhibition of CYP1A1 activity.

Discussion

Dr. Kaminsky was asked if any other metals induce heme oxygenase. He responded that other metals induce this enzyme to varying degrees. Dr. Kaminsky was asked if other constitutively expressed cytochrome P450 enzymes are diminished by arsenite. He answered that 1A2 and 1B1 appear to be affected by arsenite.

Regulation of Embryonic Neuronal Development by Chemical Mixtures From Brick, New Jersey
Carol Reinisch, Marine Biological Laboratory

High levels of PCBs are found in municipal drinking water in Brick, New Jersey, a town that also has a high prevalence of autism. The objective of this project is to examine the effects of mixtures of bromoform, chloroform, and tetrachloroethylene (PCE) on the neuronal growth of clams during embryonic development. When clam embryos are exposed to PCBs, numbers of neurons in the embryos decrease, and the rate of neurogenesis slows, an effect specific to PCBs. *Spisula solidissima*, the surf clam, presents a useful model system because the early cleavage stages of *Spisula* are easily observable and synchronous within a population. Staining with an antibody to the protein kinase A regulatory subunit, RII, showed that exposure of clams to PCE resulted in increased expression of RII in the developing embryo. Members of the p53 tumor suppressor gene family also were analyzed because PCBs are known to induce mutations in p53. Adult ganglia and neurons in clams were analyzed and the p53 family members p73, p97, and p120 were detected by immunoblotting. Expression of p73 is essential for neurogenesis; two variants of p73 were detected in clam embryos and adult clams by reverse transcription polymerase chain reaction (PCR). These unique variants of p73 in *Spisula* suggest multiple control points in gene expression during development. Expression of the p73 gene was analyzed in the developing *Spisula* embryo, and p73 mRNA has been found in the highly innervated area of the developing adductor muscle. Future experiments will assess the effect of the chemical mixture on expression of this gene at this site. Individual neurons also will be used for single-cell PCR to analyze regulation of p73.

Spisula results imply that chronic exposure to the PCE contaminants could alter normal neuronal development during embryogenesis. These studies have been extended to the vertebrate species *Danio rerio* (zebrafish). Zebrafish embryos treated with the PCE mixture show increased RII mRNA expression in the region of the developing notochord. Western blot analysis shows an increase in RII protein upon exposure of the embryos to PCE. Therefore, PCE specifically targets a neural pathway by causing a localized increase in RII production during development of the zebrafish embryo. This confirms the *Spisula* results and shows that environmental contamination affects neural development pathways in a vertebrate species. Future studies include the development of zebrafish-specific RII probes for use in quantitative PCR to identify molecular mechanisms that regulate the effects of chemical mixtures on this pathway. Additionally, p73 RNA probes will be used in *Spisula* neuronal cell cultures to determine the developmental expression pattern of p73 in *Spisula* and whether and how the chemicals present in Brick, New Jersey, drinking water affect p73 expression.

Discussion

Dr. Reinisch was asked if p73 levels were controlled at the level of protein stability rather than in message, as is true for p53. She answered that in their model, they examined extensively p53 protein levels and did not see a change in protein, only in message, but that they have not yet examined protein stability for p73. She also commented that splice variants of p73 were currently under investigation in the laboratory.

Comparative In Vitro *Immunotoxicity of Organochlorine Mixtures*
Sylvain De Guise, University of Connecticut

This project examines the effects of organochlorines (OCs) on various measures of immune system function. OCs bioaccumulate, are stable in the environment, are resistant to degradation, and mixtures have varying toxicities. In laboratory animals, OCs cause decreases in numbers of natural killer (NK) cells and T-cells and reduce T-cell cytotoxicity, antibody production, and B-cell differentiation. These chemicals also are associated with increased sensitivity to endotoxins, bacteria, and viruses. Measures of immune system function were analyzed in a wide variety of marine mammals that had been exposed to OCs. Data also were gathered to determine whether top predators such as marine mammals and humans are equally sensitive to the effects of OCs. OCs present at environmentally relevant concentrations were tested. Functional in vitro assays included mitogen-induced lymphocyte proliferation (B- and T-cells), immunophenotyping, respiratory burst, NK cell activity, and phagocytosis. These assays were performed for a wide range of marine mammals and also for humans and mice (mice were used for quality control because data for exposure of mice to these chemicals are avail-

able). Differences in these measures across species were very apparent. The mice were not predictive of the effects of OCs in humans or marine mammals. Dose response experiments comparing the effects of various PCBs on dolphin neutrophil phagocytosis to human monocytes phagocytosis showed differences in sensitivity to these chemicals. The data also were analyzed to determine if phylogeny could predict toxicity. Gene array analysis showed that phylogeny does not predict immunotoxicity across species, and in fact, the sea otter showed a more similar response to that of humans than did mice.

Work designed to predict the effects of OC mixtures on mice, killer whales, Steller sea lions, and Commerson's dolphin also was presented. The measured effects of OC mixtures on T-cell proliferation in mice and killer whales was close to those predicted. A negative correlation between predicted and measured effects was observed for Steller sea lions and Commerson's dolphins. PCB 153 and 2,3,7,8-tetrachlorodibenzo-p-dioxin affected calcium metabolism in phagocytosis in dolphins but not in the mouse model. This work shows that chemical mixtures have complex effects on measures of immunotoxicity, that top predators are not equally sensitive to the effects of OCs, and that the mouse may not be an appropriate model system for extrapolating the health risks of these compounds to humans.

Discussion

Dr. De Guise was asked whether previous exposure in vivo affected in vitro assays. He replied that he was unable to answer this question because he did not have any human or marine mammals that did not have previous exposure to OCs. A participant asked what the combined exposure was, accounting for the additive approach, from the chemical mixtures. Dr. De Guise responded that they had used a 1 + 1 approach with equivalent ppm of the different chemicals, rather than maintaining similar total concentrations. Dr. De Guise was asked if injections of OCs were compared to drinking water. He answered that adding OCs to cell culture mimics exposure at the cellular level. A participant asked if the OCs used in tissue culture are the same as metabolized OCs. Dr. De Guise responded that OCs are stable and there is very little metabolism of them.

A participant asked whether new chemicals have been studied for effects such as those described in this presentation. Dr. De Guise commented that Triclosan is a new halogenated compound that should be studied along with TTC, which is an antibiotic present at concentrations of up to 5 percent in soap. Breakdown compounds from these two chemicals are similar to those presented here.

Mechanistic Evaluation of the Toxicity of Chemical Mixtures
Allen Olmstead, North Carolina State University

Nine chemicals were chosen based on a report on surface water concentrations of a variety of xenobiotics to develop a model to predict the toxicity of chemical mixtures. Concentration-response curves for endpoints of lifespan, growth rate, and fecundity were generated for each chemical using *Daphnia magna*. The ratio of the chemicals within the mixture was maintained at the ratio reported for median detectable environmental levels. Data were compared across experiments and chemicals. The model should describe effects for any chemical and any endpoint with two different parameters. For this analysis, the chemicals are assumed to have independent effects on endpoints that contribute to the model for prediction of effects of the mixture. The model predicting mixture effects was confirmed by data for growth and fecundity. For the mixture used in this research, a mixture model combining the concepts of concentration addition and independent joint action predicted toxicity accurately. Experimental results demonstrated that for these nine chemicals, toxicity was not influenced significantly by interactions between the chemicals, but instead was dominated by a single chemical. Chlorpyrifos was the most toxic of all the chemicals and drove the mixture model to yield prediction. Leaving chlorpyrifos out of the analysis did not affect results significantly. A mixture composed of the nine chemicals at the median detected levels would pose no margin of safety for daphnids.

Discussion

Dr. Olmstead was asked how sublethal effects could be worked into the model; for example, if a chemical is toxic to the liver but not the kidney, is it possible to add on toxicity with different endpoints? Dr. Olmstead responded that this model only accounted for gross endpoints, not organ-specific toxicity. A participant commented that if the mathematical distribution of the most sensitive endpoints that cause death is equivalent across populations, the model works well. If there are subpopulations that are more or less sensitive to a given chemical, the model breaks down. He asked if the combined effects of a mixture of toxins could be assessed if the toxins have different targets. Dr. Olmstead answered that this model would require each organ to be treated separately, which is problematic because it is not possible to exclude the affected organs that cause death. Currently, he is working on adding interaction functions to the model.

Session Wrap-Up
Richard Hertzberg, U.S. EPA

The presentations from this session illustrate the difficulties inherent in calculating risk from chemical mixtures. The Superfund sites originally sparked interest in risk assessment for mixtures and showed how complicated this type of risk assessment can be, particularly when the mixture itself is not reproducible. Risk assessment for Superfund sites involved an additivity approach based on components present at the sites. Risk addition is used primarily for cancer because EPA has developed risk probabilities for cancers but not for non-cancer effects. The hazard index uses a counterpart to the additivity approach, the dose addition concept. This approach uses information on interactions (primarily binary interactions) to increase or decrease the hazard index based on the direction of interaction and the extent of its importance. This approach used a constant duration (70 years) for a "synthetic" 70-kg asexual human and examined one critical effect, one exposure route, and one chemical. Decisions on safety or remediation were made by moving down a dose response curve until potential harm was insignificant. When risk assessment must include a mixture of even two chemicals, an infinite number of combinations of safe values becomes possible. Mixtures imply interactions, which can affect exposure. Exposure, in turn, can affect toxicity; toxicity affects uptake or excretion, which further affects toxicity. Areas of research that will influence mixture risk assessment include epidemiology, toxicology, cellular responses (including genomics and proteomics), and biomarker issues. New tools for statistical analysis also will be needed because current tools may not be sufficient for analyzing risk from mixtures.

EPA also encourages research in cumulative risk assessment, which differs from mixture risk assessment because it encompasses stakeholder involvement and includes not only chemicals, but other environmental stressors. For example, exposure to excess heat or humidity may influence risk, as could dietary or nutritional status, interactions with particulates, and access to health care. It may be difficult to tell if EPA regulations have an effect on human health because vulnerable populations may have access to good health care, preventing detection of serious deleterious health effects from environmental contaminants. Conversely, less healthy populations, perhaps with inadequate access to health care, may be vulnerable to lower levels of contaminants than healthier populations.

CLOSING REMARKS
Kacee Deener, U.S. EPA

Kacee Deener thanked attendees for their participation and adjourned the meeting.

Biomarkers

Presentation Abstract

Biomarkers of Prenatal Exposure to Nonpersistent Pesticides

R.M. Whyatt[1], D.E. Camann[2], Y. Cosme[1], M. Borjas[1], L. Cruz[1], and D.B. Barr[3]
[1]Columbia Center for Children's Environmental Health, Columbia University, New York, NY; [2]Southwest Research Institute, San Antonio, TX; [3]Centers for Disease Control and Prevention, Atlanta, GA

Research is ongoing to validate a battery of biological markers of prenatal exposure to nonpersistent pesticides (NPP). Biomarker validation is needed to facilitate evaluation of health effects of NPP exposures during pregnancy. The cohort consisted of 100 African American mothers and newborns from New York City. Our prior research has shown widespread pesticide exposure during pregnancy among this minority cohort. NPP levels in 2-week integrated indoor air samples, collected continuously from the 32nd week gestation until delivery, provided the gold standard for biomarker validation. Biomarkers included NPP levels in: (1) biweekly spot maternal urine samples collected over the final 2 months of pregnancy; (2) maternal and umbilical cord blood samples; and (3) postpartum meconium. A personal air sample also was collected from the mother over 48 hours during the third trimester. Enrollment was conducted between February 2001 and April 2004 and was restricted to pregnant, nonsmoking women; 61 percent reported using some form of pest control during the third trimester, and 27 percent reported using higher toxicity methods (can spray, sprays by exterminator, or pest bombs). Analyses of 10 insecticides in 288 2-week integrated indoor air samples collected from 81 homes showed that three were detected in 99-100 percent of samples. These were the organophosphates, chloryprifos and diazinon, and the carbamate propoxur. Little within-home variability was seen in indoor air concentrations over the 2 months and the correlations between each insecticide in each of the 2-week air samples were highly significant (all r-values ≥ 0.7, $p < 0.001$, Spearman's rank). Indoor air levels of these insecticides also were highly correlated with maternal personal air levels. Preliminary results on biomarker analyses show a weak, but significant, correlation between average indoor air levels of chlorpyrifos and diazinon and levels of their respective chemical-specific metabolites in biweekly maternal urine samples (adjusted for creatinine, $r = 0.4$, $p < 0.05$). The chemical-specific metabolite of propoxur was not detected in maternal urine samples. Levels of diazinon and chlorpyrifos in blood samples collected from the mothers and newborns at delivery were highly correlated ($r = 0.9$, $p < 0.001$, $n = 28$). No association was seen, however, between these insecticides in blood (maternal or newborn) and levels in indoor air samples. Diethylphosphate (a metabolite common to both diazinon and chlorpyrifos) was detected in 20/33 (61%) of meconium samples and 3,5,6-trichloro-2-pyridinol (a metabolite specific to chlorpyrifos) was detected in 22/32 (69%) of the samples. Meconium metabolite levels were not associated with corresponding levels of the insecticide in indoor air.

Presentation Abstract

Biomarkers and Neurobehavioral Effects of Perinatal Exposure to Chlorpyrifos and Other Organophosphate Insecticides

Jay Wilkins[1], K. Dietrich[2], M. Nishioka[3], C. Weghorst[1], M. Moeschberger[1], and K. Koechlin[1]
[1]Ohio State University, Columbus, OH; [2]University of Cincinnati, Cincinnati, OH;
[3]Battelle Memorial Institute, Columbus, OH

Project Goals and Objectives:

This research project will evaluate the putative relationship between perinatal exposure to chlorpyrifos (CP) and other organophosphate (OP) insecticides and adverse neurobehavioral effects among infants and young children by employing biomarkers of exposure and susceptibility in a longitudinal design. These compounds have been demonstrated to be neurodevelopmental toxicants in animal studies.

Approach:

Cohort ascertainment involves recruitment of 176 women in their second trimester of a low-risk pregnancy into the longitudinal study, which is designed to follow only healthy full-term newborns until 2 years of age. During each pregnancy, the following data are obtained: (1) maternal exposure to CP and other OPs; (2) maternal exposure to other neurodevelopmental toxicants likely to be factors in the target populations (e.g., lead [Pb]); (3) maternal demographics and other potentially confounding family-based factors (e.g., socioeconomic status); and (4) relevant clinical information pertaining to the pregnancy and birth event (e.g., activity, pulse, grimace, appearance, and respiration scores).

Maternal exposures to the OPs of interest are assessed by analyses of urine samples that are obtained from the mother prior to delivery. Because vulnerability to the adverse effects of neurodevelopmental toxicants begins shortly after conception, blood obtained from the expectant mothers is used to determine the mother's paraoxonase (PON1) genotype, a biomarker of susceptibility to OP toxicity. After delivery, relevant data are obtained from the mother-child dyads at 3, 12, and 24 months postnatally. At 3 months, neurobehavioral data are obtained on the infant by administration of the Bayley Scales of Infant Development (BSID-II). At 12 months, control data on potential confounders will be obtained in addition to data on breastfeeding and maternal IQ. At 24 months, the primary neurobehavioral data will be obtained by a repeat administration of the BSID-II, in addition to Ireton's Child Development Inventory.

At regular intervals throughout the postnatal followup period, urine samples are collected from the infants and analyzed for dialkylphosphates, 3,5,6-trichloro-2-pyridinol, the urinary metabolite specific for CP, and 2-isopropyl-6-methyl-4-pyrimidinol, the urinary metabolite specific for diazinon. In addition, two postnatal blood samples will be obtained from the child (one at 12 months and one at 24 months). Blood Pb and the infant's PON1 genotype will be determined.

Preliminary Findings:

We are still in the process of recruiting because recruitment of pregnant women into this study has proved to be much more challenging than was anticipated initially. A total of 100 subjects have been recruited to date, but because of fetal complications and dropouts, 84 currently are enrolled actively.

Next Steps:

We will continue to recruit pregnant women and analyze blood and urine specimens obtained thus far in the study.

Presentation Abstract

Analysis of Genotoxic Biomarkers in Children Associated With a Pediatric Cancer Cluster and Exposure to Two Superfund Sites

Barry A. Finette
University of Vermont, College of Medicine, Burlington, VT

Goals and Objectives:

The objective of this research project is to evaluate the utility of specific biomarkers of effect and susceptibility for studying cancer risk in a pediatric population after genotoxic exposures.

Approach:

We will measure biomarkers of effect and susceptibility in exposed siblings of subjects in a Centers for Disease Control and Prevention-defined pediatric cancer cluster linked to transplacental and maternal exposure to contaminated groundwater from two U.S. Environmental Protection Agency-designated Superfund sites in Dover Township, NJ. Biomarkers of effect (chromosomal aberrations and hypoxanthine guanine phosphoribosyl transferase [*HPRT*] mutations) in the exposed siblings will be compared to measurements in unexposed subjects. Biomarkers of susceptibility (DNA polymorphisms for 11 carcinogen metabolizing enzymes) in the exposed siblings and unexposed subjects will be compared to subjects with cancer to determine if the latter have a higher prevalence of specific metabolic genotypes. In addition, the relationships between biomarkers of effect and susceptibility in exposed siblings and unexposed children will be examined to see if the effects of exposure are modified by any of these metabolic polymorphisms. Exposures in all subjects will be evaluated from their residential and personal histories using a computer model developed by the Agency for Toxic Substances and Disease Registry (ATSDR) to estimate exposure to different water sources over time.

Preliminary Findings:

We have completed *HPRT* mutant frequency (MF) analysis in exposed siblings of children who developed cancer and age/gender-matched unexposed children. Our results demonstrated that MFs were not different in these two groups (see Figure 1). This does not preclude the possibility that significant genotoxic differences exist, as reflected in a change in the mutational spectrum. To date, we have completed the mutational analysis on more than 150 *HPRT* T-cell isolates from 30 of the 92 individuals. Because the analysis is blind, we will not be able to interpret the results until all have been completed and the identification numbers are decoded. We have developed the techniques to generate 500-800 metaphase spreads from cryopreserved mononuclear cell samples for fluorescence *in situ* hybridization (FISH) analysis. Our FISH analysis will incorporate a two-dye, six-chromosome approach in which two individuals will study 2,000 metaphases in a double-blind approach. To date, metaphase spreads have been generated for more than 90 subjects. We have begun the recruitment of blood samples for polymorphism studies from the affected siblings (children with cancer) of subjects previously studied. We also have been studying the ATSDR Historical Reconstruction of the Water-Distribution System Serving the Dover Township Area in New Jersey for estimating exposures to differing water sources based on subjects' residential history.

Significance:

If the biomarkers tested are shown to reflect differences in genetic effects between the exposed and unexposed children and children with cancer, they will be valuable tools for assessing cancer risk in potentially exposed pediatric populations before increases in cancer incidence are evident.

Future Directions:

We will continue our blind mutational spectra analysis and chromosomal aberration and analysis from each subject. In addition, we will complete our sample acquisition of peripheral blood samples and begin quality testing for our polymorphism studies. We then will begin our studies using the ATSDR EPANET 2.0 Water-Distribution Model to estimate each subject's lifetime exposure to the various Dover Township water sources.

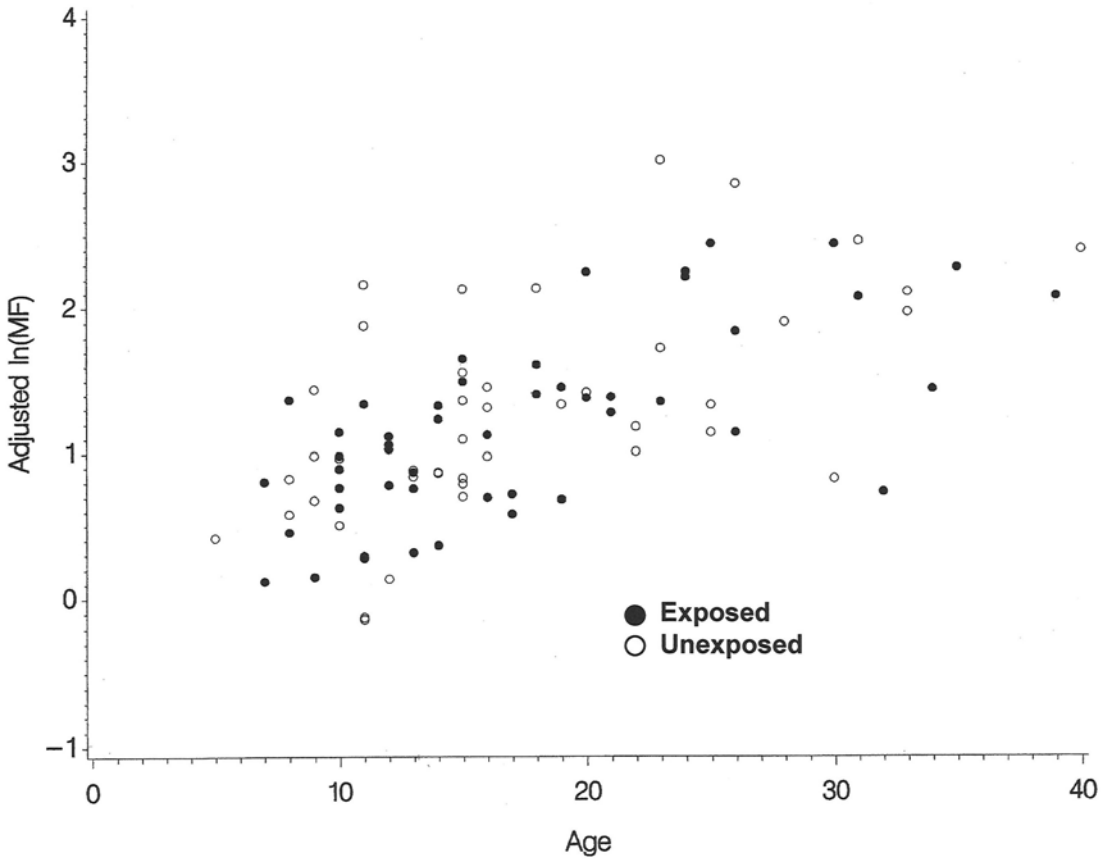


Figure 1. The relationship between MF and age in exposed siblings of children with cancer who are from a population with an elevated incidence of childhood cancer and unexposed children from neighboring communities with no increase in cancer incidence.

Presentation Abstract

Development of a Physiologically Based Pharmacokinetic and Pharmacodynamic (PBPK/PD) Model To Quantitate Biomarkers of Exposure to Organophosphorus Insecticides

Charles Timchalk and Torka S. Poet
Battelle Memorial Institute, Pacific Northwest Division, Richland, WA

This project entails development and validation of a physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model for the organophosphorus insecticide chlorpyrifos to quantitate biomarkers of dosimetry and cholinesterase (ChE) inhibition in young rats and children (see Figure 1). It is hypothesized that an age-dependent decrement in chlorpyrifos metabolism correlates with the increased sensitivity of young animals, and potentially children, to organophosphate insecticides. The experimental approach involved developing algorithms to calculate age-dependent physiological and metabolic parameters and applying them to a PBPK/PD model to adequately describe the blood and tissue time-course of chlorpyrifos and the metabolites chlorpyrifos-oxon and trichloropyridinol. Once fully developed, the model will be used to quantitate biomarkers of exposure and response (ChE inhibition) during neonatal/juvenile development (young rats and children). Coupled with model development, relevant *in vivo* and *in vitro* experiments needed to refine model parameters, validate model response, and assess the feasibility of utilizing saliva as a biomonitoring matrix for dosimetry and esterase inhibition have been conducted. Studies have focused on the development of analytical methods, acquisition of *in vivo* and *in vitro* data, and the further refinement of the PBPK/PD model. Initial analytical methods were developed for the quantitation of chlorpyrifos and major metabolites. These methods have been used to support both *in vitro* and *in vivo* experiments. *In vitro* studies were conducted to evaluate the roles that intestinal and hepatic metabolism may play in both the activation and detoxification of chlorpyrifos, and the parameter estimates obtained from these studies are being used to further refine the PBPK/PD model. Likewise, to evaluate the potential utility of saliva for biomonitoring, studies were undertaken to characterize the total salivary ChE activity and estimate the kinetic parameters of *in vitro* and *in vivo* interaction of chlorpyrifos-oxon with rat salivary ChE. These results suggest that saliva may be a useful biological matrix for monitoring chlorpyrifos exposure and response, either through measuring the metabolite levels or the degree of ChE inhibition. These data have been used for further validation of the PBPK/PD model for chlorpyrifos. The PBPK/PD model has been modified to scale allometrically (based on body weight) the age-dependent development of metabolism enzymes, and ChE enzyme activity and simulations were compared against available data. The model suggests that even though neonatal rats have lower metabolic capacity, it is adequate to detoxify chlorpyrifos at relevant environmental exposure levels. These simulations are consistent with differences in the acute toxicity response noted between neonatal and adult rats. To assess the impact of variability associated with the human chlorpyrifos-oxonase (PON1) polymorphisms in adults on the theoretical concentration of chlorpyrifos-oxon in the human brain, a Monte Carlo analysis was conducted. The results suggested that the PON1 polymorphism had the greatest impact on target tissue dosimetry at dose levels that overwhelmed other detoxification pathways.

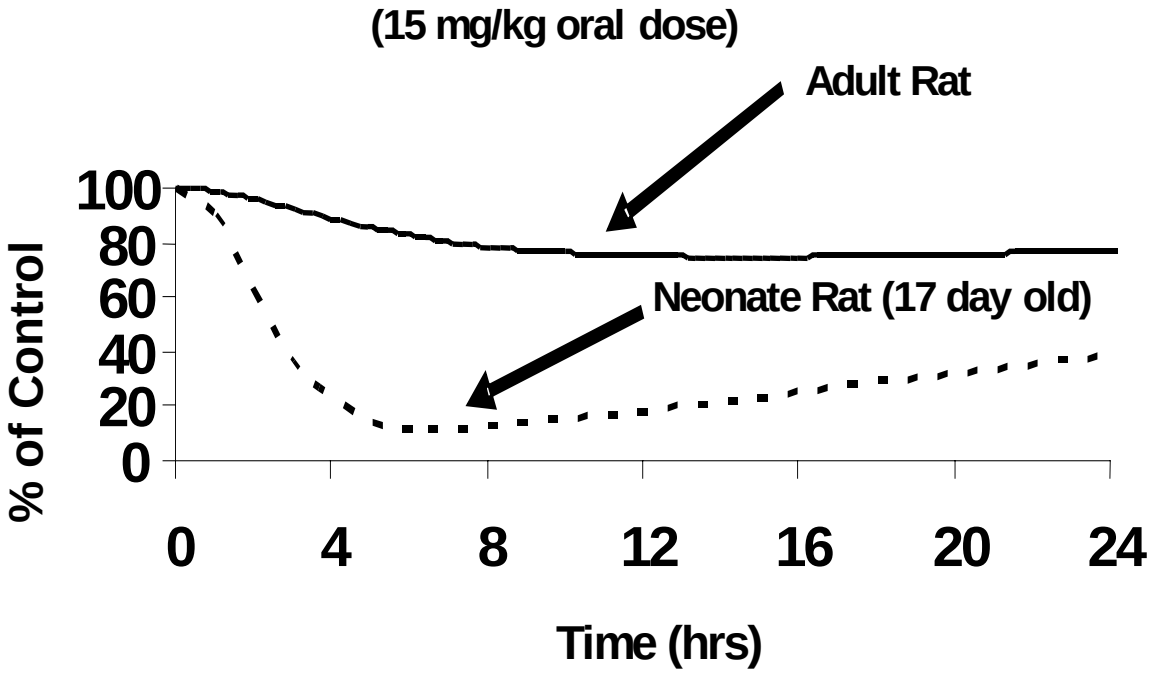


Figure 1. Simulation of brain acetylcholinesterase inhibition.

Presentation Abstract

Biomarkers of Human Exposure to Pesticides Utilizing a New PBPK/PD Model and Kinetic Data on Pesticide Metabolism in Humans

James R. Olson and James B. Knaak
State University of New York at Buffalo, Buffalo, NY

The primary objective of the proposed studies is to obtain kinetic parameters (V_{max}, K_m values) for the metabolism of model pesticides (parathion and chlorpyrifos) in the livers from humans of various ages. Age- and gender-specific kinetic parameters (V_{max}, K_m) for selected pesticides will be utilized in a multiroute, multi-chemical, physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) model (ERDEM, Exposure Related Dose Estimating Model)[1,2] to estimate the sensitivity of individuals within each age group based on biomarkers of susceptibility (i.e., paraoxonase genotype and CYP2D6 phenotypes), exposure (i.e., urinary metabolites), and effects (i.e., blood acetylcholinesterase [AChE]; AChE/butyrylcholinesterase inhibition). Results also will be examined and validated using available data from human monitoring studies. Although this work focuses on organophosphates as model compounds, the differences in the ontogeny of enzyme activity can have wide applicability to other drugs and chemicals.

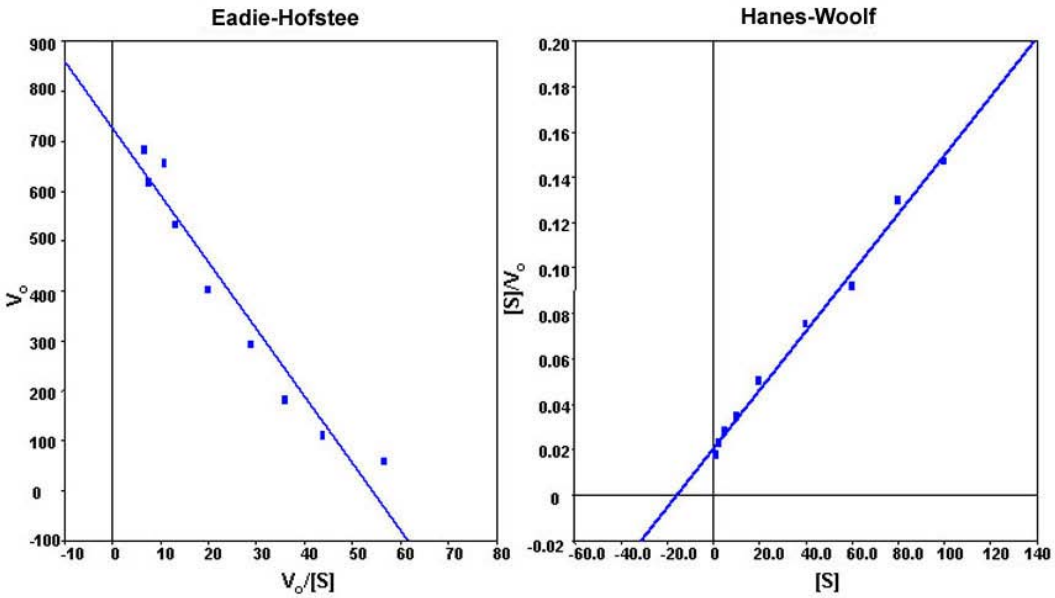
Specific aims are to: (1) characterize the levels of specific cytochrome P450s, paraoxonase genotype, uridine diphosphate-glucuronosyl-transferases, and sulfotransferase activity in the cytosol of liver specimens from humans of five age groups (0-2, 3-10, 11-20, 21-40, and > 40 years); (2) measure the kinetics (K_m, V_{max}) for the metabolism of parathion and chlorpyrifos in hepatic microsomal fractions from humans of five age groups; (3) utilize selected experimental exposure levels (i.e., 0.5 to 5.0 mg/kg of body weight) and age- and gender-specific V_{max} and K_m values for parathion and chlorpyrifos in the ERDEM to determine the sensitivity of individuals within each age group to these pesticides based on biomarkers of susceptibility, exposure, and effects; and (4) utilize human environmental exposure levels in the ERDEM to estimate biomarkers of exposure and effects. Finally, the ERDEM will be validated by comparing model biomarker estimates to monitored values (i.e., values from human monitoring studies).

Preliminary studies initially focused on measuring the kinetics (K_m, V_{max}) for the metabolism of parathion in characterized human liver microsomal specimens and recombinant human cytochrome P450 (CYPs). A sensitive and specific high performance liquid chromatography method was developed to quantify the products of biotransformation. Figure 1 illustrates the kinetic analysis of paraoxon formation from parathion (1 to 100 µM) in a characterized pool of human liver microsomes. The table within Figure 1 summarizes the kinetic parameters for the formation of paraoxon and p-nitrophenol, because the kinetic values for both products are needed in the PBPK/PD model. The kinetics of parathion metabolism also were assessed in six specimens of characterized human liver microsomes from males and females of 19 to 54 years of age. The K_m for the formation of paraoxon and p-nitrophenol ranged from 8.7 to 39.1 and from 15.2 to 55.9 µM, respectively, whereas the V_{max} values ranged from 589 to 1,495 and from 588 to 1,232 pmol/min/mg, respectively. These results support the need to include a range of kinetic parameters to reflect the inherent interindividual variability in biotransformation. The V_{max} for parathion to paraoxon formation from six characterized human liver microsomal specimens correlates with the formation of 6-beta hydroxytestosterone from testosterone, a marker for CYP3A4 activity ($r^2 = 0.96$). Studies with recombinant CYPs also indicate that CYP1A2, CYP2B6, and CYP2C19, with lower K_ms, may contribute substantially to parathion metabolism at low level human exposures. Current studies are assessing the hepatic microsomal and CYP-specific metabolism of chlorpyrifos. Estimates of the age-specific hepatic CYP content and the CYP-specific data on the kinetics of biotransformation will be utilized in the PBPK/PD and ERDEM models to improve risk assessment for these and other prototypical pesticides.

References:

1. Blancato, J.N., J.B. Knaak, F. Power, and C.C. Cary. Use of PBPK models for assessing absorbed dose and ChE inhibition from aggregate exposure of infants and children to organophosphorus insecticides. Abstract 3F-090, 10th Annual Conference of the International Society of Exposure Analysis, Asilomar Conference Center, Monterey, CA, October 24-27, 2000.

2. Knaak, J.B., C.C. Dary, F. Power, C.B. Thompson, and J.N. Blancato. Physiochemical and biological data for the development of predictive organophosphorus pesticide QSARs and PBPK/PD models for human risk assessment. *Critical Reviews in Toxicology* 2004;34(2):143-207.



Km, uM, Metabolism of Parathion to Paraoxon		17.67
Vmax, pmol/min/mg		790.35
Conversion to Hrs., 60		60
Vmax, pmol/hr/mg of Microsomal Protein		47421
Vmax, pmol/hr/g of Liver Protein		1422630
Vmax, pmol/hr/g of Body Weight		38411010
Vmax, nmol/hr/g of Body Weight		38411.01
Vmax, umol/hr/g of Body Weight		38.41
Vmax/Km		2.17
Km, uM, Conversion of Parathion to PNP		25.11
Vmax, pmol/min/mg		588
Conversion to Hrs., 60		60
Vmax, pmol/min/mg of Microsomal Protein		35280
Vmax, pmol/hr/g of Liver Protein		1058400
Vmax, pmol/hr/g of Body Weight		28576800
Vmax, nmol/hr/kg of Body Weight		28576.8
Vmax, umol/hr/kg of Body Weight		28.58
Vmax/Km		1.14

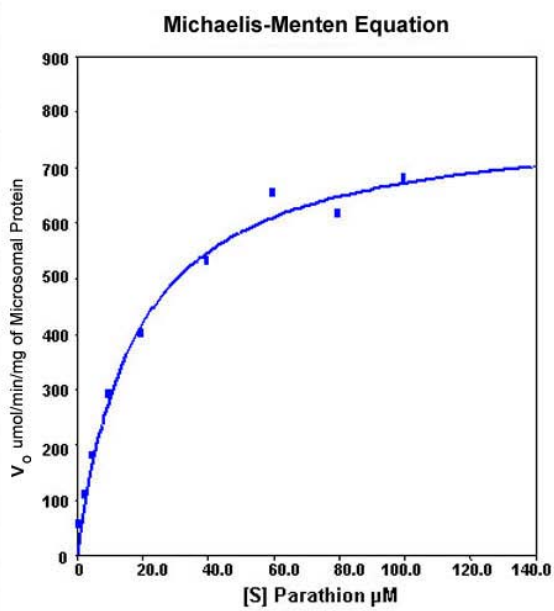


Figure 1. Paraoxon formation from parathion in pooled sample of human liver microsomes.

Presentation Abstract

Species-Specific Xenobiotic Metabolism Mediated by the Steroid and Xenobiotic Receptor, SXR

Bruce Blumberg
University of California at Irvine, Irvine, CA

Project Goals and Objectives:

The overall aim of this project is to provide a molecular basis for understanding the commonalities and differences in how humans and model animals respond to chemical exposure. We hypothesized that activation of the nuclear steroid and xenobiotic receptor/pregnane X receptor (SXR/PXR), and the consequent effects on metabolism are the major mechanisms underlying the differential susceptibility of humans and laboratory animals to environmental chemicals. The specific objectives of this research project are to: (1) characterize the commonalities and differences in the response of human and rodent SXR/PXR; (2) identify functional differences in the activation and/or regulation of SXR/PXR among humans and between commonly used strains of laboratory mice; (3) determine whether the compounds are metabolized *in vivo*; and (4) identify target genes regulated by SXR/PXR as a response to environmental chemical exposure.

Approach:

SXR homologs will be identified from a variety of model animals and the effects of a panel of xenobiotic compounds will be tested for their ability to activate the receptor. These data will be used to develop Quantitative Structure-Activity Relationship Models to predict the ability of compounds to modulate SXR/PXR, and therefore, metabolism. The genetic variation that exists in the human population at the SXR/PXR locus will be characterized, and mutations that affect the activity of the protein or its promoter will be identified. A similar analysis will identify differences in common laboratory mouse strains. This knowledge will be important for understanding the often widely different responses to the same compound observed by different laboratories. A primary hepatocyte model system will be developed from wild-type mice, mice deficient in SXR/PXR, and so-called "humanized mice" that express the human receptor in the livers of mice deficient in the mouse receptor. This will enable us to test directly both the potential for metabolism and whether a compound is, in fact, metabolized *in vitro*. This testing will be extended to the animals from which the cell system originates to determine whether the route of exposure affects metabolism. Last, target genes will be identified that are regulated by SXR/PXR in response to chemical exposure.

Findings:

Polychlorinated biphenyls (PCBs) are a family of persistent organic contaminants suspected to cause adverse effects in wildlife and humans. In rodents, PCBs bind to the aryl hydrocarbon (AhR) and PXR, inducing the expression of catabolic cytochrome P450 enzymes of the CYP1A and 3A families. We found that certain highly chlorinated PCBs are potent activators of rodent PXR but antagonize its human ortholog, SXR, inhibiting target gene induction (see Figure 1). Thus, exposure to PCBs may blunt the human xenobiotic response, inhibiting the detoxification of steroids, bioactive dietary compounds, and xenobiotics normally mediated by SXR. The antagonistic PCBs are among the most stable and abundant in human tissues. These findings have important implications for understanding the biological effects of PCB exposure and the use of animal models to predict the attendant risk.

Rifampicin (RIF) is a macrocyclic antibiotic used in the multidrug treatment of diseases caused by *Mycobacterium tuberculosis* and a wide array of infections caused by gram-positive and gram-negative bacteria. We found that RIF activation of the SXR antagonizes the activity of NF-κB, a key regulator of inflammation and the immune response. NF-κB target genes are upregulated in mice deficient in the SXR ortholog PXR. NF-κB

activation inhibits SXR activity and the expression of SXR target genes; inhibition of NF-κB activation inhibits SXR activity and the expression of SXR target genes; and inhibition of NF-κB enhances SXR activity. This mutual repression between SXR and NF-κB provides a molecular mechanism underlying the transcriptional suppression of hepatic cytochrome P450 mRNAs by inflammatory stimuli, as well as the immunosuppressant effects of RIF, thereby establishing an important relationship between xenobiotic metabolism and immune responses.

Significance of Findings:

Understanding the molecular biology of SXR/PXR allows the derivation of a commonly accepted set of principles that connect laboratory experiments, wildlife exposure data, and human risk to reduce the uncertainty about whether the underlying mechanisms of response to chemical exposure are universal or different. Our identification of highly chlorinated PCBs as rodent-specific agonists, but human-specific SXR antagonists, suggests that studies of these compounds using rodent models may not be predictive of human effects. Our finding that SXR and NF-κB signaling pathways are mutually antagonistic may provide a molecular explanation for decreased immune response commonly observed in wildlife populations that are exposed to xenobiotic chemicals, and could have implications for human exposure.

Next Steps:

SXR from various individuals and mouse strains is being sequenced intensively. These mutations will be reconstructed in the wild-type receptor and tested for functional differences in receptor activation assays. In collaboration with researchers at the National Institutes of Health Sciences, Tokyo, Japan, we are developing a "fully humanized" mouse model. Unlike the available mouse model, which expresses a human SXR transgene in a knockout mouse background, we have developed a mouse model that expresses the human cDNA under the control of the endogenous mouse promoter. This transgene is expected to be expressed in all tissues where the wild-type gene is transcribed. This model will be tested and validated as a model for human exposure to various dietary and xenobiotic chemicals.

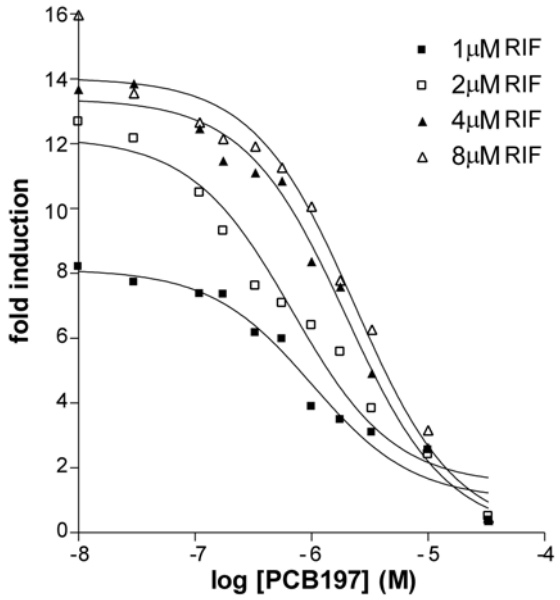


Figure 1. PCBs antagonize activation of human SXR by Rifampicin (RIF). COS7 cells were transfected with Gal-hSXR ligand-binding domain and treated with 1 μM, 2μM, 4μM, or 8 μM Rifampicin in the presence of the indicated concentrations of PCB 197.

Poster Abstract

Environmental Tobacco Smoke, Biomarkers, and Childhood Asthma

Hillary Klonoff-Cohen
University of California at San Diego, San Diego, CA

Project Goals and Objectives:

One of the greatest challenges in pediatric respiratory medicine is to identify asthmatics in early stages. Inflammatory markers increasingly are used in clinical practice as objective tools to aid in the diagnosis and monitoring of asthma. Elevated serum eosinophil cationic protein (sECP) is a marker associated with asthma and increases with tobacco smoke exposure. Urine eosinophil protein X (uEPX) significantly increases with asthma, decreasing age, and active eczema. Asthma causation has been linked to the fetal and newborn environment during development of the immune system. The deleterious effect of in utero and postnatal environmental tobacco smoke (ETS) may further complicate the diagnosis and treatment of asthma. Remarkably, the relationships between sECP, uEPX, and ETS have never been investigated in young asthmatic children. We hypothesize that in infants and children, sECP and/or uEPX may be valuable measures of eosinophil activation in asthmatics, particularly those exposed to ETS during pregnancy and postnatally (through breast milk and the environment).

Approach:

Currently, we are conducting a prospective multicenter study in Southern California to recruit 200 Caucasian, African-American, Hispanic, Asian, and/or Pacific Islander children (0-4 years) with newly diagnosed asthma, and 200 healthy children, by comparison, who are matched on age, race, sex, clinic, and hospital site. All parents participate in a telephone interview and complete two short followup questionnaires and a diary of asthma symptoms. Infant sECP, uEPX, and urinary cotinine levels are being measured at baseline, and every 4 months thereafter, for 1 year.

Preliminary Findings:

The progress made toward achievement of the objectives consists of hiring and training the appropriate personnel; constructing and pilot-testing asthma questionnaires; perfecting urine and blood collection, storage, transport, and analyses; creating a database; and finally, obtaining Human Subjects' approval in August 2004 from the U.S. Environmental Protection Agency. There are no preliminary results.

Significance of Findings:

It is expected that asthmatic infants exposed to ETS *in utero* should have the highest sECP and uEPX values, unexposed asthmatics and ETS-exposed healthy children will have intermediate values, and healthy unexposed infants will have the lowest sECP and uEPX values at baseline. These biomarkers might predict future asthmatics who have been exposed to tobacco smoke during and/or after pregnancy. Both sECP and uEPX are relatively new and simple tests that might have an immediate impact on childhood asthma. This study may have important implications for detection, prevention, and treatment of asthma.

Next Steps:

The next steps in this project include: identifying, screening, and enrolling eligible patients; and collecting urine for cotinine and EPX analyses and blood for sECP testing. We anticipate recruiting a total of 400 patients over the next 3 years. Asthma is one condition for which public health efforts are not working; in fact, research might be moving in the wrong direction, particularly in children ages 0 to 4 years, who have the highest

asthma prevalence and hospitalization rates.[1] It is hoped that this study will identify novel and non-invasive markers to diagnose asthma and assess its severity in young children. Most important, this research project will examine the effects of *in utero* environmental tobacco smoke exposure on the development of asthma in genetically predisposed infants, using inflammatory markers.

Reference:

1. Mitka M. Why the rise in asthma? New insight, few answers. *JAMA* 1999;281(23):2171–2172.

Poster Abstract

Identification of a Novel Hemoglobin Adduct in Sprague-Dawley Rats Exposed to Atrazine

Gregory P. Dooley[1], B.K. Cranmer[1], P.L. Prentiss[1], M.E. Anderson[2], and J.D. Tessari[1]
[1]Colorado State University, Fort Collins, CO; [2]Chemical Industrial Institute of Toxicology, Research Triangle Park, NC

The analysis of protein adducts may provide an effective method for the biomonitoring of environmental exposure to chemicals such as pesticides. Previous data from our laboratory suggest that the commonly applied herbicide atrazine may form a covalent adduct with hemoglobin in Sprague-Dawley (SD) rats. *In vivo* exposures of rats to 300 mg/kg of atrazine resulted in significantly higher globin adducts than *in vitro* experiments with rat whole blood, suggesting that metabolism of atrazine is important for adduct formation. Mass spectrometry data indicate an approximately 109 addition (see Figures 1 and 2) to the beta chain, which may correspond to a reaction with a deprotonated diaminotriazine metabolite. Using globin isolated from SD rats exposed to atrazine, we investigated the nature of the covalent binding of metabolized atrazine to the beta chain of globin. High performance liquid chromatography of rat globin was used as a means to separate the alpha and beta chains in about 30 minutes. Collected fractions were analyzed with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) to confirm the identity and purity of the globin chain fraction. The beta chains were digested with trypsin and the digest analyzed with MALDI-TOF-MS to identify possible differences in mass due to the addition of a covalent triazine adduct in atrazine-exposed rats compared to controls.

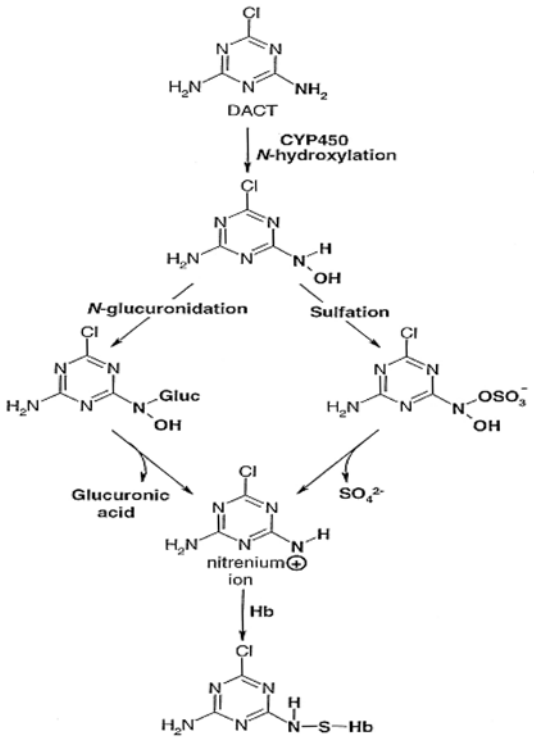


Figure 1. Proposed mechanism of Hb adduct formation after DACT undergoes *N*-hydroxylation by CYP450.

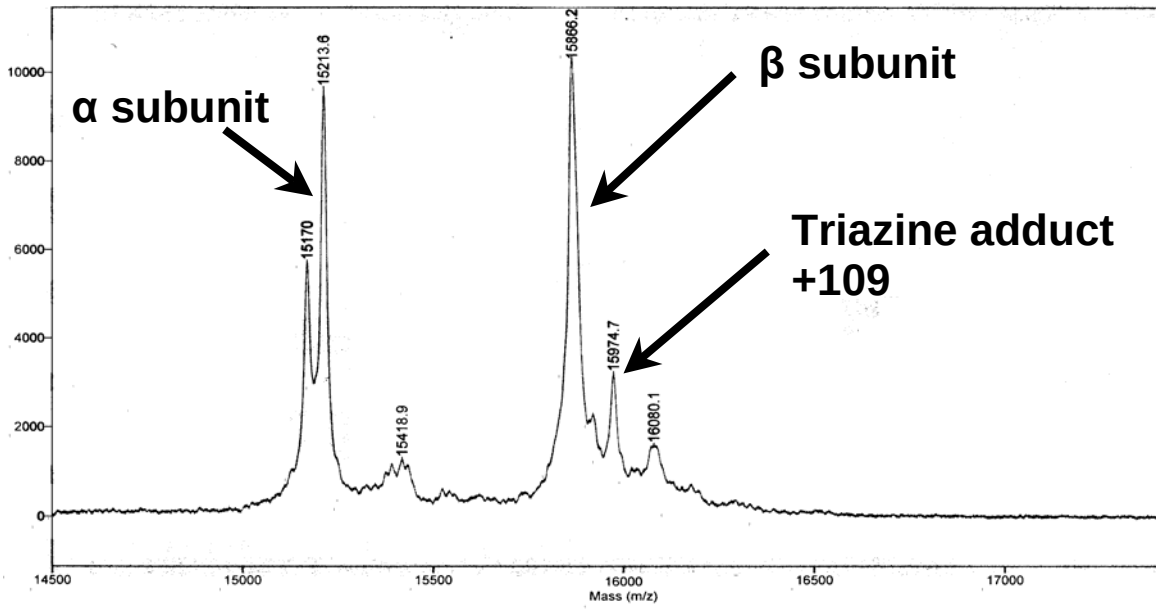


Figure 2. Results of MALDI-TOF-MS analysis of globin isolated from whole blood of male Sprague-Dawley rats exposed to 300 mg/kg atrazine.

Poster Abstract

The Pregnancy Environment and Child Health (PEACH) Study of Intrauterine and Postnatal Exposure to Halogenated Compounds and Childhood Atopy

Kevin Brooks[1], Hanem Hassan[1], Sridhar Samineni[1], Venu Gangur[1], John Riebow[2], and Wilfried Karmaus[1]
[1]Michigan State University, East Lansing, MI; [2]Michigan Department of Community Health, Lansing, MI

Despite many years of intense research, the etiology of allergic (atopic) disorders (asthma, eczema, and hay fever) remains poorly understood. The role of intrauterine priming is in dispute, particularly whether endocrine disrupting chemicals (EDCs) alter fetal immune responses. The Pregnancy Environment and Child Health (PEACH) Study (see Figure 1) is using time-sensitive measurements in a period of rapid changes integrated into a complex design. By enrolling primiparae and measuring their serum EDC concentration in the first trimester of pregnancy, unbiased measures of exposure were established. At delivery, placental samples are used to determine fetal EDCs. Cord blood samples are collected to determine the neonatal cytokine response of mononuclear cells. Lifestyle and diet during pregnancy, as well as perinatal events, are ascertained by interviews and medical records. Two weeks postnatally, breast milk samples were collected to determine EDCs, cytokines, and immunoglobulins. Information from pediatric charts and interviews at 6 and 12 months postnatally is used to establish atopic manifestations in the offspring. Pregnancy and infancy are marked by a series of rapid endocrine and immunologic changes. Each step depends on the one before; therefore, a design that facilitates the analyses of a cascade of events is needed. Regarding exposure, the extent to which maternal concentration of EDCs, such as organochlorines, predicts placental and breast milk concentrations will be assessed. Regarding immune response, the association between maternal and placental EDCs, and cord blood (fetal) immune markers will be estimated. The immunologic benefits of breast milk and the increased risk caused by organochlorine have long been debated. The PEACH Study will allow us to disentangle the protective from the potentially harmful properties. Finally, the impact of maternal and early childhood predictors on the development of atopic disorders in infancy will be addressed. Such a holistic approach is attainable because of an improved technique to quantify both exposure and outcome using specific and time-sensitive markers. We believe that this holistic approach will help to overcome the scientific uncertainties produced by fragmented studies focusing on exposure-outcome relationships at single points in time. Initial results and interpretations of the preliminary data will be presented. In the future, the PEACH Study will include further followup of this cohort to characterize gene-environment interactions.

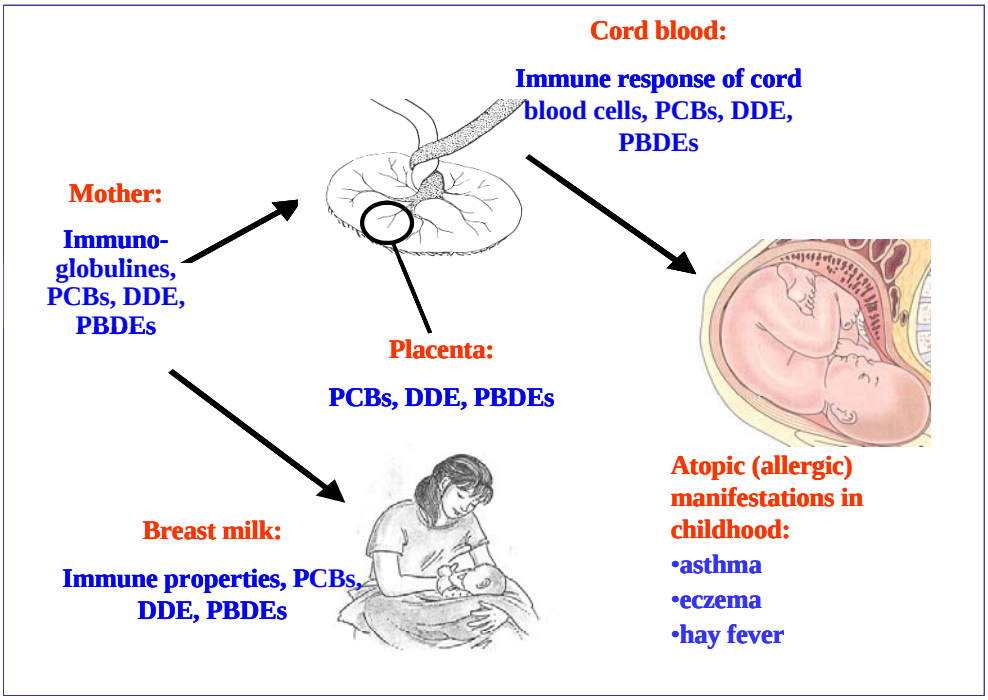


Figure 1. The conceptual schema of the PEACH Study.

Poster Abstract

Meconium Analysis: A Promising Tool To Detect Fetal Exposure to Environmental Toxicants

Enrique M. Ostrea, Jr., Dawn M. Bielawski, Noberto C. Posecion, Jr., Melissa L. Corrion, and Jeremy J. Seagraves
Wayne State University, Detroit, MI

Project Goals and Objectives:

The objective of this project is to develop an analysis of meconium, an infant's first stools, as a sensitive, diagnostic tool to detect fetal exposure to environmental toxicants. This project aims to: (1) compare the prevalence and amount of fetal exposure to environmental toxicants through the analysis of meconium, cord blood, and neonatal hair; and to determine the degree of agreement among these methods; and (2) determine the relationship between maternal exposure to environmental toxicants during pregnancy, as determined by serial analyses of maternal hair and blood and the positivity rate and concentrations of environmental toxicants in meconium, cord blood, and neonatal hair.

Approach:

Pregnant women were recruited at midgestation from a rural site in the Philippines, where our preliminary survey showed significant residential and agricultural use of pesticides and an herbicide (see Table 1). Maternal hair and blood were obtained upon recruitment (A) and at birth (B). Infant hair, cord blood, and meconium were collected at birth. Solid-phase extraction was used to isolate carbamate (propoxur), organophosphates (malathion, chlorpyrifos, and diazinon), organochlorines (lindane and DDT), pyrethroids (bioallethrin, cyfluthrin, cypermethrin, and transfluthrin), and chloroacetanilide herbicide (pretilachlor) from meconium. Liquid-liquid extraction was used to isolate parent pesticides from blood and hair and several metabolites from all matrices. Extracts were analyzed using gas chromatography/mass spectrometry in selected ion monitoring mode to measure pesticide and metabolite concentrations.

Preliminary Findings:

Analysis of meconium detected the highest fetal exposure rate (percent positive) to the various toxicants (see Table 1). Maternal exposure was determined most frequently by maternal hair analysis; only propoxur was present in maternal blood. Lindane and transfluthrin were not detected in any matrix, whereas all other compounds were quantified in at least one. Pesticide metabolites were not seen in meconium or cord blood.

Significance of Findings:

Preliminary results suggest that prenatal exposure to environmental toxicants is detected best by the analysis of meconium, because the prevalence of pesticides and herbicides are highest in that matrix. Propoxur has been detected in every matrix and occurs more frequently than any other compound. We have found that the high fetal exposure rate to propoxur in the study population may be caused by misuse of the pesticide spray Baygon® (that contains propoxur and cyfluthrin), because of inadequate labeling of the product. In response to a letter to S.C. Johnson Company, manufacturers of Baygon®, two representatives visited our laboratory to discuss our results. The principal investigator is dedicated to educating health care professionals in the Philippines and in the United States to reduce or prevent future exposures.

Next Steps:

We are continuing to enroll subjects to reach our goal of recruiting 750 mother-infant dyads. Once all of the samples have been analyzed, we will have sufficient information to compare statistically the prevalence and concentrations across matrices and to determine the relationship between positive maternal and fetal samples. We also are involved in a collaborative effort to submit a proposal for "Building Health Professional Capacity To Address Children's Environmental Health," which will facilitate training of health care professionals internationally in reducing exposure of children to pesticides, heavy metals, and environmental tobacco smoke.

Table 1. Prevalence of exposure to environmental toxicants as detected in maternal and infant matrices.

MATRIX	Maternal Hair A	Maternal Hair B	Infant Hair	Maternal Blood A	Maternal Blood B	Cord Blood	Meconium
No. of Samples Analyzed	469	361	345	466	361	346	342
Parent Pesticide (Rate of Use)	**Prevalence of Exposure (Percent Positive)**						
Propoxur (73% [a])	12.37%	18.01%	0.29%	0.64%	5.54%	3.47%	39.18%
Diazinon (12% [b])	0%	0%	0%	0%	0%	0%	0.29%
Lindane[c]	0%	0%	0%	0%	0%	0%	0%
Transfluthrin (11% [a])	0%	0%	0%	0%	0%	0%	0%
Malathion (13% [b])	1.71%	0%	0%	0%	0%	0%	0.58%
Chlorpyrifos (6%[a], 37%[b])	0.21%	0.55%	0.29%	0%	0%	0%	0%
Bioallethrin (26%[a])	12.58%	12.47%	0%	0%	0%	0%	0.29%
Pretilachlor (28%[b])	0.21%	0.28%	0%	0%	0%	0%	1.46%
DDT[c]	0.43%	0.83%	0%	0%	0%	0%	0.88%
Cyfluthrin (73%[a])	0%	0%	0%	0%	0%	0%	0.29%
Cypermethrin (31%[b])	0%	0%	0%	0%	0%	0%	3.51%

[a]Residential use, according to survey.
[b]Agricultural use, according to survey.
[c]Not included in pesticide survey.

Exposure Assessment

Presentation Abstract

Estimating Human Health Risk From Dermal Exposure to Contaminated Soils

Annette L. Bunge
Colorado School of Mines, Golden, CO

Project Goals:

The overall goal of this research project was to determine experimentally the mechanisms, and to develop methods for making reasonable predictions, of dermal absorption from contaminated soils. Ideally, the computational procedure would require only known or easily determined input parameters, such as the conditions of the exposure (e.g., contact time, mass of soil adhering to skin, and chemical concentration on the soil); characteristics of the soil (e.g., organic carbon and water contents); and properties of the absorbing chemical (e.g., lipophilicity, molecular size, and vapor pressure).

Approach:

The approach was to study chemical transfer from soil to skin or polymeric membranes acting as skin surrogates. The study measured penetration through skin and membranes mounted in diffusion cells, as well as uptake into membranes, from soils contaminated with varying amounts of chemical, pure powdered chemical, and saturated aqueous solutions. The factors studied included contaminant concentration on the soil (C_{soil}), soil organic carbon content, and the applied mass of soil per exposed area (M_{soil} /A). All soils in this study were dry.

Preliminary Findings:

Absorption was independent of M_{soil} /A, as long as the surface was almost completely covered and absorption did not reduce contaminant concentration (C_{soil}) too much. Apparently, contaminant on the soil particles above those with direct skin contact (i.e., the monolayer illustrated in Figure 1) contribute little to the uptake in skin or membranes. This is consistent with fast absorption relative to contaminant transport within the dry soil. As a result, the percent of the applied dose that absorbs, decreases when M_{soil} is increased above the amount needed for a monolayer. Equilibrium uptake measurements in polymeric membranes indicate that soils have a solubility limit (S_{soil}). Absorption into membranes and skin was proportional to C_{soil}, as long as C_{soil} was less than S_{soil}.

Significance of Findings:

Most studies of contaminated soil have measured dermal absorption from one soil at one C_{soil} for one M_{soil}/A. Experimental values for C_{soil} and for M_{soil} /A are almost always much greater than would occur in actual exposures. Despite this, percent absorption numbers calculated from these results are the basis of the default absorption values for contaminated soils in the 2001 Superfund guidance. The results of this study indicate that these percent absorption values may underestimate the real absorption potential.

Next Steps:

Further study is needed on the effect of M_{soil} /A when it is too small to cover the skin surface completely, as well as the effects of soil organic carbon content and moisture. There are differences in skin as compared to polymeric membranes in the amount absorbed from soil, relative to an aqueous solution of the same compound. The cause of this difference could be skin hydration, difference in soil contact with the skin surface, or something else. Understanding this difference would provide important further insight into the mechanism of dermal absorption from contaminated soils.

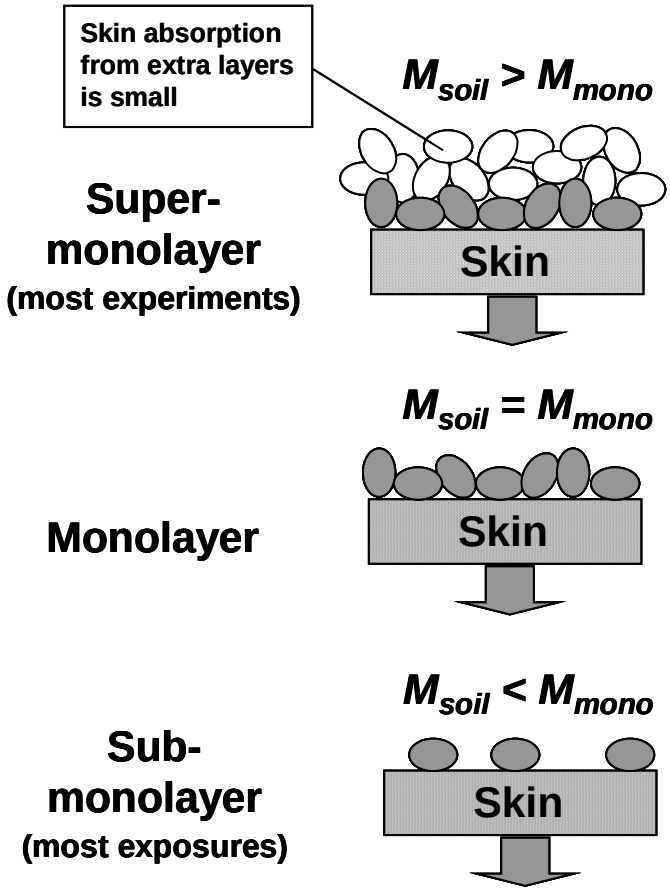


Figure 1. Schematic diagram illustrating the effect of M_{soil}/A on dermal absorption as observed in experiments compared with estimates for actual environmental exposures.

Presentation Abstract

A Longitudinal Approach of Assessing Aggregate Exposure to Organophosphate Pesticides in Children

Chensheng (Alex) Lu
Emory University, Atlanta, GA

Objectives:

This study has three overall objectives. First, this study is designed to establish children's organophosphorus (OP) pesticide and pyrethroid exposure levels in a longitudinal fashion and to determine the contribution of pesticide residues in children's diets to these levels. Second, the temporal and interindividual variability of total OP pesticide exposures in children will be characterized in relation to both residential pesticide use and dietary intake. Third, this study will examine children's OP pesticide exposure from multiple sources via several unique pathways and will assess the relative contribution of these pathways and sources to total OP pesticide and pyrethroids body burden.

Approach:

A combination of biological monitoring and multipathway sampling techniques is proposed, including foods, as complementary approaches to exposure assessment in this project. Currently, a cohort of 23 children ages 3-12, who only consume conventional diets, were enrolled in a 12-month sampling period, in which two daily spot urine and saliva samples are being collected from this cohort for 7 consecutive days for every 3 months. Also collected are their 1-day 24-hour duplicate food samples. In this food sample collection day, four spot urine and saliva samples were collected from each child. Selected OP pesticides and pyrethroids and their urinary metabolites will be measured in food, urine, and saliva samples. In two of four sampling periods (summer and fall seasons), subjects are asked to participate in a dietary intervention study in which 5 additional sampling days are added to the middle of the original 7 sampling days. During the 5 additional sampling days, organic diets, primarily fresh produce and juices, were provided for consumption.

Preliminary Findings:

Only a small fraction of total data is available for now. The most significant finding from this study so far is the decrease of urinary metabolites of OP pesticides that are commonly found in or on foods during the 5-day organic diet consumption period. Some of the metabolite levels reached the instrumental detection limits at the end of the 5-day period. These levels were increased as the cohort returned to their normal conventional diets, and eventually reached the same levels prior to the introduction of organic diets. This decreasing trend is not obvious for pyrethroids metabolites.

Next Steps:

The data will continue to be analyzed as they become available. The relationship between pesticide residues found on or in children's diets and the pesticides or their metabolite levels in saliva and urine samples will be examined. This study will be duplicated in an area wherein the contribution of pesticide exposure from residential use may be more significant than dietary intakes. With the design of this study, we may be able to determine the pharmacokinetics of selected pesticides in children through oral ingestion using a compartmental modeling approach.

Presentation Abstract

Longitudinal Study of Children's Exposure to Pyrethroids

Ye Hu, M. Spruill, J.H. Raymer, M. Gardner, J. Deese-Spruill, J. Knight, T. Marrero, and M. Rice
Research Triangle Institute, Research Triangle Park, NC

Project Goals and Objectives:

The specific aims for this study are to: (1) investigate the time course of the redistribution of pyrethroids in various media after application and factors affecting the redistribution; (2) investigate the functional relationships across time between environmental media, personal measurements, and biological media; (3) estimate aggregate exposure after application and the importance of each exposure pathway; and (4) investigate the difference between the time course of pyrethroid metabolism between adults and children.

Approach:

A longitudinal study is being conducted to investigate 15 homes that use pyrethroids indoors and with stay-at-home children younger than 3 years old. Each of the homes was followed intensively for 7 days after pesticide application. They then were followed once a week in the first month after application, and every other month for the following 11 months. Environmental samples (e.g., surface wipes, air samples, and toy wipes), food samples, personal samples (e.g., hand wipes and whole body dosimeter), and video and urine samples were collected from children and one stay-at-home parent.

Preliminary Findings:

A method has been developed to extract urine from disposable diapers that contain polyacrylate granules for analysis of pyrethoid pesticide metabolites and creatinine. Pyrethroid metabolites 3-(2,2-dichlorovinyl)-2,2-dimethyl-(1-cyclo-propane) carboxylic acid (DCCA), 3-(2,2-dibromovinyl)-2,2dimethyl-(1-cyclo-propane) carboxylic acid (DBCA), and 3-phenoxybenzoic acid (3-PBA) were analyzed using liquid chromatography/mass spectrometry and evaluated for recoveries in the urine released from the diapers. The study found calcium chloride dihydate to be satisfactory in releasing urine and metabolites from the polymers. The percent recoveries for the three tested pyrethroid metabolites were mostly in the range of 65-130. The percent recoveries for creatinine were in the range of 71-133. The detection limit for each of the three metabolites was 0.1 μg/L. The end of the sample collection phase is approaching. Eight subjects already have finished the 1-year followup with full sets of samples. Four subjects missed 1 to 2 visits, and one missed 3 visits. The remaining two are expected to finish the followup with full sets of samples.

Significance of Findings:

The method that we developed for analyzing pesticide metabolites from gel-containing diapers makes it possible to use commercial diapers to collect urine samples from young children who are not toilet trained. Several new sample collection methods, including commercial diapers and whole body dosimeter, have been used in this study. High compliance rates indicate these methods have the potential to be used for large-scale and/or longitudinal studies.

Next Step:

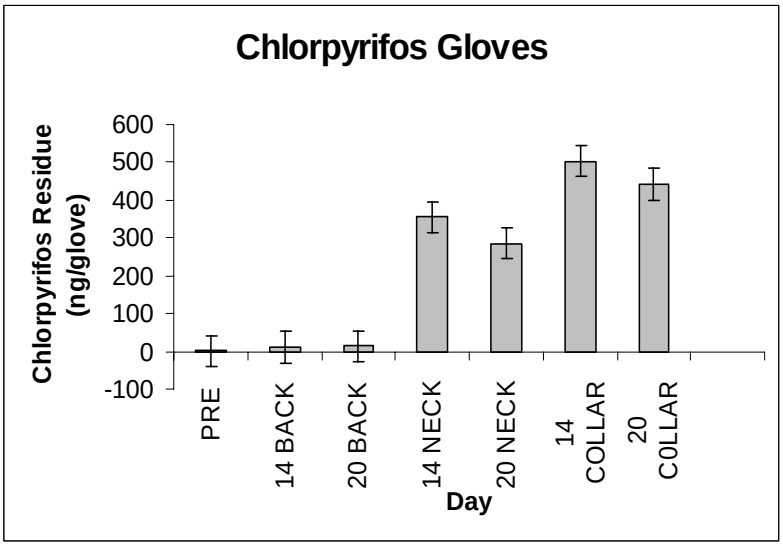
The main focus of our next step includes sample analysis and statistical analysis. The anticipated results include: (1) a longitudinal characterization of permethrin concentrations in a multipathway exposure environment for young children; (2) an information base from which to develop a relationship of within- and across-home variation; and (3) development of adult-child urinary metabolite profiles over time.

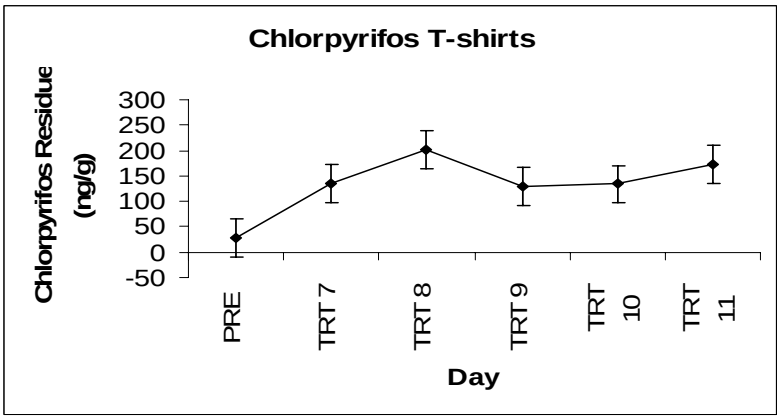
Presentation Abstract

Assessing Levels of Intermittent Exposures of Children to Flea Control Insecticides From the Fur of Dogs

Janice E. Chambers, J.E. Moran, M.K. Davis, and J.S. Boone
Center for Environmental Health Sciences, College of Veterinary Medicine,
Mississippi State University, Mississippi State, MS

Because there is substantial interaction between children and their pet dogs, and because dogs frequently are treated with flea control insecticides, it is likely that children will be exposed to these insecticides after contact with their pet dogs. The project was designed to determine the levels of dislodgable insecticide residues obtained from the fur of dogs treated with flea collars, the level of residues that could be transferred to a child from the dog, and the levels of urinary metabolites in children and adults in contact with the dogs. Fur was sampled by rubbing the dog with a white cotton glove. The flea collars contained organophosphorus insecticides, either chlorpyrifos or tetrachlorvinphos (TCVP). The protocol studied dislodgable insecticide residues from the dog's back, the neck without the collar, and the neck with the collar in place. One collar contained chlorpyrifos, and 11 samples were taken over the 6-month period. Twenty-four replications (i.e., separate dogs and children) were included. Within about a week, the values reached a plateau that was maintained during the sampling period. The average levels of chlorpyrifos transferred to the glove in a 5-minute rubbing were about 15, 300, and 450 ng for the back, neck without the collar, and the neck with the collar, respectively. T-shirts worn by the child for 4 hours on the day prior to the urine samples showed levels of chlorpyrifos of about 28 ng/g prior to the collar placement, and 150 ng/g following placement of the collar. During this same study, the levels of 3,5,6-trichloropyridinol (TCP) in the first morning-void urine samples were measured in a child (either sex; age range 3-12 years) and an adult (either sex) in the household. TCP was present in the urine of the children (14 ng/mL) and adults (8 ng/mL) prior to the placement of the flea collar on the dogs. TCP concentrations were higher in the children's urine than in the adult urine, both on a concentration basis and a concentration adjusted for creatinine. There were no increases in urinary TCP following the placement of the collar on the dog (see Figure 1). Peak TCVP residues from the rubbing of the dogs' fur were about 0, 8, and 24 mg from the back, neck, and neck plus collar, respectively. T-shirts showed about 29 ng TCVP/g prior to placement of the collar and 1,816 ng/g after placement of the collar. Urinary metabolites of TCVP are being quantified presently. Residues of chlorpyrifos and TCP before placement of the flea collar on the pet dog reflects the widespread use of chlorpyrifos. The biomonitoring data indicate that there appears to be no enhanced exposure to chlorpyrifos in either adults or children from their contact with pet dogs wearing a chlorpyrifos containing flea collar.





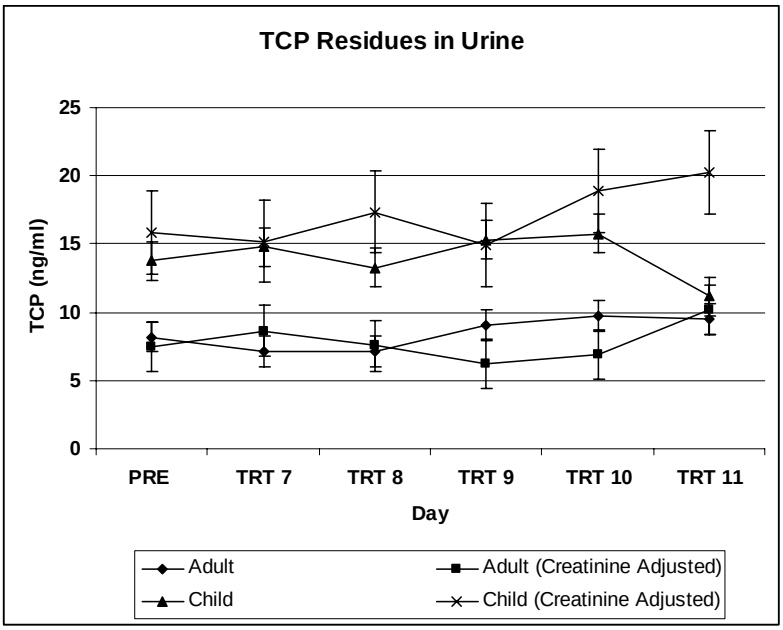


Figure 1. Residues of chlorpyrifos on gloves used to rub the fur of dogs or on T-shirts worn by children and residues of trichloropyridinol (TCP) in the urine of children and adults before and after placement of chlorpyrifos-containing flea collars on pet dogs.

Presentation Abstract

Ingestion of Pesticides by Children in an Agricultural Community on the U.S./Mexico Border—Variations in Behaviors and Metabolite Levels

Stuart L. Shalat[1], Natalie C.G. Freeman[1], Kirby C. Donnelly[2], James A. Calvin[2], Kathleen Black[1], Marta Zimenez[1], and Dana Barr[3]

[1]Environmental and Occupational Health Sciences Institute, Robert Wood Johnson Medical School, Piscataway, NJ; [2]Center for Environmental and Rural Health, Texas A&M University, College Station, TX; [3]National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA

A pesticide assessment of 60 infants and toddlers was conducted in a colonia near Loredo, TX, over a 2-year period. The objectives were to evaluate children's exposure to pesticides over four growing seasons through the use of pesticide metabolites in urine and pesticides from hand rinses, and to quantify the variation of behaviors of children between 6 and 60 months old from videotapes, questionnaires, and time/activity diaries obtained over several visits. Urine samples were collected from first morning voids the day after videotaping and analyzed for dialkyl phosphate pesticide metabolites by the Centers for Disease Control and Prevention (CDC). Children's hands were cleaned prior to the start of the 4-hour videotaping session and hand rinses, using isopropyl alcohol, were collected immediately after the taping session and analyzed for a suite of organophosphate pesticides. The videotapes were transcribed for both the left and right hands using the Virtual Timing Device software. Activity data also were obtained from questionnaires and diaries completed by the parents.

Seasonal variations in metabolite levels were found, and during some sampling periods, diethyl phosphate, diethylthiophosphate, and dimethyl phosphate metabolites were substantially higher than those reported for the National Health and Examination Survey's 6-11 year-old children.[1] Variations in hand loadings of pesticides across seasons also were observed. Age differences in children's behaviors were found both in videotaped observations and from parental reports. Whereas infants do more mouthing than older children, their limited mobility and oversight by parents reduce access to potentially contaminated surfaces and objects compared to toddlers. There were clear transitions in the occurrence of some behaviors as children aged. For example, soil consumption was most frequently reported for children between 12-23 months old, and the amount of time spent outdoors and concomitant contacts with dirt and grass increased with age. At the same time, hygiene habits also improved with age, which may reduce the potential exposures associated with increased mobility and independence. Much of the elevated metabolite levels can be attributed to seasonal use of pesticides in adjacent farm areas. Evaluation of the data is ongoing; however, preliminary analysis suggests that variations in metabolite levels are related to hand loadings of pesticides in conjunction with children's behaviors. In the future, we hope to follow these children as they enter school and evaluate the neurodevelopmental influences of pesticide exposure.

Reference:

1. CDC. *Second National Report on Human Exposure to Environmental Chemicals.* National Center for Environmental Health. Publication No. 03-0022. The Centers for Disease Control and Prevention, Atlanta, GA. January 2003.

Presentation Abstract

Vulnerability of Young Children to Organophosphate Pesticides Through Intermittent Exposures in Yuma County, Arizona

Mary Kay O'Rourke[1], N.C. Freeman[2], A. Aguirre[3], M. Nishioka[4], and M.D. Lebowitz[1]
[1]University of Arizona, Tucson, AZ; [2]University of Florida at Gainesville, Gainesville, FL; [3]Western Arizona Area Health Education Center, San Luis, AZ; [4]Battelle Memorial Institute, Columbus, OH

Young children in Yuma County, AZ, appeared to have elevated urinary biomarkers indicative of organophosphate pesticide (OP) exposure. A multimedia exposure assessment indicated cumulative intake (nine OPs) from inhalation and water ingestion to be about 2 percent. About one-third of the exposure originated from ingestion of food and beverage; the remaining 68 percent was modeled as secondary ingestion via hand-to-mouth transfer of house dust. In addition to modeling exposure, this project evaluated the hand-to-mouth transfer portion of the cumulative intake model through evaluation of microactivity and intermittent hand wipes. A total of 210 children were recruited from three agricultural communities (Somerton, Gadsden, and San Luis, AZ). Metal content (Pb, Mn, Cd, and 26 others) of house dust did not indicate any elevated exposure risks. Pesticide metabolites from urine samples and OP Enzyme-Linked Immunosorbent Assay analysis of house dust were used to assign children to exposure groups. After initial recruitment and screening, *promotoras* had difficulty "selling" and implementing the remainder of the complex study design. The next portion of the design required multimedia exposure assessment, biomarker collection, dermal wipes, and videotapes for microactivity. Forty-six households agreed to participate. Videotaped observations (3.5 hours) were obtained for 36 children 24-60 months old (mean 42.9 ± 8.3). There were 16 girls and 20 boys in the observation study, and there was no difference in the age distribution by gender (boys 42.9 ± 8.9 months; girls 43.1 ± 7.6 months). Videotapes were transcribed using the VTD software, a continuation of the VideoTraq software, developed at Stanford University.

Boys and girls spent similar amounts of time in most rooms of the home. Boys spent significantly ($p \leq 0.01$) more time outdoors and in other locations than did girls. In terms of contact, boys exhibited more soil and hard floor contact, whereas girls spent more time touching their hair and skin, plush toys, and paper products. Twenty-six children from Yuma County completed the entire protocol. Hand wipes were analyzed for several OP and pyrethroid pesticides, and hand wipe results were compared with videotaped locations and biomarker content. The joint use of behavioral data and hand wipe loadings identified potential sources of exposure in and around the children's homes. Fifty hand wipes were collected from the 26 children. The percent of children with pesticides found in first hand wipes ranged from 27 percent (chlorpyrifos) to 54 percent (cis-permethrin), and 58 percent (trans-permethrin) to 73 percent (diazinon). A comparison of the pesticide hand loadings of children with one hand wipe with the average value for the children with multiple wipes exhibited no difference in median levels for the four pesticides: diazinon, chlorpyrifos, and cis- and trans-permethrin. Comparison of pesticides obtained from first and second hand wipes for the 13 children with repeated samples was conducted for the four pesticides. There was no significant difference in the loadings on the children's hands from the first and second wipes for diazinon and chlorpyrifos. Mean differences in pesticide loadings were 2.2 ng/hand wipe and 0.4 ng/hand wipe, respectively. One child exhibited extreme loading of cis- and trans-permethrin (2,210 and 3,857 ng/wipe, respectively) collected on the second wipe. (This child was filmed playing in the fields.) Removal of these outliers resulted in mean differences in cis-permethrin and trans-permethrin for the remaining 12 children of 3.9 and 1.3 ng/hand wipe, respectively. When children's hands were wiped more than two times during the video session, little difference was seen between wipes. Currently, we are evaluating the relationships among frequent intermittent contact with contaminated surfaces, modeled exposures, and metabolite yields. To date, the results suggest reduction of play near home entry will reduce exposure for young children; playing in fields contributes to exposure directly (as expected); and contact with surfaces in the home quickly reloads hands to a consistent "loading."

Table 1. Differences in pesticide content from the hands of 13 children with multiple hand wipes.

	First Wipe	All Wipes
Diazinon	3.2 (1.0 - 32.8)	2.6 (0.7 - 17.6)
Chlorpyrifos	1.0 (1.0 - 7.9)	1.0 (1.0 - 3.2)
Cis-Permethrin	2.5 (0.5 - 7.5)	1.8 (0.5 - 36.0)*
Trans-Permethrin	1.9 (1.5 - 29.2)	4.7 (0.5 - 70.1)*
Malathion	2.5 (3.7 - 26.2)	5.0 (1.3 - 36.0)

Mann-Whitney test: no significant differences
Samples below detection limit given value of one-half limit of detection (LOD)
*Excluding outliers

Table 2. Stepwise logistic regression results for children's activity variables contributing to elevated metabolite levels using above and below the LOD as the dependent variable.

		Exp. (B)	95th CI for Exp. (B)	P-Value
TCPY	$R^2 = 0.395$			
Mouthing in Eating Area		1.164	1.003 - 1.251	0.047
Hard Toy Contact in Entry/Hall		1.027	1.002 - 1.352	0.028
DEDTP	$R^2 = 0.276$			
Food Contact		1.002	1.000 - 1.003	0.048
Hard Toy Contact in Bedroom		1.018	1.001 - 1.035	0.035
DETP	$R^2 = 0.203$			
Mouthing		1.033	1.004 - 1.062	0.027
DEP	$R^2 = 0.403$			
Food Contact Outdoors		41.67	3.565 - 486.9	0.003
Hard Surface Contact in Living Room		1.040	1.002 - 1.080	0.018
DMDTP	$R^2 = 0.226$			
Plush Toy Contact in Entry/Hall		1.136	1.024 - 1.260	0.016
DMTP	$R^2 = 0.162$			
Water Contact		10.80	1.164 - 100.2	0.036

Poster Abstract

Measurements and Models of Longitudinal Dietary Intake of Pyrethroid and Organophosphate Insecticides by Children

P. Barry Ryan
Emory University, Atlanta, GA

Exposure to pesticides in the environment has been associated with acute toxic effects and long-term health outcomes. Evaluation of such exposures has been problematic, however, for two main reasons. First, exposures occur through all pathways, including vapor-phase and particle inhalation, dermal contact with contaminated soil or dust, and inadvertent and purposeful ingestion of materials contaminated with these substances. Additionally, the specific pathways followed by these contaminants are many and complicated. Second, there has been difficulty in ascertaining the effect of such pollutants on both exposure and effect. Because many pathways are involved and individuals have many activities in which they partake, exposures are highly variable and not easy to predict.

Biomarkers of exposure offer clear evidence that exposure to a specific compound or class of compounds has taken place, and thus represent a very powerful tool in the exposure assessor's arsenal. Urinary biomarkers of exposure have been especially useful in gathering information on exposures to these contaminants. Nonspecific markers of general exposure, namely dialkyl phosphates, which represent metabolites from approximately 75 percent of all organophosphates, have been used for organophosphate insecticides. Specific markers for individual organophosphate insecticides also have been measured, in which the nonphosphate "leaving group" component of the molecule is analyzed. Similar work has been done using urinary metabolites of pyrethroid insecticides. Metabolites of both Type I and Type II pyrethroids have been evaluated.

Key questions now must focus on the relationship between the measured biological marker of exposure and the exposures experienced by the individual. This research project focuses on the question: Do urinary biomarkers of exposures to these two classes of insecticides reflect exposures experienced in the environment? Because children may be at greater risk than adults for a given exposure level, they are the primary group of interest. In this study, we will recruit a cohort of children ages 2-6 years, and follow them for a period of 18 months, monitoring each child and his or her residence at least three times. The residence of each child will be monitored for pesticide exposure through collection and analysis of soil, house dust, and air samples. Each of these environmental media will be analyzed for the total content of the two classes of pesticides by evaluating specific compounds selected for their common use in residential settings. In addition, the family of the child will be asked to keep a dietary record for 1 week prior to the monitoring period, in which the foods eaten by the child will be recorded. Food items will be purchased for the monitoring period in duplicate, with one-half going to the family for preparation for the child's diet and the remainder kept for analysis within the laboratory. The analysis plan calls for direct comparison between measured concentrations in the environmental media and biomarkers of exposure to these compounds.

Currently, we are developing analytical procedures in our laboratory designed to enable us to monitor multiple classes of pesticides simultaneously. Figure 1 shows the results of the gas chromatography/electron capture detector analysis of 20 pesticides drawn from three classes: persistent organchlorines, organophosphates, and pyrethroids.

The small peak barely discernable between *p,p*-DDT (M) and methoxychlor (N) is likely with resmethrin, but we are not yet confident with the identification. Technical endosulfan consists of the α and β isomers in a ratio reported from 4:1 to 7:3. The pyrethroids (bioallethrin, cyhalothrin, permethrin, cyfluthrin, cypermethrin, and deltamethrin) have various isomers that nearly co-elute, giving rise to a complex chromatogram for each.

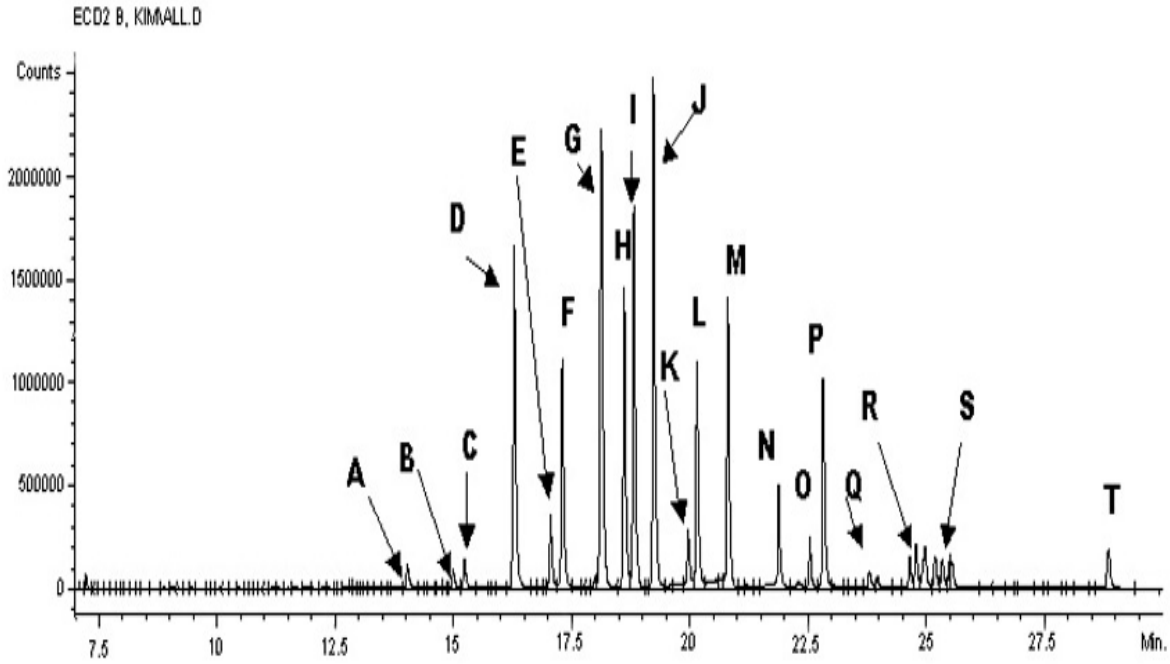


Figure 1. Chromatogram for mixed calls pesticides—Emory University Environmental Exposure Assessment Group, October 2004.

Identifiers for Chromatogram:

A - Phorate	F - Chlorpyrifos	K - Endosulfan β	P - λ Cyhalothrin
B - Terbufos	G - Bioallethrin	L - *o,p*-DDT	Q - Permethrin Isomers
C - Diazanon	H - *o,p*-DDE	M - *p,p*-DDT	R - Cyfluthrin Isomers
D - Methyl Parathion	I - Endosulfan α	N - Methoxychlor	S - Cypermethrin Isomers
E - Malthion	J - *p,p-DDE*	O - Azinophos Methyl	T - Deltamethrin Isomers

Poster Abstract

Study of Exposure and Body Burden of Children of Different Ages to Pesticides in the Environment

James H. Raymer, G. Akland, Y. Hu, T. Marrero, M. Spruill, K. Briggs, and B. Childs
RTI International, Research Triangle Park, NC

During the past several years, there have been numerous studies that have shown that there can be differences in the exposures to environmental pollutants experienced by children and adults in similar environments. These differences are derived from a number of factors including activities, diet, routes of exposure, and differences in the way children metabolize and excrete pollutants. A study at RTI has examined both exposure to various pesticides and the resulting doses experienced by children of different ages and adults sampled from both rural and urban homes. The main objective of this study was to test the hypothesis that children have significantly higher environmental exposures and resulting doses than do adults living in the same home. The study was conducted in two areas of Minnesota: Minneapolis/St Paul/Rice and Goodhue counties in the east and locations in and around Moore county in the western part of the state. Samples of indoor air, personal (breathing zone) air, diet, surface wipe, surface press, house dust, dermal rinse, and pajamas ("body suits") were collected to define potential exposure. In some cases, volatile organic compounds and exhaled breath samples were collected. The target pesticides/herbicides were atrazine, chlorpyrifos, diazinon, and parathion, although each sample was screened for the presence of other organophosphorus and chlorinated pesticides and these were quantified if present. Urine samples were analyzed for both free and conjugated pesticides/metabolites. Using this information, the dose experienced by individuals of different ages resulting from the measured exposure will be estimated as will potential differences in metabolism as reflected by differing ratios of parent compound to metabolite in the different age groups. A total of 22 homes and 62 participants in the east were sampled in 2000 and 19 homes and 49 participants in the west were sampled in 2001. Summaries of the data and interpretations of the data will be presented.

Susceptibility and Vulnerability

Presentation Abstract

Bioaccumulative Toxics in Native American Shellfish

Felix Basabe
Swinomish Tribe, LaConner, WA

Project Goals, Objectives, and Approach:

The goals of this project are to: (1) ascertain whether the Swinomish people (see Figure 1) who consume subsistence-harvested shellfish are exposed to bioaccumulative toxics to the degree that they potentially cause chronic and acute health risks; (2) determine and communicate the health risks; (3) develop mitigation measures; and (4) confirm prominent health conditions and develop hypotheses related to found contaminants.

The central hypothesis of this project states that Swinomish people are exposed to low-level, chronic bioaccumulative toxics when participating in subsistence gathering and consumption of shellfish. The project aims to evaluate the validity of this hypothesis by quantifying the toxic levels in two species of clams (*Saxidomis giganteus* and *Protocatha staminea)*, one species of crab (*Cancer magister)*, and sediment samples; translating those levels to an effective "dose" based on physical parameters, appropriate consumption levels, and eco-cultural considerations; and assessing the risks posed to consumers by these doses. Outreach and education measures in the Swinomish community and other potentially affected Puget Sound area tribal communities are being designed and implemented.

Preliminary Findings, Significance, and Next Steps:

Preliminary findings indicate that some of the shellfish beds are less contaminated than others. Initial risk assessment results are complete for polychlorinated biphenyl congeners and dioxins in clams. Averages listed in Table 1 are based on the mean risk across all butter clam samples and all steamer clam samples (all sites combined and averaged as a representative sample). Risks were calculated using three potential ingestion rates; Swinomish-specific ingestion rates currently are being quantified. Risk assessments of the other samples and contaminants currently are under way. If significant risks are elucidated, risk mitigation measures will be developed, and prominent health conditions will be confirmed to develop hypotheses related to found contaminants.

For results pertaining to the outreach and education component, the Swinomish Environmental Educator presented the Tox in a Box® educational toolkit in local middle and high schools and in the Swinomish preschool and Birth-to-Six programs. Evaluation forms from students and teachers provided positive feedback, and all of the teachers asked for secondary visits. Establishing annual presentations in the schools that focus on chemicals and the local environment currently are being planned. The Environmental Educator also collaborated with teachers in organizing and conducting field trips to wetlands on the reservation.

The first annual meeting of the tribal advisory group convened in 2003, the purpose of which was to share project design and implementation methodology and to provide assistance, where feasible. Representatives from nine Puget Sound area tribes attended. Discussion focused on collection and analysis methodology, possible funding sources for other tribes, and potential collaborations between tribes regarding toxic issues. Future meetings will disseminate research findings and discuss mitigation options.

Figure 1. Historic photo of Swinomish tribal members beach-seining.

Table 1. Mean risk across all butter clam samples and all steamer clam samples.

	1 g/day	100 g/day (3.3 oz.)	454 g/day (1 lb/day)
Butter Clam Cancer Risk (Average)	2.50E-07	2.50E-05	1.14E-04
Steamer Clam Cancer Risk (Average)	2.30E-07	2.30E-05	1.04E-04
Butter Clam Hazard Index (Non-Cancer Risk) (Average)	1.60E-03	1.60E-01	7.26E-01
Steamer Clam Hazard Index (Average)	1.40E-03	1.40E-01	6.36E-01

Presentation Abstract

The Effects of the World Trade Center Disaster on Pregnant Women and Their Infants

Trudy Berkowitz
Mount Sinai School of Medicine, New York, NY

The destruction of the World Trade Center (WTC) in New York City on September 11, 2001, released a toxic atmospheric plume that contained soot, benzene, polycyclic aromatic hydrocarbons, heavy metals, pulverized glass and cement, and alkaline particulates. Many of these substances have been linked in previous research to adverse perinatal outcomes and neurodevelopmental impairment in children, as well as to asthma and other respiratory diseases. The destruction of the WTC also was a source of enormous psychological trauma. The effects of traumatic stress on the health of pregnant women, and possibly their children, was expected to be substantial and may have interacted with the effects of airborne toxicants.

The specific aims of this project are to: (1) determine exposures to pollutants among pregnant women who were present at or adjacent to the WTC on September 11, 2001, or shortly thereafter; (2) document levels of stress and anxiety, including post-traumatic stress disorder symptoms (PTSS) in the exposed women; and (3) evaluate the effects of these pollutants, as well as stress and anxiety, on perinatal outcomes and childhood development. We established a cohort study of 187 women who were pregnant and present in one of five exposure zones near the WTC on or about September 11, 2001 (see Figure 1). Most of the women in the WTC cohort were white, married, ages 30 years or older, and college graduates. As a comparison group, we evaluated 2,367 private patients who delivered at Mount Sinai Medical Center during the same time period and who were not known to have been in lower Manhattan on September 11, 2001. No significant differences were seen between the two groups in the frequency of preterm births (9.9% in the WTC cohort versus 9.2% in the Mount Sinai cohort, $p = 0.76$) or low birth weight (8.2% versus 6.8%, $p = 0.47$). The WTC cohort, however, had a two-fold increased risk of small-for-gestational age infants (defined as a birthweight below the 10th percentile for gestational age), even after adjusting for potentially confounding factors. Furthermore, preliminary data suggest that infants of mothers in Zones 1 and 2 scored more poorly on some tests of early cognitive development at 9 months and, more importantly, had significantly lower scores on the Mental Development Index of the Bayley Scales of Infant Development at 2 years of age. We also have observed an inverse relationship between PTSS and head circumference at birth among women who were in their first or second trimesters on September 11, 2001. These findings suggest that the WTC disaster had a detrimental impact with respect to pregnancy outcome and early childhood development. Further analyses are needed to determine whether these effects were caused by toxicant exposures, the associated psychological trauma, or both. The children continue to have follow-up assessments for growth and neurodevelopment.

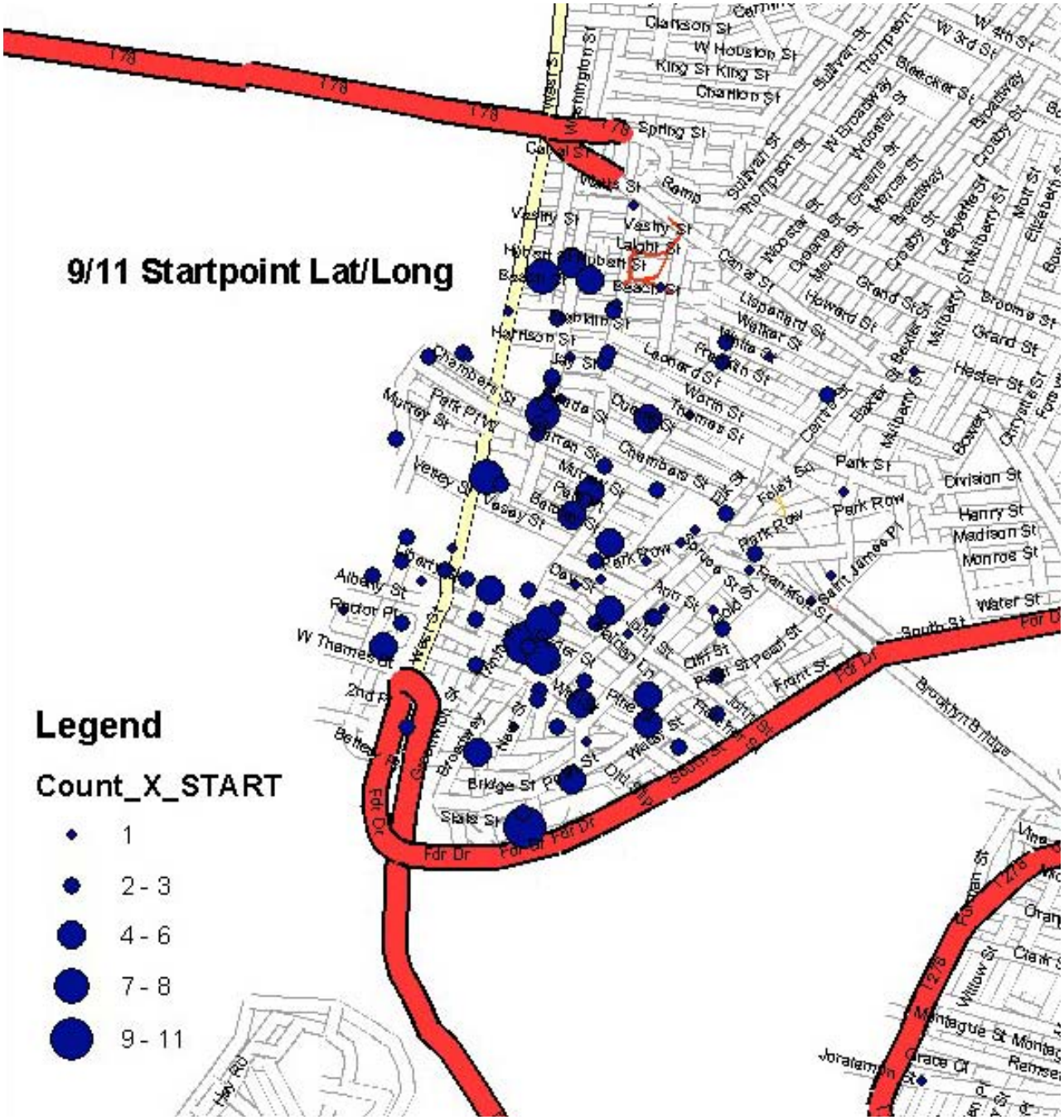


Figure 1. Location of 166 pregnant women who were in Zones 1-3 at 9 a.m., e.s.t., on September 11, 2001. The WTC is the blank trapezoid just south of Vesey Street. (Mount Sinai WTC Pregnancy Study.)

Presentation Abstract

Genetic Basis of the Increased Susceptibility of Children to Inhaled Pollutants

Terry Gordon, Al Gunnison, and Lung Chi Chen
New York University School of Medicine, New York, NY

Project Goals and Objectives:

The objective of this project is to determine the biological mechanism(s) underlying the increased susceptibility of children to inhaled pollutants. We hypothesize that there is a genetic basis for the differential response of neonatal and adult rodent lungs to inhaled pollutants. By testing this hypothesis, we will: (1) quantify the contribution of genetic versus environmental factors; (2) identify candidate genes that play a critical role in the molecular pathways leading to the increased susceptibility of the neonatal lung; and (3) compare these genes to those involved in adult lung toxicity.

Approach:

Pulmonary injury and inflammation have been examined in eight inbred strains of neonatal mice exposed to ozone. For comparison, adverse pulmonary changes also were examined in male and female adult mice.

Preliminary Findings:

A time-course study in neonatal mice demonstrated that the greatest adverse effects occurred on days 15 and 16 after birth. Clear interstrain differences in response to ozone were observed in neonatal mice: SJL, C3H/HeJ, and BALB/C mice were the most sensitive to ozone exposure, and AKR and 129 mice were the most resistant to ozone exposure. Examination of the absorbed ozone dose in the lungs of different strains of neonatal mice (using ozone generated from oxygen-18) demonstrated that dose was not a factor in the strain-dependent differences in response. Importantly, the response of neonatal mice was greater than that observed in ozone-exposed male and female adult mice, particularly in the SJL and C3H/HeJ strains of mice.

Significance of Findings:

The strain-dependent differences in the response of neonatal mice exposed to ozone strongly suggest that genetic determinants play an important role in the enhanced sensitivity of the juvenile lung to ambient air pollutants. This research will enable us to determine which genetic factors contribute to the susceptibility of the juvenile lung to ozone and to quantify the relative contribution of genes versus the environment in the adverse effects of inhaled ozone.

Future Steps:

This project will focus on the genetic factors that control the increased susceptibility of neonatal mice to the adverse effects of ozone. These studies will include microarray experiments to identify strain-dependent differences in gene expression, as well as quantitative linkage analysis experiments to pinpoint regions of the murine genome that contain genes responsible for the increased susceptibility of the neonatal lung exposed to ozone.

Presentation Abstract

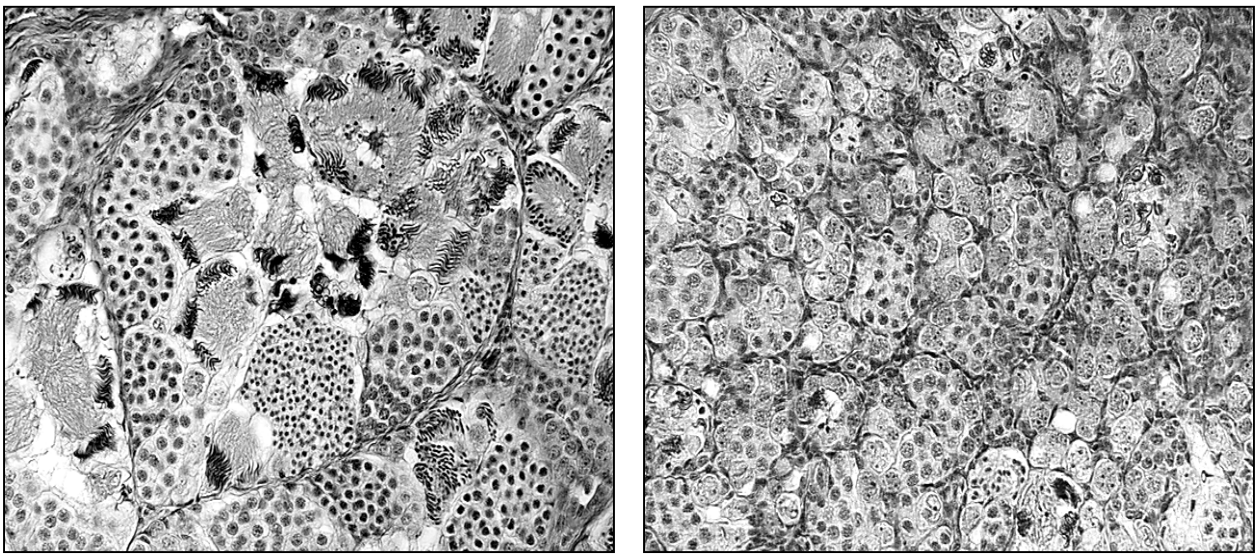
Impact of Phthalates on the Male: Frog and Rabbit Models

Shannon K. Lee, Gwendolyn A. Owens, Ty T. Higuchi, Jennifer S. Palmer, Carol L. Moeller, Ginger E. Sammonds, John D. Tessari, and D.N. Rao Veeramachaneni
Colorado State University, Fort Collins, CO

The objective of this research project is to test the hypothesis that exposure to dibutyl phthalate (DBP) during differentiation of the reproductive system, even at relatively low concentrations, alters reproductive function in adults. This hypothesis is being tested in two animal models: an amphibian, the South African clawed frog *Xenopus laevis*, and a nonrodent mammal, the rabbit. The former facilitates transdermal exposure and evaluation of an easy-to-monitor developmental process (metamorphosis), while the latter facilitates longitudinal evaluations of hormones, semen parameters, and sexual capacity. Furthermore, rabbits, unlike rodents, have a relatively long infantile period of reproductive development that more closely approximates the human situation. Dermal route of exposure is particularly pertinent to children's vulnerability to environmental toxicants, because children have a greater ratio of surface area-to-body weight than adults.

Xenopus embryos were exposed to 0, 0.1, 0.5, 1, 5, 10, and 15 ppm DBP during the first 4 days of life, when major organs differentiate and developmental effects were evaluated. DBP, at a concentration as low as 0.1 ppm, inhibited the growth of tadpoles. The lowest concentrations that caused death or a malformation in 50 percent of the population were found to be 14.5 ppm and 0.98 ppm, respectively. In another experiment, *Xenopus* tadpoles were exposed 0, 0.1, 0.5, 1, 5, and 10 ppm DBP between 21 and 54 days of life, when sexual differentiation and metamorphosis occur, and effects on reproductive development and spermatogenesis were evaluated at 33 weeks of age. Four to 6 percent of DBP-treated frogs had only one testis and 2 to 4 percent had retained oviducts. In all DBP-treated groups, seminiferous tubule diameters and number of germ cell nests per tubule were lower, and the number of tubules with no germ cell nests was higher ($p < 0.05$). The percent of secondary spermatogonial nests significantly decreased ($p < 0.05$) in 1.0, 5.0, and 10.0 ppm groups. In addition, dysgenetic tubules and degenerating or hypoplastic germ cell nests were observed in DBP-treated frogs (see Figure 1). Collectively, these observations indicate that DBP at environmentally relevant concentrations has the potential to cause irreversible damage to amphibian populations by affecting survival, development, growth, and spermatogenesis.

In a preliminary study, male rabbits were exposed *in utero* (gestation days 15-29) or during adolescence (postnatal weeks 4 to 12) to 0 or 400 mg DBP/kg body wt/d, and reproductive development and function were assessed after puberty at 25 weeks of age. The most pronounced effects were in rabbits exposed *in utero*. There was a 43 percent reduction in ejaculated sperm and a doubling in the incidence of abnormal sperm (16 to 30%). One of the 17 DBP-treated males had hypospadias, hypoplastic prostate, aplastic bulbourethral gland, and cryptorchid testes with carcinoma *in situ*-like cells. One of the 11 rabbits exposed during adolescence was unilaterally cryptorchid. In the ongoing definitive studies, male rabbits were exposed to 0, 5, 50, or 500 mg DBP/kg body wt/d during the two critical windows of development as in the preliminary study, and a variety of endpoints to assess sexual function are being evaluated. Analyses of tissue residues of monobutyl phthalate, the metabolite of DBP, are in progress.



Frog Testis—Control **Frog Testis—10 ppm DBP**

Figure 1. Testicular histology of *Xenopus laevis* frogs (both pictures were photographed at the same magnification).

Presentation Abstract

Mutations in Steroid 5-Alpha Reductase Type 2 and the Severity of Hypospadias

Jeanne M. Manson
Children's Hospital of Philadelphia, Philadelphia, PA

Project Goals and Objectives:

The purpose of this project is to evaluate gene-environment interactions for the risk and severity of hypospadias. Our hypothesis is that allelic variants in genes controlling androgen action and metabolism (steroid 5-alpha reductase type 2 [SRD5A2] and the androgen receptor) will be associated highly with the risk for and severity of hypospadias. Parental exposure to environmental agents during pregnancy may further increase the risk in a fetus with a susceptible genotype, resulting in a gene-environment interaction.

Approach:

This is the first study in a large, outbred population to investigate gene-environment interactions on the risk for this birth defect. Families of case infants of less than 1 year of age presenting for diagnosis and/or surgical repair of hypospadias in a pediatric urology clinic are recruited into the study. Families of affected control infants with renal anomalies are recruited from the same population. Parents are administered questionnaires to obtain information on reproductive and obstetrical history and exposures to drugs and environmental agents. Buccal swabs are collected from the mother, father, and infant, and DNA is extracted for an evaluation of candidate genes.

Preliminary Findings:

Results-to-date on approximately 350 families indicate that there are no differences between groups for maternal or paternal occupational exposures, whereas case mothers reported significantly more exposures to paints and stains, and case fathers to pesticides at home, than their respective controls. The most common genotype finding in the study population was a V89L missense mutation in exon 1 of the SRD5A2 gene. There was a highly significant association between the severity of hypospadias and the frequency of this mutation. There were no differences between case and control groups for CAG and GGC repeat lengths on the androgen receptor. To date, there is no significant gene-environmental interaction between this mutation and environmental exposures.

Significance of Findings:

This is the first study to identify a candidate gene for the severity of hypospadias and to find an association between exposures to paints, stains, and pesticides and the risk for hypospadias.

Next Step:

We will continue to enroll subjects into the study and evaluate genetic and environmental risk factors and their interactions for the risk for hypospadias.

Presentation Abstract

Prenatal Exposures of Children to Polybrominated Diphenyl Ethers: The Collection of Animal and Human Data Along With the Development and Validation of a PBPK Model

James Raymer, Amy C. Licata, and Ed Garner
RTI International, Research Triangle Park, NC

Exposures to environmental contaminants *in utero* and during childhood create the potential for a variety of adverse health effects, including abnormal or disturbed development of systems, such as the neurological system and the endocrine system. There is growing concern that the increasing incidence of ailments, such as adult and childhood cancers, reproductive and developmental anomalies, and behavioral deficits might be linked to environmental exposures early in life. Little is known about *in utero* exposures to most environmental chemicals, including the polybrominated diphenyl ethers (PBDEs). These PBDEs are known to have neurological effects and are suspected of having endocrine disruption capability. Ongoing work at RTI International has focused on the development of a physiologically based pharmacokinetic (PBPK) rat model for the PBDEs 2,2',4,4'-tetrabromomodiphenyl ether and 2,2',4,4',5-pentabromodiphenyl ether (see Figure 1). We are testing the hypothesis that these PBDEs can be used to estimate fetal exposures to PBDEs in humans. An analytical method is being developed and is based on extraction followed by gas chromatography with either electron capture detection or negative chemical ionization mass spectrometry. The method will be used to determine tissue:saline partition coefficients for PBDEs in maternal blood, plasma, liver, adipose, placenta, amniotic fluid, kidneys, muscle, gastrointestinal tract, and fetuses. Single and repeated dosing experiments (i.e., intravenous and oral gavage) are in progress to help develop the model. This presentation describes the dosing studies, aspects of the analytical method, and the initial PBPK model being developed.

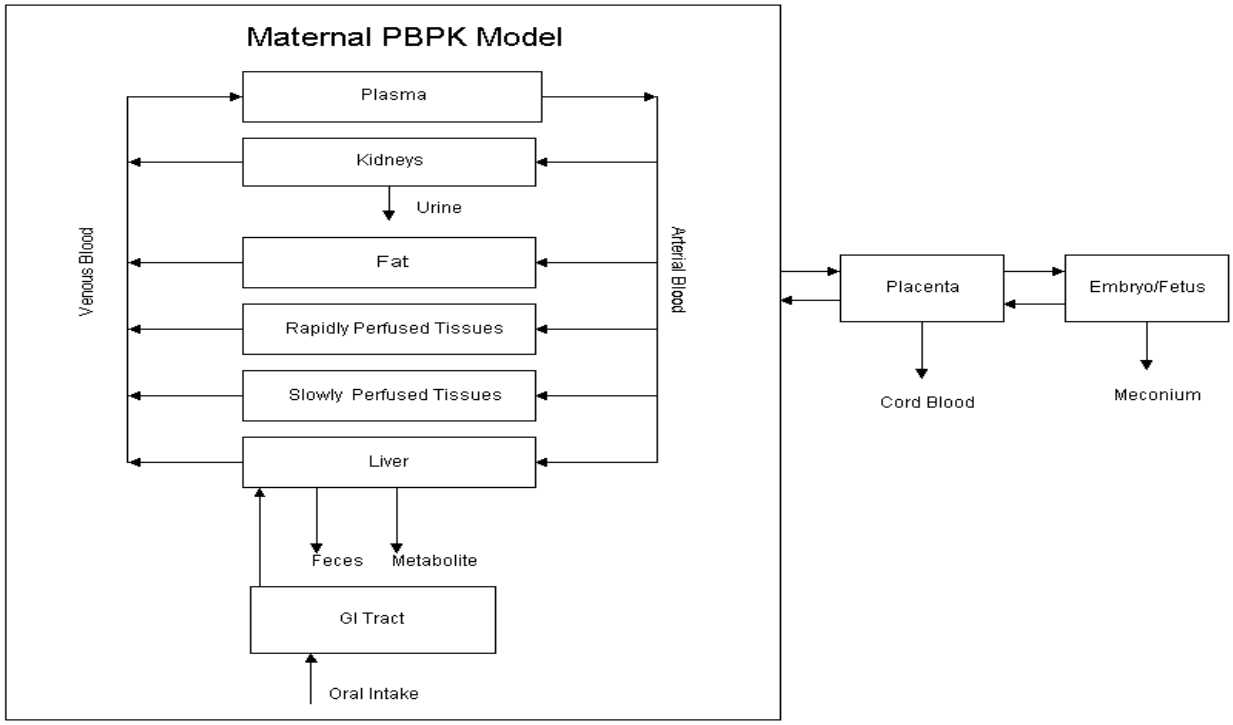


Figure 1. Schematic diagram of the initial PBPK model.

Presentation Abstract

Strain-Dependent Susceptibility to Transplacentally Induced Murine Lung Tumors

Mark S. Miller[1], Mian Xu[1], Joseph E. Moore[1], Nancy D. Kock[1], Garret B. Nelson[2], Stephanie T. Dance[1], Sandra Leone-Kabler[1], Alan J. Townsend[1], and Jeffrey A. Ross[2]
[1]Wake Forest University School of Medicine, Winston-Salem, NC; [2]U.S. EPA, Research Triangle Park, NC

Objectives:

Organ- and strain-specific differences in the levels of toxicant metabolism and/or DNA repair may determine the relative susceptibility of the developing organism to genetic damage that leads to the initiation of cancer. Several studies have shown that the developing organism is very sensitive to chemical and physical carcinogens, suggesting that exposure of pregnant women to environmental toxicants may place the embryo and fetus at higher risk for the induction of cancer. Despite this higher sensitivity and increased vulnerability, few studies have examined the mechanisms of cancer causation and toxic responses to environmental chemicals during gestation. The main goal of this research project is to elucidate the biochemical and molecular mechanisms that determine oncogenic damage and modulate susceptibility to chemical carcinogens during the sensitive period of fetal development.

Approach:

The levels of Phase I and Phase II enzyme activity in fetal tissues and their effects on the metabolism of MC in different strains of mice and F1 crosses between these mice were determined. In addition, the levels of DNA adducts and the rate of DNA repair (assessed by the decrease in DNA damage over time) were measured to determine if the observed differences in lung tumor incidence result from differences in the amount of damage to DNA that is induced by exposure to the chemical carcinogens or is the result of differences in the ability of the lung cells to repair the damage once it has occurred. We are in the process of assessing the tumors for mutations in specific genes that are associated with cancer in human patients, in particular the Ki-*ras* oncogene.

Preliminary Findings:

The results demonstrated that parental C57BL/6 mice are relatively resistant to the induction of lung tumors following transplacental exposure to 3-methylcholanthrene (MC). C57BL/6 mice exhibited only a 11 percent incidence of lung tumors, whereas Balb/c mice and crosses between the two parental strains demonstrated a 100 percent tumor incidence. Counting only lung lesions that were discrete, individual nodules, the numbers of tumors per mouse in Balb/c, [C57BL/6 x Balb/c]F1, [Balb/c x C57BL/6]F1, and C57BL6 mice were 5.8 ± 3.7, 5.0 ± 3.3, 4.8 ± 2.9, and < 0.1, respectively. Thus, Balb/c and DBA/2 strains of mice appear to have the dominant phenotype of tumor susceptibility. These differences in tumor incidence were not due to differences in induction of metabolic enzymes responsible for the activation (cytochromes *P4501A1* and *1B1*) or detoxification (glutathione *S*-transferases) of MC, the levels of DNA damage induced by the chemical carcinogen, or the repair of the damaged DNA.

Significance:

These studies highlight the important interactions between genetic and environmental factors in determining individual susceptibility to environmental toxicants during development. The results provide preliminary evidence suggesting the presence of a novel, unidentified gene locus that modulates sensitivity to chemically induced lung cancer specifically following exposure during the sensitive fetal period.

Next Steps:

Future studies will focus on identifying the gene locus responsible for the differential sensitivity to lung tumor induction during fetal exposures.

Poster Abstract

Reducing Uncertainty in Children's Risk Assessment: Development of a Quantitative Approach for Assessing Internal Dosimetry Through Physiologically Based Pharmacokinetic Modeling

James V. Bruckner, Kyu-Bong Kim, Satheesh Anand, Jeffrey W. Fisher, Srinivasa Muralidhara, Michael G. Bartlett, Catherine A. White, and Hyo-Joong Kim
College of Pharmacy, University of Georgia at Athens, Athens, GA

The overall goal of this project is to reduce uncertainties inherent in risk assessments of pesticides in infants and children. The primary objective is to develop and validate a physiologically based pharmacokinetic (PBPK) model that can be used to predict blood and target organ concentrations of parent compounds and major metabolites over time, after inhalation or oral pesticide exposures to children of different ages. Attention now is focused on deltamethrin (DLM), a type II pyrethroid insecticide of relatively high neurotoxic potency. Methods available for analysis of DLM in biological samples were time consuming and not sensitive enough to monitor the chemical's complete time-course after dosing. We developed and validated a rapid, sensitive high performance liquid chromatography procedure for: (1) separation of DLM and 3-phenoxybenzoic acid (PBA), one of DLM's major metabolites; and (2) quantification of DLM and PBA in both blood and solid tissues. To date, pharmacokinetic studies have been conducted in adult male and 10-day-old male Sprague-Dawley rats. A series of doses of DLM in glycerol formal were given by gavage. As shown in Figure 1, blood DLM levels were substantially higher after a 10 mg/kg oral dose in pups than in adults. (AUC_0^{6hr} in pups was 6.7-fold higher). The pups exhibited tremors and choreoatheosis and died within 6 hours, but the adults were asymptomatic other than for transient salivation. Blood PBA levels were markedly lower in the pups, apparently reflecting their lower capacity to metabolize DLM. Bioavailability was only approximately 17 percent in adults, while halflife was approximately 28 hours. DLM metabolism also was characterized in the liver and plasma of adult rats. The results indicate that DLM is metabolized primarily by liver cytochrome P450 and to a lesser extent by plasma and liver carboxylesterases. K_m and V_{max} values for each enzyme and tissue were determined *in vitro* by measuring the disappearance of parent compound from plasma and liver microsomal preparations. These metabolic parameters will be utilized in construction of a PBPK model for DLM in adult rats. Once this model is validated, metabolic and physiological parameters measured in immature rats of different ages will be used to extend the adult model to young rats. These age-specific parameters obtained for immature rats can be used subsequently for PBPK modeling of additional pyrethroids and other chemicals. Age-specific parameters for children, including metabolic constants measured *in vitro*, can be inputted into the models that generate predictions of internal doses of chemicals for a variety of exposure scenarios.

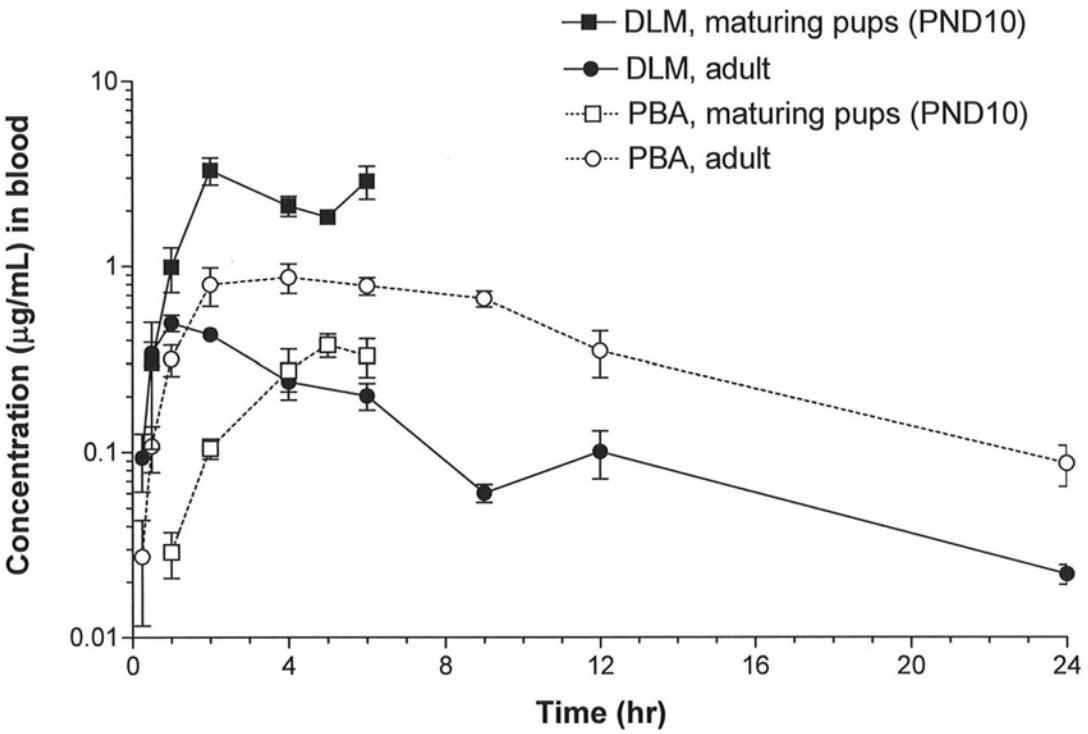


Figure 1. Blood concentration versus time profiles of DLM and PBA, a major metabolite, after an oral dose of 10 mg DLM/kg to adult rats and 10-day-old pups. The pups died after 6 hours. Data are ± standard error of the mean for three animals.

Poster Abstract

Determinants of Fetal Male Germ Cell Vulnerability to Phthalate Esters

Elena Kleymenova and Kevin W. Gaido
CIIT Centers for Health Research, Research Triangle Park, NC

Phthalate esters represent a class of environmental endocrine-active chemicals known to alter development of the male reproductive tract in rodents. Phthalates are used widely as softeners of consumer plastics and in solvents used in personal care, residential construction, and automotive products. Children and women of reproductive age have significantly higher urine concentrations of monobutyl phthalate, a monoester metabolite of reproductive toxicant di(n-butyl)phthalate (DBP), compared to the general population. Exposure *in utero* to DBP results in the development of genetically abnormal multinucleated gonocytes (MNG) in the fetal rat testis.

Goal:

Our goal is to identify key molecular and cellular events associated with germ cell development in the rat testis that are targets for endocrine active chemicals following *in utero* exposure.

Approach:

Timed pregnant Sprague-Dawley rats were exposed during late gestation to various dose levels of DBP, and testes were collected on gestation days 17 through 21 and postnatal days 1, 2, and 5. Cell proliferation, apoptosis, and Sertoli-germ cell interactions in the exposed fetal testes were evaluated using hematoxylin and eosin or immunostained tissue sections.

Findings:

MNG occur at low frequency during normal development of the rat testis. However, *in utero* exposure to DBP resulted in a significant increase in the number of these abnormal germ cells in the fetal testis on gestation day 21 (see Figure 1). Although there was a trend indicating that the 50 mg/kg dose level increases the incidence of MNG, statistical significance was achieved only at the 100 mg/kg dose level. Fetal MNG were neither proliferating nor undergoing apoptosis. Seminiferous cords in DBP-exposed fetal testes had abnormal morphology, and cell proliferation was significantly decreased at the 50 mg/kg/day dose level. Immunostaining revealed collapsed vimentin cytoskeleton of Sertoli cells and abnormal contact between fetal Sertoli cells and gonocytes in exposed testes. Electron microscopy confirmed a lack of normal contact between Sertoli cells and gonocytes and retracted (caused by collapsed cytoskeleton) cytoplasmic processes of Sertoli cells in exposed testes.

Significance:

In contrast to the adult and postnatal exposure, *in utero* exposure to DBP does not induce germ cell apoptosis, but decreases cell proliferation in the fetal rat testis in a dose-dependent manner. Adverse cellular responses to DBP in the fetal testis can be detected at lower doses compared to those causing gross pathological changes. A coincidental increase in the number of MNG and loss of normal contact between fetal Sertoli and germ cells suggest that abnormal interactions between these cells during fetal life play a role in the development of MNG.

Future Directions:

Changes in global gene expression in gonocytes following *in utero* exposure to DBP will be analyzed using laser capture microdissection combined with RNA microarrays. Proteins involved in Sertoli-germ cell interactions will be evaluated in fetal and postnatal testes using immunocytochemistry and Western blot analysis.

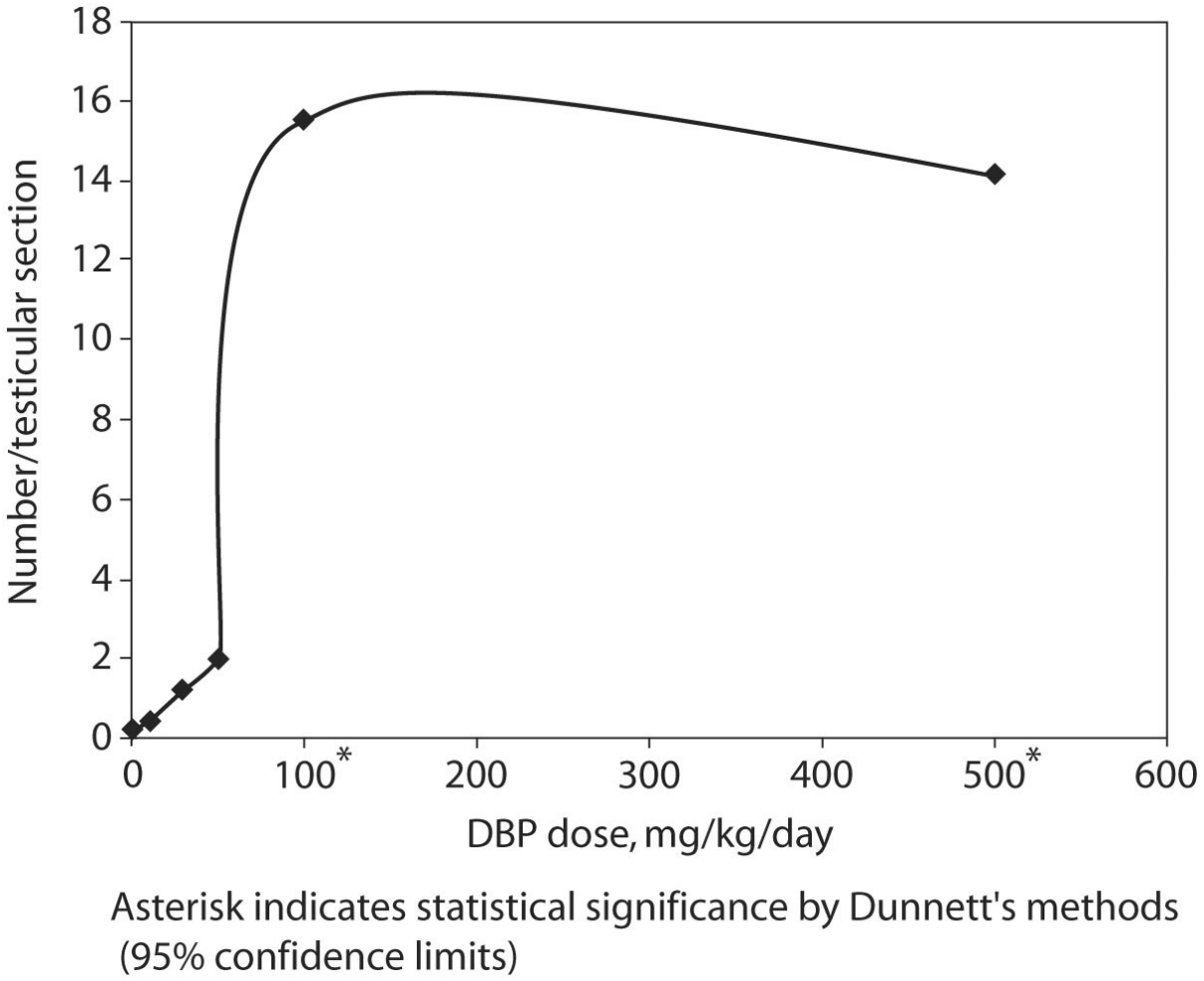


Figure 1. Dose-response to DBP exposure *in utero*: multinucleated gonocytes.

Poster Abstract

Neurobehavioral and Neuropathological Deficits in the Rat Offspring Following Maternal Exposure to Nicotine and Chlorpyrifos, Alone and in Combination

Mohamed B. Abou-Donia, Ali Abdel-Rahman, Anjelika M. Dechkovskaia, Larry B. Goldstein, Sarah H. Bullman, and Wasiuddin A. Khan
Duke University Medical Center, Durham, NC

Goals and Objectives:

The goals of the proposed studies are to characterize neurological deficits in rat offspring at various stages of the development after maternal exposure to nicotine and chlorpyrifos, alone and in combination during gestation days (GD) 4-20. Our hypothesis is that combined exposure to nicotine and chlorpyrifos during the critical periods of fetal development disrupts the structural organization of the cholinergic system and interferes with the neural transmission in the central nervous system (CNS), resulting in neurological deficits. The objective is to use an integrated approach of biochemical, neuropathological, and behavioral evaluations to characterize CNS deficits in the offspring.

Approaches:

Timed pregnant Sprague-Dawley rats (300-350 g) were treated daily with nicotine (1 mg/kg, subcutaneous, in normal saline) or chlorpyrifos (0.1 mg/kg, dermal, in ethanol) or a combination of nicotine and chlorpyrifos from GD 4-20. Control animals were treated with saline and ethanol. Signs of general toxicity in the mothers and the offspring were observed during the period of experiment. The offspring on postnatal days (PND) 30, 60, and 90 were evaluated for biochemical changes in cholinesterase in the plasma and brain regions, and ligand-binding densities for $\alpha4\beta2$ and $\alpha7$ nicotinic acetylcholine receptors in the cortex. Neuropathological evaluations on PND 30 were conducted using hematoxylin and eosin (H and E) and for PND 60 and 90 using cresyl violet staining. Glial fibrillary acidic protein immunostaining was used to assess astrocytic changes in the brain. Neurobehavioral evaluations for beam-walk time and score, incline plane, and forepaw grip strength were conducted on PND 90 offspring.

Findings:

On PND 30, male offspring from the mothers treated with nicotine alone gained significantly less weight compared to control offspring. On PND 30, male pups showed a significant increase in the acetylcholinesterase (AChE) activity in the brainstem (~ 134-148% of control) and cerebellum (~ 299-345% of control) in all treated groups. PND 30 female offspring showed a significant increase in the AChE activity in the brainstem of the chlorpyrifos-alone group and in the cerebellum of the pups from the combination of nicotine and chlorpyrifos. There was no change in the plasma butyrylcholinesterase (BChE) activity of male or female pups on PND 30. Histopathological evaluation by H and E staining on PND 30 showed an increased neuronal cell death in the cerebellum granular cell layer of the female offspring from nicotine or nicotine in combination with chlorpyrifos. An increase in glial fibrillary acidic protein (GFAP) immunostaining was observed in the CA1 subfield of the hippocampus and cerebellum on PND 30 female offspring from the mothers treated with either nicotine or nicotine in combination with chlorpyrifos. Male offspring from the same mothers showed a similar increase in GFAP expression, but to a lesser extent than females.

On PND 60, the female offspring from chlorpyrifos-treated mothers showed a significant increase (~ 183% of control) in plasma BChE activity. Male offspring from mothers treated with either chlorpyrifos or nicotine alone showed a significant increase in the AChE activity in the brainstem, whereas female offspring

from mothers treated with either nicotine or a combination of nicotine and chlorpyrifos showed a significant increase (~ 134% and 126% of control, respectively) in AChE activity in the brainstem. No significant changes were observed in the ligand-binding densities for α4β2 and α7 nicotinic acetylcholine receptors in the cortex. Histopathological evaluation using cresyl violet staining showed a significant decrease in surviving Purkinje neurons in the cerebellum of the offspring from nicotine-treated mothers. An increase in GFAP immunostaining was observed in the cerebellum of the offspring from the mothers treated with nicotine.

On PND 90, both male and female offspring from mothers treated with nicotine and chlorpyrifos, alone or in combination, showed significant impairments in beam-walk time, incline plane, and forepaw grip time. Male offspring showed greater deficits in behavioral performance than the female offspring. Female offspring from mothers treated with a combination of nicotine and chlorpyrifos showed a significant increase in plasma BChE activity. Brain regional AChE activity showed differential changes in male and female offspring. The brainstems and cerebellums of female offspring from mothers treated with nicotine or chlorpyrifos, alone or in combination, showed a significant increase, whereas the brainstems of male offspring from mothers treated with nicotine alone or a combination of nicotine and chlorpyrifos showed a significant increase in AChE. Ligand-binding densities for α4β2 and α7nAChR did not show any significant changes in the cortex in both male and female offspring from treated groups. Histopathological evaluations using cresyl violet staining showed a significant decrease in surviving Purkinje neurons in the cerebellum. An increase in GFAP immunostaining was observed in cerebellum white matter and the granular cell layer of the cerebellum.

Significance:

Cigarette smoking and environmental exposure to chlorpyrifos during pregnancy could lead to developmental toxicity in the offspring at different ages in life. These data suggest that maternal exposure to nicotine and chlorpyrifos, alone and in combination, produces differential effects on brain regional AChE activity, significant decrease in the surviving neurons in the CA1 subfield of the hippocampus on PND 30 and in the cerebellum on various developmental stages, and an increased expression of GFAP in the cerebellum of adult offspring. A significant neurobehavioral deficit in male and female adult offspring suggests that maternal exposure to nicotine or chlorpyrifos at realistic doses may produce these abnormalities at adulthood.

Next Step:

A dose-response study and the mechanisms of neuronal deficits after developmental exposure to nicotine and chlorpyrifos during different stages of the CNS development warrant further investigation.

Poster Abstract

Neurokinin Receptors and Environmental Lung Injury

Gary W. Hoyle
Tulane University Health Sciences Center, New Orleans, LA

Respiratory irritants encountered as pollutants in ambient air and in occupational settings damage the lung and induce inflammation of the respiratory tract, respiratory symptoms, and decrements in lung function. Lung injury and inflammation caused by respiratory irritants also may exacerbate lung diseases such as asthma and chronic obstructive pulmonary disease. Inhalation of irritants stimulates sensory nerves that innervate the lung to release chemicals known as tachykinins. Tachykinins regulate irritant-induced inflammation and lung injury by binding to proteins called tachykinin receptors (also known as neurokinin receptors) that are present on a variety of cell types in the lung. Tachykinin receptors, in turn, activate signaling cascades that produce the downstream effects on lung injury and inflammation. Tachykinins released from sensory nerves can have protective or detrimental effects on lung injury, depending on the context of the noxious stimulus. We hypothesize that these divergent responses are mediated by differential signaling pathways that are activated by tachykinin receptors after irritant exposures. Our studies are focused on identifying and characterizing these signaling pathways. The objectives are to: (1) characterize the role of sensory nerves, tachykinins, and tachykinin receptors in modulating lung injury and inflammation in mice exposed to ozone (an irritant pollutant in ambient air) or hexamethylene diisocyanate biuret trimer (HDI-BT; an irritant compound that is a component of polyurethane spray paints); (2) determine the effects of increasing the number of tachykinin receptors in lung cells on inflammation and injury caused by ozone or HDI-BT; and (3) characterize signaling pathways downstream from tachykinin receptor activation that mediate the divergent responses to ozone and HDI-BT.

The objectives are being addressed using mouse models, in which the amount of sensory innervation of the lung or the number of tachykinin receptors is manipulated, as well as cell culture models, in which intracellular signaling pathways can be dissected. In ozone-exposed mice, sensory nerves stimulated inflammatory processes, including the influx of inflammatory cells and production of proinflammatory growth factors. To study signaling pathways involved in this process, we developed a model in which tachykinin receptor activation in cultured lung cells turns on a proinflammatory program within the cell. This model was used to identify intracellular signaling events that activate this proinflammatory response. In mice exposed to HDI-BT, sensory nerves induced a protective effect against lung injury and inflammation. Likewise, in mice manipulated to increase the number of tachykinin receptors in the lung, inflammation and lung injury induced by HDI-BT inhalation were reduced. We developed a model in which cultured lung cells are protected from HDI-BT-induced cell death by tachykinin receptor activation, and we characterized some basic aspects of this mechanism (see Figure 1). These results indicate that tachykinins can activate two main intracellular signaling pathways, one of which is beneficial and one of which is detrimental. Future studies will focus on understanding the nature of these pathways more completely at the molecular level and on developing methods by which the protective pathway can be activated preferentially as a means of reducing susceptibility to irritant-induced lung injury.

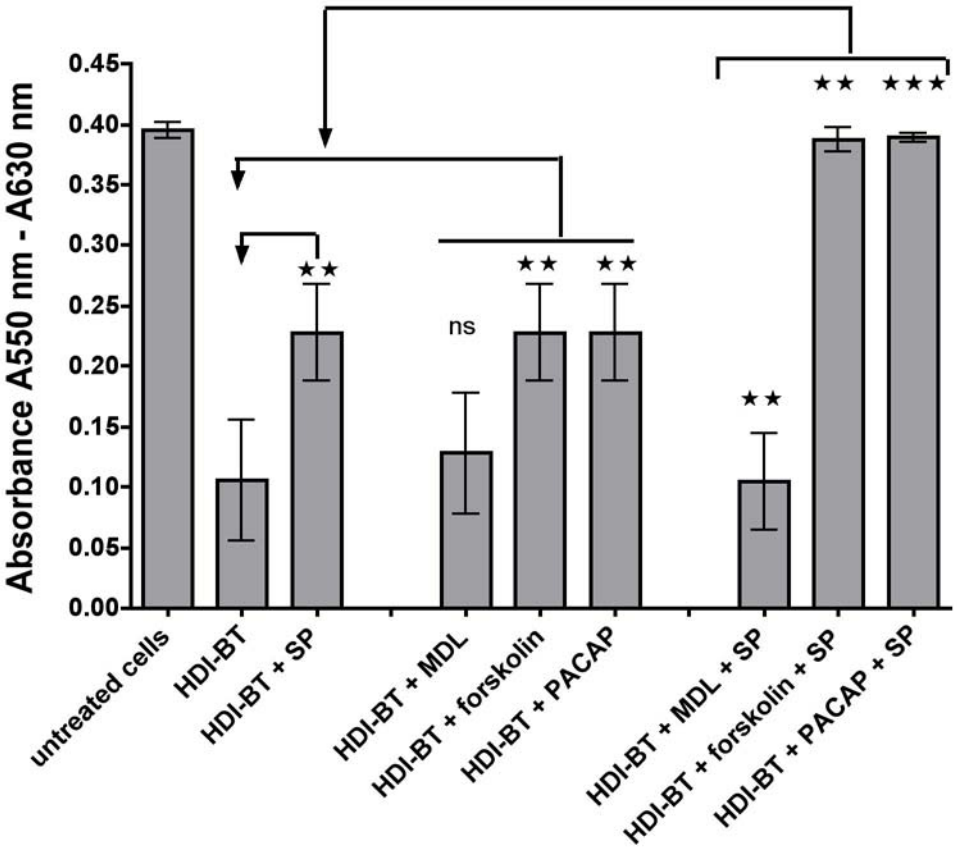


Figure 1. The protective effect of substance P on HDI-BT-induced toxicity is mediated by cyclic adenosine monophosphate (AMP). A549 human lung epithelial cells were treated in various ways, and cell viability was measured using a microtiter assay. Treatment of cells with 8 mg/mL HDI-BT caused a decrease in cell viability, and this effect was significantly inhibited by treatment with 100 nM of the tachykinin substance P. Treatment with 26 µM MDL-12, 330A (adenylate cyclase inhibitor) inhibited the protective effect of substance P, whereas 240 µM forskolin or 22 µM pituitary adenylate cyclase activating polypeptide 1-38 (adenylate cyclase activators) mimicked the protective effect. Bars represent mean ± standard deviation of eight wells. ns: not significant; **: $p < 0.001$; ***: $p < 0.0001$. These results implicate cyclic AMP as an important signaling molecule in tachykinin-mediated protection from HDI-BT-induced cytotoxicity.

Chemical Mixtures

Presentation Abstract

PAH/Metal Mixtures—Human *In Vitro* Mutagenicity Studies

Laurence Kaminsky and Erin Bessette
New York State Department of Health, Wadsworth Center, and the University at Albany, State University of New York, Albany, NY

Polycyclic aromatic hydrocarbons (PAHs) and heavy metals are often environmental co-contaminants that could interact to alter PAH carcinogenicity. The goals of this project are to assess the extent to which the four most hazardous environmental metal contaminants affect the putative carcinogenicity of the five most hazardous environmental PAH contaminants. The aim of this project is to determine how one of these metals, arsenite, affects the bioactivation that is essential for expression of the carcinogenicity of the PAHs. This bioactivation is catalyzed primarily by a form of cytochrome P450, CYP1A1. The heavy metal, arsenite, and the PAH, benzo[k]fluoranthene (BKF), were used as prototypes to investigate, in human liver cancer HepG2 cells, mechanisms whereby the bioactivation of BKF by human CYP1A1 could be diminished by arsenite-mediated decreases in CYP1A1 induction by BKF. To determine whether arsenite downregulates CYP1A1 transcription, quantitative realtime reverse transcriptase-polymerase chain reaction assays and luciferase reporter gene expression assays were used with HepG2 cells treated with BKF and arsenite, separately and as a mixture. BKF (0.5 μM) and arsenite (5 μM) markedly decreased BKF-mediated induction of CYP1A1 mRNA by 45 percent (see Figure 1). Plasmids containing the CYP1A1 promoter region (pHu-1A1-FL) were induced 7.4-fold over the BKF administration vehicle by BKF (0.5 μM), whereas arsenite (1, 2.5, or 5 μM) decreased reporter gene expression by 46 percent, 45 percent, and 61 percent, respectively. The plasmid, pHu-1A1-Δ 100-FL, which is a truncated version of Phu-1A1-FL, with the xenobiotic response element sites at positions -1061 and -981 removed, showed greater responsiveness relative to pHu-1A1-FL by 1.7-fold (suggesting that a negative response element also was removed). BKF (0.5 μM) and arsenite (1, 2.5, or 5 μM) decreased reporter gene expression by 0 percent, 27 percent, and 39 percent, respectively. Thus, arsenite did not affect BKF induction directly through these xenobiotic response element sites. Arsenite is stable for at least 48 hours in the HepG2 cells medium with respect to its ability to diminish CYP1A1 BKF induction, and it did not affect the stability of CYP1A1 mRNA. Another construct that contained the sequence of a xenobiotic response element site was transfected into HepG2 cells. It was induced by BKF administration, but the induced levels were not affected by simultaneously administered arsenite. The result implies that the effects of arsenite were not mediated through the aryl hydrocarbon receptor pathway. We conclude that arsenite affects the transcriptional regulation of the BKF-mediated induction of CYP1A1, which could diminish PAH carcinogenicity by decreasing its bioactivation by CYP1A1.

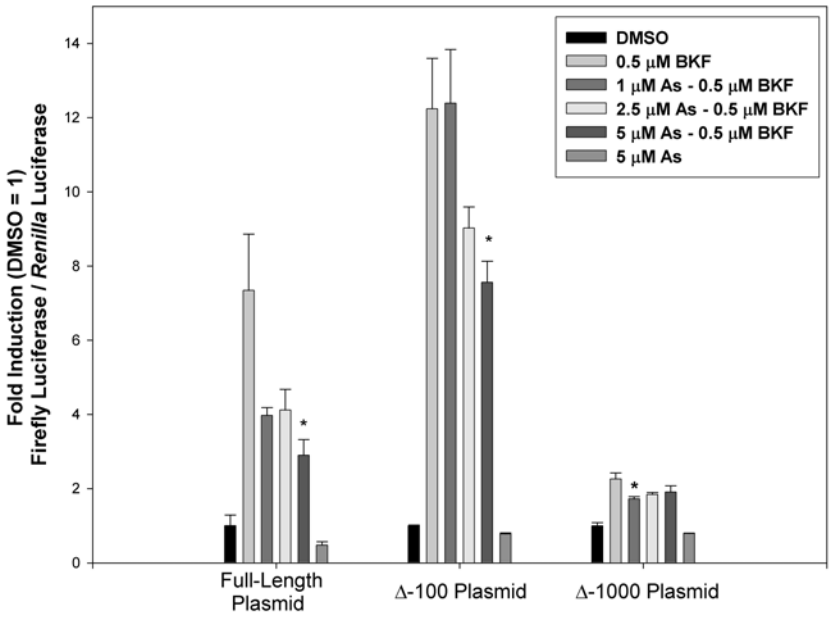


Figure 1. BKF-responsiveness of pHu1A1-FL, pHu1A1-Δ 100-FL (Δ -100), or pHu1A1-Δ 1000-FL (Δ-1000) experimental plasmid when cotransfected with pRL-CMV into HepG2 cells. After 24-hour transfection, the cells were treated with dimethylsulphoxide (DMSO) vehicle; 0.5 μM BKF; 0.5 μM BKF and 5 μM arsenite; or 5 μM arsenite alone. Cells were harvested 24 hours post-treatment, and firefly luciferase experimental reporter activity was determined in the cell lysates followed by normalization using *Renilla* luciferase activity. Each transfection was performed in triplicate. Values are reported as the mean ± standard error (n = 3). The mean DMSO values (n = 3) were normalized to 1 by multiplying by 4.2 (full-length plasmid), 82.6 (Δ-100 plasmid), or 249.6 (Δ-1000 plasmid). Deletion of the 100-bp fragment and the 1,000-bp fragment was performed using QuikChange site-directed mutagenesis kits. (*Significantly different from BKF-induced level, $p \leq 0.05$.)

Presentation Abstract

Regulation of Embryonic Neuronal Development by Chemical Mixtures From Brick, New Jersey

Jill A. Kreiling, Rachel L. Cox, and Carol L. Reinisch
Marine Biological Laboratory, Woods Hole, MA

The goal of this project is to use an embryonic model to assess the neurological effects of environmental contaminants that were found in Brick, NJ. Our interests lie in the identification of exposure-induced changes occurring at the molecular level during development of the nervous system. Specifically, altered expression patterns of various proteins and their corresponding transcripts, including transcription factors (*p53* and its family members), and kinases are being analyzed.

Initially, surf clam (*Spisula solidissima*) embryos were used, a unique marine model well suited for this study by virtue of a highly synchronized and rapidly developing nervous system. Using bromoform, chloroform, and tetrachloroethyelene (BCE), a ternary mixture of contaminants, we tested the hypothesis that BCE exposure specifically alters neural signaling pathways during development in *Spisula*.

Our results[1] showed that BCE exposure increases the expression of RII, a developmentally expressed isoform of the regulatory subunit of protein kinase A (PKA). Specifically, BCE exposure induces a global and localized increase in RII, at the site of innervation for the primordial gill and ciliated velar epithelium. A resultant enhanced ciliary activity in the velum causes increased larval swimming speed. Importantly, these effects occur only with the ternary mixture; each component, tested individually or in pairs, has no significant effect on RII levels or ciliary activity.

The results documented in *Spisula* demonstrate the selectivity of the Brick, NJ, contaminants for specific targets in the developing embryo. PKA is part of a ubiquitous signaling pathway known to be involved in learning and memory across species. Therefore, the results imply that chronic exposure to the BCE contaminants in drinking water could alter normal neuronal development during embryonic development in humans.

Our studies using the *Spisula* model served to underpin our current research in the aquatic species *Danio rerio* (zebrafish). The prevalence of PKA in neural tissue and the highly conserved nature of the protein across species indicate the relevance of this pathway to comparative toxicogenomic analysis. Following the *Spisula* model, we extended our experimental program to zebrafish, a species for which the entire genome is accessible.

Preliminary confocal studies using the mytilus RII antibody in zebrafish show a more intense staining of neural cell bodies localized to the region of the developing spinal chord in BCE exposed versus normal embryos (see Figure 1). These results demonstrate that exposure to BCE specifically targets a neural pathway by causing a localized increase in RII production during development. The data thus far confirm the results in *Spisula* and show that the direct effect of environmental contamination on neural development is reproducible in zebrafish, a vertebrate species.

Further analysis will require production of specific molecular probes based on sequences of genes involved in the RII signaling pathway. Using zebrafish, a model species well established for its utility in high-throughput genetic screens, we will continue to define the impact of chemical exposure on development of the vertebrate nervous system.

Reference:

1. Kreiling J.A., R.E. Stephens, C.L. Reinisch. A mixture of environmental contaminants increases cAMP-dependent protein kinase A *Spisula* embryos. *Environmental Toxicology and Pharmacology* (in press, 2005).

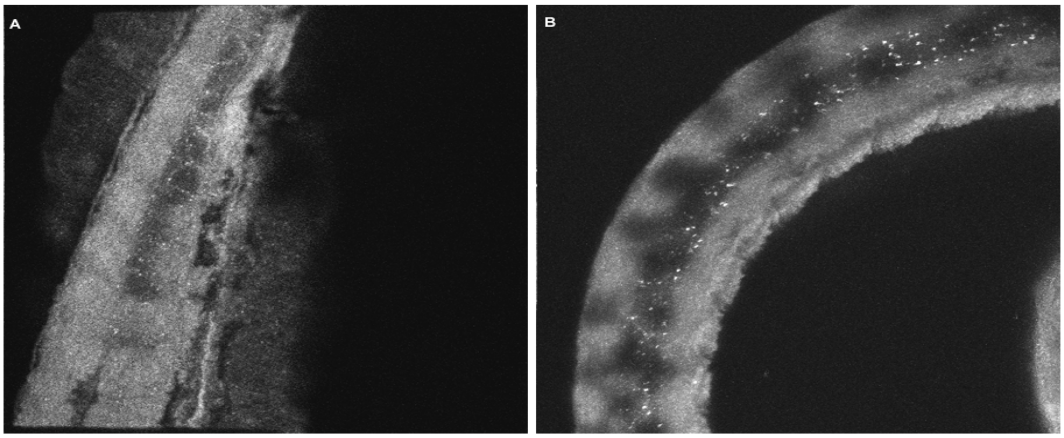


Figure 1. Forty-eight hour zebrafish embryos labeled with an antibody to mytilus RII and a rhodamine conjugated secondary antibody. (A) Control. (B) Triple mixture treated. Note the increased staining in the cell bodies along the central region of the treated embryo.

Presentation Abstract

Comparative *In Vitro* Immunotoxicity of Organochlorine Mixtures

Sylvain DeGuise
University of Connecticut, Storrs, CT

Program Goals and Objectives:

This project studies the immunotoxicity of mixtures of organochlorines (OCs) at relatively low concentrations in mice, humans, and marine mammals. The objectives of this project are to: (1) assess the interactions of OCs in mixtures; (2) assess differences between species; (3) validate the *in vitro* exposure model in mice; and (4) attempt to assess exposure at the cellular level using antibodies.

Approach:

The mixtures tested represent all of the possible combinations using five individual compounds: polychlorinated biphenyl (PCB) International Union of Applied Chemistry numbers 138, 153, 169, 180; and 2,3,7,8-tetrachlorodibenzodioxin. These mixtures are used in *in vitro* immunological assays, wherein functions of exposed cells are compared to those of unexposed control cells. The significance of *in vitro* exposure to determine immunotoxicity compared to traditional *in vivo* exposures will be validated in mice. We also tested a new rapid and economical method of assessing exposure to PCBs at the cellular level using antibodies and flow cytometry.

Preliminary Results:

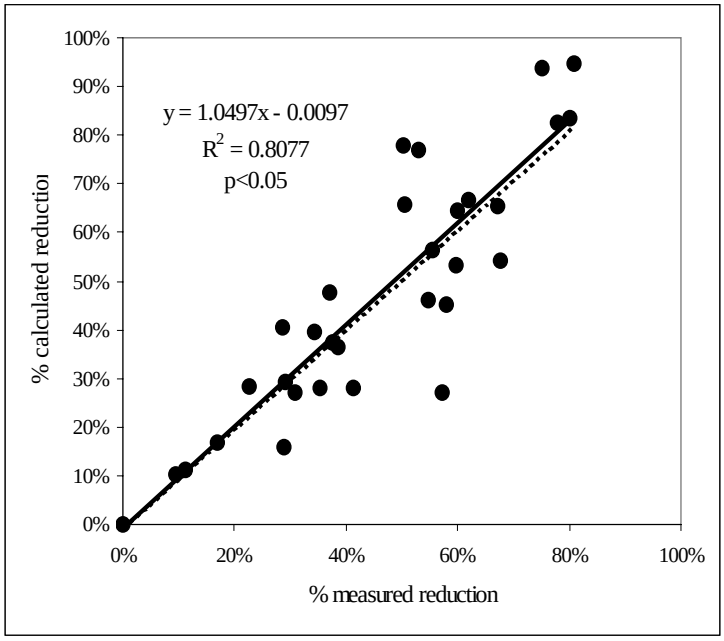
Modulation (decreases and increases) of T-cell proliferation was observed in several mixtures. B-lymphocyte proliferation was reduced by all mixtures in mice, but either was not affected or increased in marine mammals. Phagocytosis was modulated (usually decreased) by organochloride (OC) mixtures in neutrophils and monocytes in humans, in 9/9 and 6/7 marine mammal species, respectively, whereas no effects were detected in mice. Respiratory burst either was increased or reduced in marine mammals, but not affected in mice. Natural Killer activity was not affected by OCs in beluga whales. OCs modified the relative proportion of human lymphocyte populations in culture. Our results demonstrate complex (synergistic and antagonistic) interactions between congeners (see Figure 1), sometimes mediated specifically by the noncoplanar congeners. Toxicity varied considerably between species, and phylogeny failed to predict toxicity. The traditional toxicity equivalence (TEQ) and mouse models failed to predict effects in other species. The use of antibodies to assess exposure at the single cell level was not successful.

Significance of Findings:

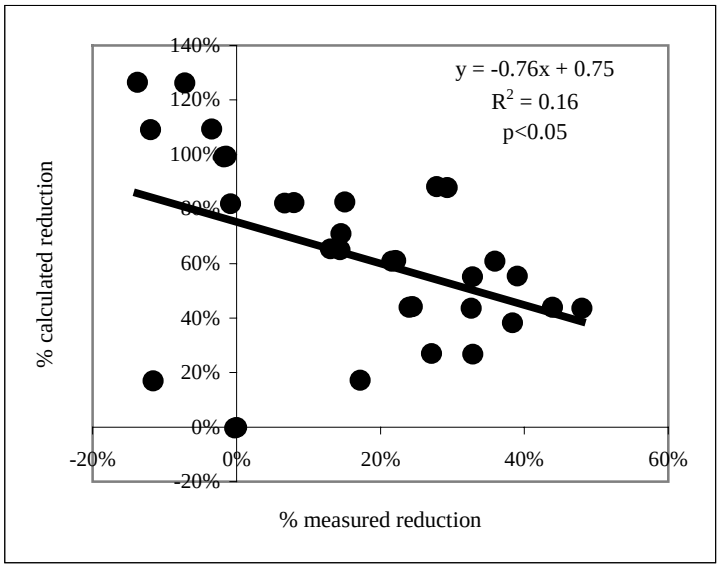
The development and validation of *in vitro* assays will provide an attractive and economical alternative for regulatory purposes. It also will provide an opportunity for studies in species (such as humans, marine mammals, and endangered species) for which controlled *in vivo* exposures are impractical for logistic, economic, and ethical reasons. The clarification of the complexity of the interactions (i.e., synergistic/antagonistic properties) between OCs will be important in the assessment of the immunotoxicity of mixtures. Our results shed light on new mechanisms of immunotoxicity and highlight the inability of the widely used mouse model and TEQ approach to predict accurately the effects of exposure to mixtures of OCs in other species. Our model will be useful for risk assessment and management for wildlife and humans.

Next Steps:

The validation of the *in vitro* approach in mice will be performed shortly. The continuing (opportunistic) sampling and analysis of marine mammal blood will allow the completion of assays for the species and functions for which we do not yet have sufficient statistical power.



Bottlenose dolphin



Northern fur seal

Figure 1. Correlation between the effects predicted (calculated) from those of the compnents of mixtures and those measured on experimental exposures. Significant positive correlations (such as in bottlenose dolphins) suggest additive effects; negative correlations (as in northern fur seals) suggest more complex interactions.

Presentation Abstract

Mechanistic Evaluation of the Toxicity of Chemical Mixtures

Allen Olmstead
North Carolina State University, Raleigh, NC

In this study, nine chemicals were chosen from a recent report on surface water concentrations of a variety of xenobiotics to test the hypothesis that the toxicity of chemical mixtures could be estimated using a model based on the toxicity of the individual chemicals. Concentration-response curves for the endpoints of lifespan, growth rate, and fecundity were generated for each chemical experimentally using the crustacean, *Daphnia magna*. From this data, a mathematical model for the combined toxicity of mixtures was generated that combined the concepts of concentration addition and independent joint action. Toxicity of a mixture was modeled at various levels at which the ratio of the chemicals within the mixture was maintained at that reported for median detectable environmental levels. Toxicity of the mixture was determined experimentally and compared to model predictions. The model predicted the most sensitive endpoint and defined the lowest toxic-effect level of the mixture. For this mixture of chemicals, results demonstrated that toxicity was not influenced significantly by interactions among the chemicals, and toxicity tended to be dominated by a single constituent. According to model predictions, the median detectable environmental concentrations of chemicals constituting this mixture provide no margin of safety.

Poster Abstract

Lumped Chemical Approach for Fate and Transport Modeling of Organic Pollutant Mixtures

Kenneth F. Reardon, Jin Chul Joo, Matthew Hoelscher, Charles D. Shackelford, and Amy Pruden
Colorado State University, Fort Collins, CO

Project Goals and Significance:

Accurate risk assessment and effective risk management rely on knowledge of the transport and fate of contaminants. Whereas this is a difficult task for single chemicals, it is much more challenging for mixtures. The primary goal of this project is to model the transport and fate of organic chemical mixtures using lumping analysis. With this approach, chemicals are grouped into "pseudocompounds," which then are modeled in place of the original mixture components. The user of this method has the flexibility to choose his or her desired position on the tradeoff between simplicity and accuracy. A secondary goal is to evaluate microbial community changes and changes in biodegradation rates along the flow path of a model aquifer system. Four objectives will be addressed: (1) measure mixture biodegradation kinetics and expand/refine our previous lumped chemical model for biodegradation kinetics; (2) determine mixture transport properties; (3) evaluate mixture transport and fate in soil columns (including microbial population analysis); and (4) develop lumped chemical modeling for the transport and fate of the organic chemical mixture.

Approach:

The 12-chemical mixture used in this research was chosen with the following criteria: prevalence at Superfund sites, high rankings on the Agency for Toxic Substances and Disease Registry list, at least moderate solubility, and documented biodegradability. The mixture chemicals are alkyl-substituted benzenes (benzene, toluene, and M-xylene); alkyl-substituted phenols (phenol, P-cresol, and 2,4-dimethyl phenol); ketones (acetone, 2-butanone, and 2-hexanone); and chlorobenzenes (chlorobenzene, 1,4-dichlorobenzene, and 1,2,4-trichlorobenzene). We have developed a stable microbial community from a soil inoculum and are using several model soils, each with different organic matter content. Experiments followed by grouping and modeling are performed separately for sorption and biodegradation, and the two models then are linked and tested in column experiments.

Preliminary Findings:

Experiments evaluating sorption of the mixture chemicals have yielded extensive data on the effects of organic matter content for both polar and nonpolar compounds, and have provided evidence that mixture effects are minimal (e.g., the sorption of one chemical is not influenced by the presence of others). Statistical methods were used to group the mixture chemicals, and these groups changed in a logical manner as the soil organic matter increased (see Figure 1). Similarly, experiments with the mixed microbial culture were used to group the chemicals according to their biodegradation kinetics. In this case, significant mixture effects were noted: some compounds are degraded more quickly in the mixture, but the degradation of others is inhibited by the presence of other mixture components.

Significance of Findings:

These results will provide the basis for the model based on "pseudocompounds," which will facilitate risk assessment and management. In addition, the data generated regarding sorption and biodegradation of chemical mixtures have basic scientific value.

Next Steps:

Current experiments are aimed at determining the robustness of the chemical groups determined for sorption, and at measuring the biodegradation model parameters for each group. Following that, column experiments will be performed to integrate the biological and abiotic effects. In these experiments, molecular biological methods will be used to track changes in the microbial community along the columns.

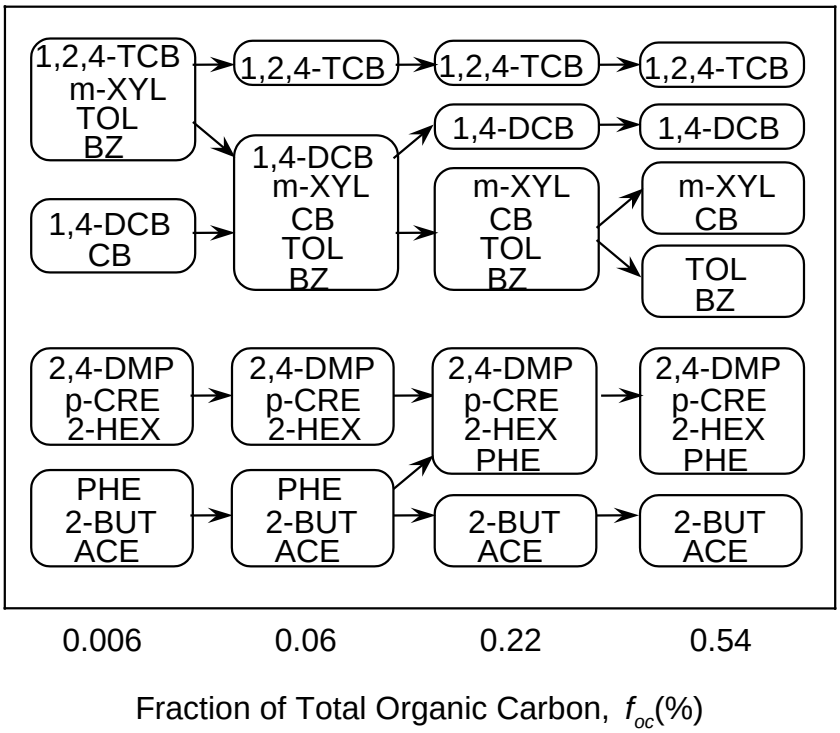


Figure 1. Optimal grouping of chemical mixture components into pseudocompounds as a function of soil organic matter content.

Poster Abstract

Total Metal Ion-Synthetic Chelating Agent Interactions in Aqueous Media

Alan T. Stone
G.W.C. Whiting School of Engineering, The Johns Hopkins University, Baltimore, MD

Project Goals and Objectives:

The choice of a chelating agent for a particular industrial or household application is based on perceived efficacy. This choice has environmental consequences. When present in effluent waters, some chelating agents are much better at solubilizing adsorbed or precipitated toxic metal ions than others. Different toxic metal ion-chelating agent complexes, once they have formed, react at different rates and through different pathways. In this presentation, structure-reactivity relationships among chelating agents pertaining to the aqueous chemistry of chromium(III) (Cr[III]) and manganese(III) (Mn[III]) are explored. The goal of this project is to predict particularly problematic metal ion-chelating agent combinations.

Approach:

Capillary electrophoresis enables us to identify and quantify chelating agents, their breakdown products, and their metal ion complexes in unprecedented detail, thereby adding a new dimension to the development of structure-reactivity relationships.

Preliminary Findings:

Dissolved Cr(III). At several locations in the mid-Atlantic, soils and sediments are contaminated with Cr(III)-containing solids. We have shown that common industrial and household builders (e.g., ethylene diamene tetracetic acid [EDTA]) can solubilize amorphous Cr(III) (hydr)oxide solids. Oxidation by Mn(III, IV) (hydr)oxides represents a possible environmental sink for these Cr(III)-chelating agent complexes. Cr(III) complexes with the inorganic ligands chloride and sulfate are oxidized by Mn(III, IV) (hydr)oxides far more rapidly than complexes with common builders (see Figure 1). Chelating agents that occupy six coordination positions around the central Cr(III) atom yield the slowest rates. One-to-one ratio complexes with nitrilotriacetate, for example, react two orders-of-magnitude more rapidly than 1:2 complexes with iminodiacetate. Cr(III) complexes with EDTA did not exhibit degradation during month-long experiments.

Dissolved Mn(III). Manganese is not usually considered a contaminant, but industrial activities can cause its accumulation. The chlorination of estuarine water for cooling water operations is one situation in which this can take place—cooling water pipes can become coated with Mn(III, IV) (hydr)oxides. Manganese in multiple oxidation states also is present naturally within soils and sediments. Our experiments focus on the (hydr)oxides "birnessite," which contains both Mn(III) and Mn(IV), and "manganite," which consists solely of Mn(III). The ability of more than two dozen organic chemicals to solubilize Mn(III,IV) (hydr)oxides has been investigated. Within timescales of 1 week or less, pyrophosphoric acid, methylenediphosphonic acid, and phosphonoacetic acid react solely via a process of ligand-assisted dissolution, which does not change the Mn oxidation state; Mn(III) is solubilized, whereas Mn(IV) is left in the precipitated state. (Note: phosphonoacetic acid is an antiviral pharmaceutical that might be present in wastewater.) The scale-control chemical 2-phosphonobutane-1,2,4-tricarboxylic acid, as well as citric acid and phosphonoformic acid (another antiviral pharmaceutical), also generate dissolved Mn(III). Depending on pH and medium composition, however, they also yield appreciable amounts of dissolved Mn(II), generated by a process termed reductive dissolution.

Significance of Findings:

Our Cr(III) experiments remind us that the environmental impact of synthetic organic chemicals should not be considered in isolation. Particular structures especially are effective at solubilizing Cr that may have entered the environment decades ago. Functional groups and structure also control the balance between source and sink terms (i.e., oxidation by Mn), and therefore control steady-state concentrations. Our experiments examining the production of dissolved Mn(III) indicate that naturally occurring metals can be rendered toxic through interaction with synthetic organic chemicals. Principles developed in this research enable us to distinguish chelating agents capable of dissolving Mn(III) from those that are not.

Next Steps:

Additional experiments are under way, in which additional chelating agent classes are explored. By systematically changing organic chemical structure, we are identifying pathways of both intramolecular and intermolecular redox reaction involving metal ion-organic chemical complexes. Fundamental chemical information of this kind should allow us to identify metal ion-organic combinations that yield undesirable synergistic effects.

$$2Cr^{III}L + 3MnO_2(s) + 2H_2O = 2HCr^{VI}O_4^- + 3Mn^{2+} + 2H^+ + L$$

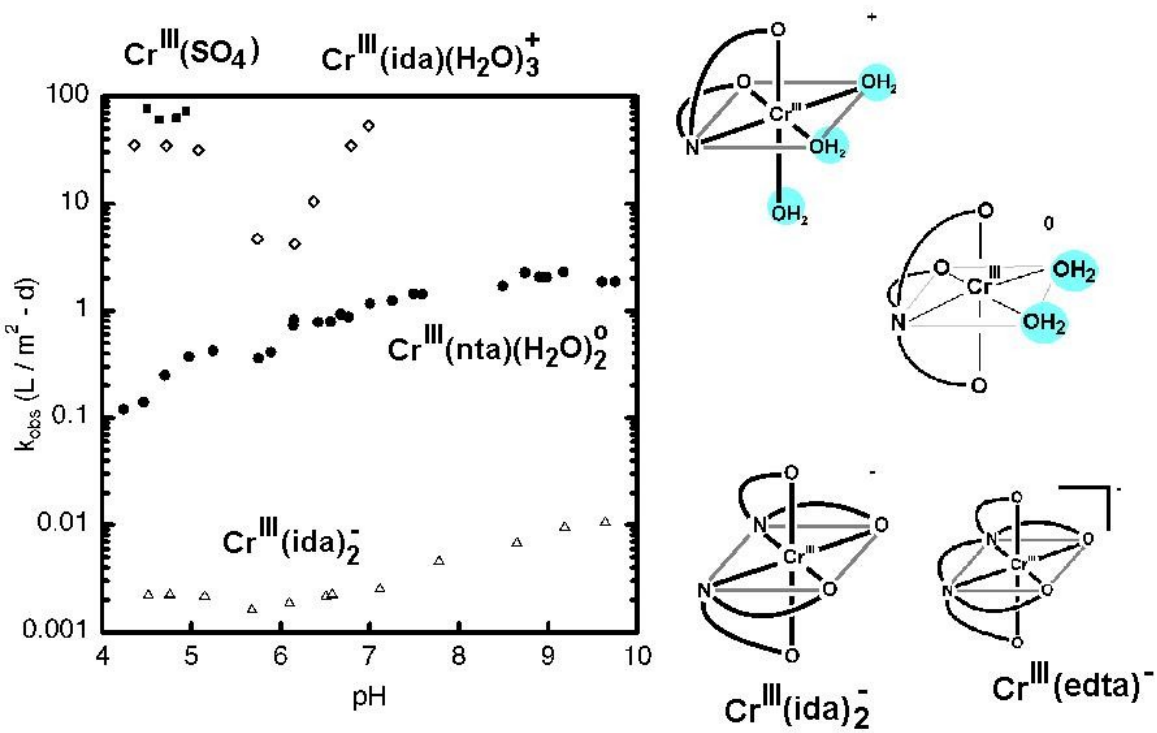


Figure 1. Effect of Cr(III) speciation on rates of oxidation by a mixed Mn(III, IV) (hydr)oxide phase as a function of pH.

Poster Abstract

Patterns and Prediction: Molecular Analysis of Hydrocarbon-Degrading Microbial Populations and Their Function in Real Contaminant Mixture Environments

Natsuko Hamamura, S.H. Olson, Katherine J. Schultz, David M. Ward, and William P. Inskeep
Montana State University, Bozeman, MT

Project Goals and Objectives:

The primary goal of this project is to link the biodegradation patterns of complex petroleum hydrocarbon mixtures with the distribution and function of specific microbial populations important in contaminated soil environments.

Approach:

Seven soil types obtained from geographically distinct areas (MT, AZ, OR, IN, OK, and VA) were examined for biodegradation activity. Biodegradation experiments were conducted in soil batch vessels contaminated with 2 percent (w/w) crude oil or diesel fuel spiked with [1-^{14}C] hexadecane. Chemical changes were monitored by gas chromatography/mass spectrometry and $^{14}CO_2$ analysis. Microbial populations were analyzed using 16S rDNA-based denaturing gradient gel electrophoresis (DGGE). Functional diversity was elucidated using group-specific polymerase chain reaction (PCR) and PCR-DGGE primers targeting phylogenetically distinct groups of alkane hydroxylase genes (*alkB*). The expression of *alkB* genotypes were examined by reverse-transcriptase PCR followed by DGGE analysis. Cultivation of hydrocarbon-degrading isolates was performed with selected soil samples.

Preliminary Findings:

All seven soil types showed comparable crude oil degradation activities, wherein more than 80 percent of added crude oil and approximately 40 to 70 percent of added ^{14}C-hexadecane was depleted after 40 days of incubation (see Figure 1). Preferential utilization of *n*-alkanes relative to branched alkanes generally was observed. Concomitant with crude-oil degradation, diverse microbial populations were observed across soil types using 16S rDNA-DGGE profiles; however, similar hexadecane-degrading, *Rhodococcus*-like populations were found in three soil types. DGGE profiles of diesel-degrading soils showed similar profiles to those receiving crude-oil amendments. Successional patterns of several distinct *alkB* genotypes were detected, corresponding to changes in hydrocarbon chemistry during crude-oil degradation.

Significance of Findings and Next Steps:

Contamination of soil-water systems with crude oil results in the emergence of dominant microbial populations, but there is remarkable diversity in the types of organisms selected across geographically diverse soil types. Contamination of the same soil type with different hydrocarbon mixture types, crude oil versus diesel fuel, resulted in similar microbial population patterns in two soil types tested, whereas one soil type showed different degradation activity towards two mixture types. These results suggest that soil type has a significant impact on patterns of population distribution and succession. The functional gene approach enabled us to identify functionally active populations that were indistinguishable at the 16S rDNA level and to link biodegradation activity with specific microbial populations. These findings improve our understanding of microbial population dynamics and function in real contaminated soil-water systems. Examination will be expanded to other hydrocarbon mixtures containing additives or distinct components such as pentachlorophenol and polynuclear

aromatic hydrocarbons, which may have a significant impact on microbial response to hydrocarbon contamination. In addition, simpler synthetic mixtures will be used to elucidate the potential function of identified phylogenetic populations.

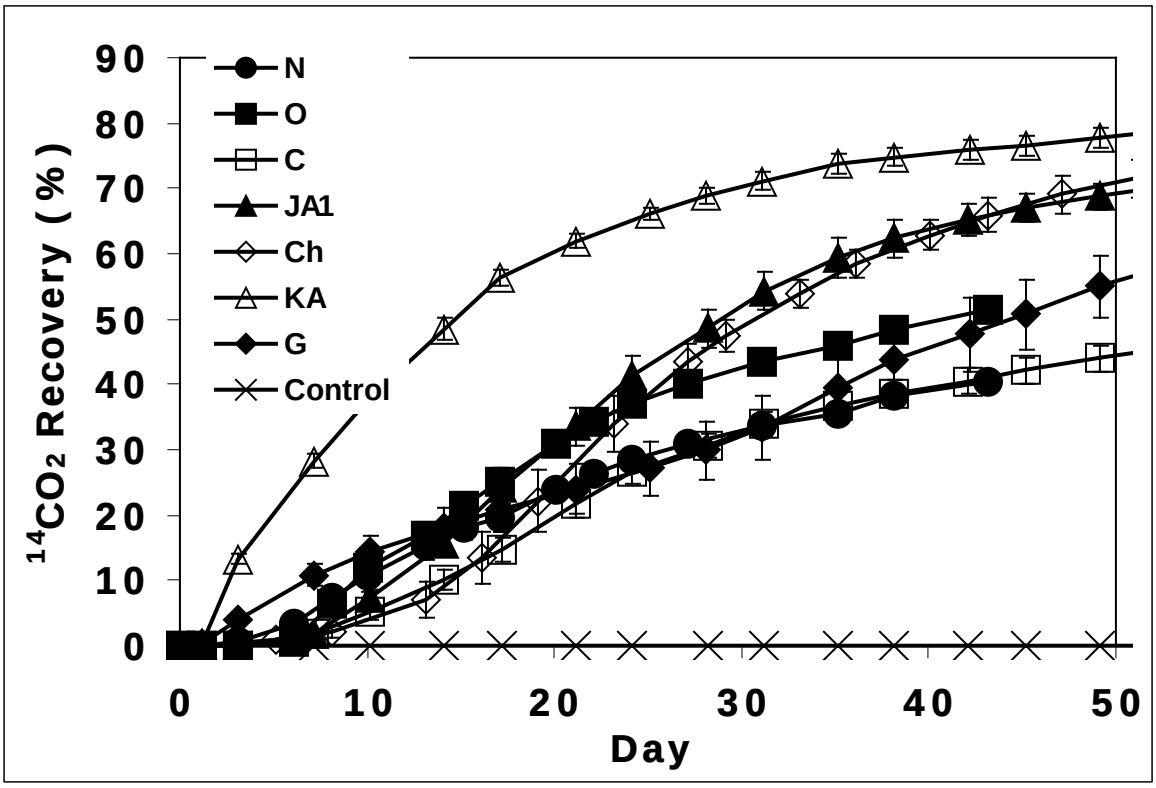


Figure 1. Mineralization of ^{14}C-hexadecane in seven different crude oil amended (2%) soils. The corresponding microbial populations present in these soils were evaluated using molecular techniques.

U.S. EPA 2004 Science To Achieve Results (STAR) Progress Review Workshop – Human Health Symposium

Loews Philadelphia Hotel
1200 Market Street
Philadelphia, PA 19107

October 28 – 29, 2004

Agenda

Thursday, October 28, 2004

8:00 – 9:00 a.m	**Welcome and Keynote**	
	8:00 – 8:10 a.m.	**Welcome – Becki Clark, Division Director, Environmental Sciences Research Division, NCER**
	8:10 – 8:30 a.m.	**STAR Human Health Research Overview – Chris Saint, Assistant Center Director, NCER**
	8:30 – 9:00 a.m.	**Region 3 Health Science Issues – Michael Kulik, Branch Chief, Environmental Innovations, EPA Region 3**
9:00 a.m. – 12:10 p.m.	**Biomarkers Session**	
	9:00 – 9:20 a.m.	**Introduction to Session/EPA Research – Pauline Mendola, Epidemiologist, Human Studies Division, NHEERL**
	9:20 – 9:45 a.m.	**STAR Grantee – R828609 – *Biomarkers of Prenatal Exposure to Nonpersistent Pesticides*** Robin Whyatt, Columbia University
9:45 – 10:00 a.m.	**Break**	
	10:00 – 10:25 a.m.	**STAR Grantee – R828611 – *Biomarkers and Neurobehavioral Effects of Perinatal Exposure to Chlorpyrifos and Other Organophosphate Insecticides*** Jay Wilkins, Ohio State University

	10:25 – 10:50 a.m.	**STAR Grantee – R830757 –** ***Analysis of Genotoxic Biomarkers in Children Associated With a Pediatric Cancer Cluster and Exposure to Two Superfund Sites*** Barry Finette, University of Vermont
	10:50 – 11:15 a.m.	**STAR Grantee – R828608 –** ***Development of a Physiologically Based Pharmacokinetic and Pharmacodynamic (PBKD/PD) Model To Quantitate Biomarkers of Exposure to Organophosphorus Insecticides*** Charles Timchalk, Battelle Memorial Institute
	11:15 – 11:40 a.m.	**STAR Grantee – R830683 –** ***Biomarkers of Human Exposure to Pesticides Utilizing a New PBPK/PD Model and Kinetic Data on Pesticide Metabolism in Humans*** James Olson and James Knaak, SUNY Buffalo
	11:40 a.m. – 12:05 p.m.	**STAR Grantee – R830686 –** ***Species-Specific Xenobiotic Metabolism Mediated by the Steroid and Xenobiotic Receptor, SXR*** Bruce Blumberg, University of California—Irvine
	12:05 – 12:10 p.m.	**Session Wrap-Up – Pauline Mendola, Epidemiologist, Human Studies Division, NHEERL**
12:10 – 1:30 p.m.	**Lunch (on your own)**	
1:30 – 4:45 p.m.	**Exposure Assessment Session**	
	1:30 – 1:50 p.m.	**Introduction to Session/EPA Research – Jim Quakenboss, Environmental Scientist, NERL**
	1:50 – 2:15 p.m.	**STAR Grantee – R826684 –** ***Estimating Human Health Risk From Dermal Exposure to Contaminated Soils*** Annette Bunge, Colorado School of Mines
	2:15 – 2:40 p.m.	**STAR Grantee – R829364 –** ***A Longitudinal Approach of Assessing Aggregate Exposure to Organophosphate Pesticides in Children*** Chensheng (Alex) Lu, Emory University
2:40 – 3:00 p.m.	**Break**	
	3:00 – 3:25 p.m.	**STAR Grantee – R829397 –** ***Longitudinal Study of Children's Exposure to Pyrethroids*** Ye Hu, RTI International

	3:25 – 3:50 p.m.	**STAR Grantee – R828017 –** ***Assessing Levels of Intermittent Exposures of Children to Flea Control Insecticides From the Fur of Dogs*** Janice Chambers, Mississippi State University
	3:50 – 4:15 p.m.	**STAR Grantee – R827440 –** ***Ingestion of Pesticides by Children in an Agricultural Community on the U.S./Mexico Border—Variations in Behaviors and Metabolite Levels*** Natalie Freeman, University of Florida
	4:15 – 4:40 p.m.	**STAR Grantee – R827443 –** ***Vulnerability of Young Children to Organophosphate Pesticides Through Intermittent Exposures in Yuma County, Arizona*** Mary Kay O'Rourke, University of Arizona
	4:40 – 4:45 p.m.	**Session Wrap-Up – Jim Quakenboss, Environmental Scientist, NERL**
4:45 – 5:00 p.m.	**Break**	
5:00 – 6:30 p.m.	**Poster Session**	

Exposure Assessment Posters

R829396 - Measurements and Models of Longitudinal Dietary Intake of Pyrethroid and Organophosphate Insecticides by Children, Barry Ryan, Emory University
R827444 - Study of Exposure and Body Burden of Children of Different Ages to Pesticides in the Environment, Michelle McCombs, RTI International

Susceptibility and Vulnerability Posters

R830800 - Reducing Uncertainty in Children's Risk Assessment: Development of a Quantitative Approach for Assessing Internal Dosimetry Through Physiologically Based Pharmacokinetic Modeling, James Bruckner, University of Georgia
R830766 - Determinants of Fetal Male Rat Germ Cell Vulnerability to Phthalate Esters, Elena Kleymenova, CIIT Centers for Health Research
R829399 - Neurobehavioral and Neuropathological Deficits in the Rat Offspring Following Maternal Exposure to Nicotine and Chlorpyrifos, Alone and in Combination, Wasiuddin Khan, Duke University
R830685 - Neurokinin Receptors and Environmental Lung Injury, Gary Hoyle, Tulane University

Biomarkers Posters

R830826 - Environmental Tobacco Smoke, Biomarkers, and Childhood Asthma, Hillary Klonoff-Cohen, University of California, San Diego
R828610 - Identification of a Novel Hemoglobin Adduct in Sprague-Dawley Rats Exposed to Atrazine, Gregory P. Dooley, Colorado State University
R830825 - The Pregnancy Environment and Child Health (PEACH) Study of Intrauterine and Postnatal Exposure to Halogenated Compounds and Childhood Atopy, Wilfried Karmaus, Michigan State University
R829395 - Meconium Analysis: A Promising Tool To Detect Fetal Exposure to Environmental Toxicants, Dawn Bielawski, Wayne State University

Chemical Mixtures Posters

R829355 - Lumped Chemical Approach for Fate and Transport Modeling of Organic Pollutant Mixtures, Kenneth Reardon, Colorado State University
R829356 - Total Metal Ion-Synthetic Chelating Agent Interactions in Aqueous Media, Alan Stone, The Johns Hopkins University
R829357 - Patterns and Prediction: Molecular Analysis of Hydrocarbon-Degrading Microbial Populations and Their Function in Real Contaminant Mixture Environments, William Inskeep, Montana State University

Friday, October 29, 2004

8:00 – 11:35 a.m.	**Susceptibility and Vulnerability Session**	
	8:00 – 8:20 a.m.	**Introduction to Session/EPA Research – Brenda Foos, Toxicologist, Office of Children's Health Protection**
	8:20 – 8:45 a.m.	**STAR Grantee – R829467 – *Bioaccumulative Toxins in Native American Shellfish*** Felix Basabe, Swinomish Tribal Community
	8:45 – 9:10 a.m.	**STAR Grantee – R830827 – *The Effects of the World Trade Center Disaster on Pregnant Women and Their Infants*** Trudy Berkowitz, Mount Sinai School of Medicine
	9:10 – 9:35 a.m.	**STAR Grantee – R830755 – *Genetic Basis of the Increased Susceptibility of Children to Inhaled Pollutants*** Terry Gordon, New York University
9:35 – 9:50 a.m.	**Break**	
	9:50 – 10:15 a.m.	**STAR Grantee – R829429 – *Impact of Phthalates on the Male: Frog and Rabbit Models*** Rao Veeramachaneni, Colorado State University
	10:15 – 10:40 a.m.	**STAR Grantee – R828599 – *Mutations in Steroid 5-Alpha Reductase Type 2 and the Severity of Hypospadias*** Jeanne Manson, Children's Hospital of Philadelphia
	10:40 – 11:05 a.m.	**STAR Grantee – R830756 – *Prenatal Exposures of Children to Polybrominated Diphenyl Ethers: The Collection of Animal and Human Data Along With the Development and Validation of a PBPK Model*** Ed Garner, RTI International
	11:05 – 11:30 a.m.	**STAR Grantee – R829428 – *Strain-Dependent Susceptibility to Transplacentally Induced Murine Lung Tumors*** Mark Miller, Wake Forest University

	11:30 – 11:35 a.m.	**Session Wrap-Up – Brenda Foos, Toxicologist, Office of Children's Health Protection**
11:35 a.m. – 12:00 noon	**Upcoming EPA Research Initiatives – Paul Gilman, Assistant Administrator for the Office of Research and Development at EPA, U.S. EPA Science Advisor**	
12:00 – 1:00 p.m.	**Lunch (on your own)**	
1:00 – 3:05 p.m.	**Chemical Mixtures Session**	
	1:00 – 1:20 p.m.	**Introduction to Session/EPA Research – Richard Hertzberg, Biomathematician, NCEA**
	1:20 – 1:45 p.m.	**STAR Grantee – R827180 – *PAH/Metal Mixtures—Human* In Vitro *Mutagenicity Studies*** Laurence Kaminsky, New York State Department of Health
	1:45 – 2:10 p.m.	**STAR Grantee – R829359 – *Regulation of Embryonic Neuronal Development by Chemical Mixtures From Brick, New Jersey*** Carol Reinisch, Marine Biological Laboratory
	2:10 – 2:35 p.m.	**STAR Grantee – R829361 – *Comparative* In Vitro *Immunotoxicity of Organochlorine Mixtures*** Sylvain De Guise, University of Connecticut
	2:35 – 3:00 p.m.	**STAR Grantee – R829358 – *Mechanistic Evaluation of the Toxicity of Chemical Mixtures*** Allen Olmstead, North Carolina State University
	3:00 – 3:05 p.m.	**Session Wrap-Up – Richard Hertzberg, Biomathematician, NCEA**
3:05 – 3:30 p.m.	**Meeting Wrap-Up – Kacee Deener, Environmental Health Scientist, NCER**	
3:30 p.m.	**Meeting Adjourns**	

U.S. EPA 2004 Science To Achieve Results (STAR) Progress Review Workshop—Human Health Symposium

Loews Philadelphia Hotel
1200 Market Street
Philadelphia, PA 19107

October 28–29, 2004

Participants List

Margaret Adgent
U.S. Environmental Protection Agency

Alvaro Alvarado
U.S. Environmental Protection Agency

Stan Barone
U.S. Environmental Protection Agency

Felix Basabe
Swinomish Tribal Community

Julie Becker
Thomas Jefferson University

Trudy Berkowitz
Mount Sinai School of Medicine

Dawn Bielawski
Wayne State University

Bruce Blumberg
University of California—Irvine

Kevin Brooks
Michigan State University

Michael Brown
U.S. Environmental Protection Agency

James Bruckner
University of Georgia

Annette Bunge
Colorado School of Mines

Richard Callan
Association of Schools of Public Health Fellow

Ray Chalmers
U.S. Environmental Protection Agency

Janice Chambers
Mississippi State University

Rebecca Clark
U.S. Environmental Protection Agency

Roger Cortesi
U.S. Environmental Protection Agency

Allen Davis
Association of Schools of Public Health Fellow
U.S. Environmental Protection Agency

Kacee Deener
U.S. Environmental Protection Agency

Sylvain DeGuise
University of Connecticut

Michael Dellarco
U.S. Environmental Protection Agency

Greg Dooley
Colorado State University

Nigel Fields
U.S. Environmental Protection Agency

Barry Finette
University of Vermont

Brenda Foos
U.S. Environmental Protection Agency

Natalie Freeman
University of Florida

C. Edwin Garner
RTI International

Paul Gilman
U.S. Environmental Protection Agency

Terry Gordon
New York University

Richard Hertzberg
U.S. Environmental Protection Agency

Gary Hoyle
Tulane University

Ye Hu
RTI International

William Inskeep
Montana State University

Nancy Jafolla
U.S. Environmental Protection Agency

Laurence Kaminsky
New York State Department of Health

Wilfried Karmaus
Michigan State University

Nagu Keshava
U.S. Environmental Protection Agency

Wasiuddin Khan
Duke University Medical Center

Elena Kleymenova
CIIT Centers for Health Research

Hillary Klonoff-Cohen
University of California—San Diego

James Knaak
State University of New York at Buffalo

Michael Kulik
U.S. Environmental Protection Agency

Susan Laessig
U.S. Environmental Protection Agency

Barbara Levinson
U.S. Environmental Protection Agency

Chensheng (Alex) Lu
Emory University

Jeanne Manson
Children's Hospital of Philadelphia

Errol Mazursky
Association of Schools of Public Health Fellow
U.S. Environmental Protection Agency

Michelle McCombs
RTI International

Teresa Mendez-Quigley
Women's Health and Environmental Network

Pauline Mendola
U.S. Environmental Protection Agency

Mark Miller
Wake Forest University

David Mustra
U.S. Environmental Protection Agency

Allen Olmstead
North Carolina State University

James Olson
State University of New York at Buffalo

Mary Kay O'Rourke
University of Arizona

Devon Payne-Sturges
U.S. Environmental Protection Agency

James Quackenboss
U.S. Environmental Protection Agency

Kenneth Reardon
Colorado State University

Carol Reinisch
Marine Biological Laboratory

Lawrence Robinson
Philadelphia Health Department

Magda Rodriguez-Hunt
U.S. Environmental Protection Agency

P. Barry Ryan
Emory University

Chris Saint
U.S. Environmental Protection Agency

Deborah Segal
U.S. Environmental Protection Agency

Alan Stone
Johns Hopkins University

Hugh Tilson
U.S. Environmental Protection Agency

Charles Timchalk
Battelle Memorial Institute

Ritu Tuteja
Association of Schools of Public Health Fellow
U.S. Environmental Protection Agency

Jeff Tuttle
U.S. Environmental Protection Agency

D.N. Rao Veeramachaneni
Colorado State University

Robin Whyatt
Columbia University