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Proceedings

Human Health Symposium— A STAR Progress Review Workshop

April 9-10, 2003
Washington, DC



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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 by conducting most of the Agency's research within its own laboratories and through an extramural grants program. ORD has identified research to improve human health risk assessment and management, with a high priority on emerging concerns such as aggregate and cumulative exposure and susceptible human populations.

As part of its research program, ORD's National Center for Environmental Research (NCER), through its Science to Achieve Results (STAR) grants program, supports human health research to improve the ability to assess the 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 (i.e., air, water, soil, food) and multiple pathways (i.e., breathing, eating, skin contact)
- ❖ Characterizing the unique environmental threats faced by sensitive sub-populations such as children
- ❖ 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 solicitations, NCER has developed a diverse portfolio of extramural research in the area of human health risk assessment, including grants dealing with children's vulnerability to toxic substances in the environment, biomarkers to assess exposure and toxicity in humans, the impact of exposure to chemical mixtures, gene-environment interactions, environmental justice, and research in community-based intervention.

This Human Health Symposium—a STAR Progress Review Workshop—brought together EPA-funded scientists, public sector scientists, and policymakers to share their 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 for mitigating 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 participating 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 risk assessment 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). For more information on all research areas in EPA's STAR program, please go to <http://www.epa.gov/ncer>.

U.S. Environmental Protection Agency Human Health Symposium—A STAR Progress Review Workshop

Washington, DC
April 9–10, 2003
Meeting Summary

Wednesday, April 9, 2003

Overview

The U.S. Environmental Protection Agency's (EPA) *Human Health Symposium—A STAR Progress Review Workshop* convened EPA-funded scientists, public sector scientists, and policymakers 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 two simultaneous breakout sessions. Symposium topics included exposure assessment, environmental justice, statistics, molecular epidemiology, biomarkers, toxicology, and chemical mixtures.

General Forum: Welcome and the National Children's Study

Welcome and Opening Remarks

Jack Puzak

Jack Puzak, Acting Director of NCER, welcomed participants to the EPA Human Health Symposium and provided a brief overview of the structure of the EPA and NCER. Under EPA's Office of Research and Development (ORD), NCER provides extramural grants through the Science to Achieve Results (STAR) program. Established in 1995, the STAR program's mission is to provide research opportunities to universities and nonprofit groups congruent with EPA's research programs and to ensure the best quality of science in areas of greatest importance to the Agency. The STAR program also awards graduate fellowships that aim to encourage promising students to obtain advanced degrees and to pursue careers in environmental fields. NCER awards approximately 100 to 175 research grants and 100 to 150 fellowships annually and manages approximately 1,000 active research grants and fellowships. There is significant cross-agency and interagency involvement with solicitation planning, writing, and review for STAR grant selection. Through competitive solicitations, the STAR research program awards approximately \$100 million annually. Additional funds are awarded through joint solicitations with other agencies. Research proposals undergo an external peer review and an internal programmatic review (which provides program and regional input) to ensure that the highest priority projects are funded. High-priority research areas include particulate matter, drinking water, clean water, global change, children's health, endocrine disruptors, ecological risk, human health, pollution prevention and new technologies, and economics and decision sciences. Annual and final progress reports for the research grants and fellowships are posted on the NCER Web Site at <http://epa.gov/ncer/results/>. Through the Web Site, the EPA communicates research results, obtains feedback, and posts announcements.

Research Needs Related to the National Children's Study

Carole Kimmel

Carole Kimmel, Senior Scientist in EPA's National Center for Environmental Assessment (NCEA), provided an overview of the National Children's Study (NCS), a longitudinal study of the effects of environmental pollutants on child health and development. The 1998 Presidential Task Force on Environmental Health Risks and Safety Risks to

Children was charged with developing strategies to reduce the risk of environmental exposure to children. The Task Force, co-chaired by the Secretary of Health and Human Services, the Administrator of the EPA, and seven additional Cabinet Members and senior staff, in consultation with several agencies and public-private partnerships, endorsed the NCS in January 2000. The Children's Health Act of October 2000 gave the Director of the National Institute of Child Health and Human Development authority to plan and implement the NCS. In this study, environment is broadly defined to include physical, chemical, biological, and psychosocial factors. The study's mission is to investigate the basic mechanisms of developmental disorders and environmental factors, both risk and protective. Nine concepts form the basis for the study:

- ❖ High-quality longitudinal study of children, their families, and their environment
- ❖ National in scope
- ❖ Environment defined broadly to include chemical, physical, behavioral, social, and cultural factors
- ❖ Study common range of "environmental" exposures and less common outcomes
- ❖ Study the relationship between the environment and genetic expression
- ❖ State-of-the-art technology—tracking, measurement, data management
- ❖ Consortium of multiple agencies
- ❖ Extensive public-private partnerships
- ❖ National resource for future studies.

Hypotheses for the study cover such areas as undesirable outcomes of pregnancy, neurobehavioral effects, injury, asthma, obesity, and physical development. Anticipated measures of exposure for the study include environmental samples (air, water, dust, soil); biomarkers for chemicals (e.g., blood, breast milk, hair, tissue, urine); interview and history; serology and medical data; housing and living characteristics; family and social experiences; and neighborhood and community characteristics. Anticipated outcome measures include fetal growth and outcome of pregnancy; birth defects and newborn exam; growth, nutrition, and physical development; medical condition and history; cognitive and emotional development; and mental, developmental, and behavioral conditions. Pilot studies for the NCS began in 2000 and primarily were initiated by the EPA. These initial studies addressed general questions about exposures and outcome measures and provided information on technology and data collection.

The NCS is considering questions regarding sample design, including representativeness, pre-pregnancy versus early pregnancy, subpopulations and oversampling, recruitment and retention, response rate, sufficient range, and proportions of exposures and outcomes. The NCS also is considering a hybrid model that includes office-based models, medical-center models, and household area-based models. These models will be examined for their respective strengths, costs, and statistical power.

Several workshops and respective reports and white papers are planned for topics on community outreach and communications, fetal growth and development, assessment of neonatal/infant growth and development, prescription and over-the-counter drug and dietary supplement exposures, ethics, and technology.

Research needs of the NCS include best methods to assess growth and development, best exposure measures, best analytical tools, and best statistical/analytical tools for longitudinal analysis of exposure-outcome links and interactions.

The NCS currently is strengthening its methods development efforts. The projected timeline is to:

- ❖ Finalize hypotheses and develop a study design in mid-2003
- ❖ Select initial centers or alternatives and pilot test core protocol in mid-2005
- ❖ Begin full study with vanguard centers in late 2005
- ❖ Enroll additional centers in 2005–2007
- ❖ Observe preliminary results available from pregnancy in 2008–2009
- ❖ Analyze data as collection continues; publish results throughout 2007–2030.

Breakout Session I: Exposure Assessment and Environmental Justice

Exposure Assessment

Pesticide Exposures of Preschool Children Over Time (PEPCOT) Design and Recruiting

Nancy Wilson

Nancy Wilson, a former EPA employee who is now affiliated with the Battelle Memorial Institute, discussed the design and recruiting progress made thus far in the Pesticide Exposure of Preschool Children Over Time (PEPCOT) Study. The purpose of this study is to understand how recent changes in pesticide use affect children's exposure to these chemicals. Examples of changes in pesticide use include the phaseout in residential use of chlorpyrifos and diazinon and the increased use of alternative pesticides. Specifically, the project's objectives are to estimate the longitudinal changes in aggregate exposures to targeted pesticides for selected preschool children in the same age group over 4 years and to estimate the variability of the exposures among preschool children living in the same homes and among those living in different homes.

The PEPCOT approach was to recruit 50 3-year-old children and their younger siblings (two children per household). The children's aggregate exposures at home to selected organophosphorus (OP) and pyrethroid (PY) insecticides and acid herbicides were measured for 24 hours once yearly for 3 years during a spring, summer, or fall sampling period. Concentrations of pesticides in indoor and outdoor air, house dust, play area soil, food, and beverages served to each child, children's hand wipes, and urine were measured. Parents were requested to record child time-activity diaries for the 24-hour sampling period. The child survey collected information for the past year on the types and frequency of foods eaten, medical conditions, and doctor visits. The environmental and personal measurements and child activity diary information were collected and entered into a database.

Study participants were recruited through telephone screening and respondent referrals. Flyers were posted in clinics, social service offices, child care centers, and similar service locations. To be eligible, households had to include two children aged 3 years and younger by March 2003, and be located within 1 hour's driving time of Battelle's Durham, North Carolina, office. Once recruited, the adult participants were interviewed by telephone and signed an informed consent form.

Field sampling consisted of an initial survey, microenvironmental media and personal samples, and the child activity diary and food survey. The initial survey covered household characteristics through field sampler observation; a parent premonitoring questionnaire on family characteristics (e.g., occupation, education, pesticide use); and a parent postmonitoring questionnaire on child and household activities (e.g., pesticide use, foods, and food preparation). The microenvironmental media sampling collected breathable particles of indoor and outdoor air, house dust and play area soils, and a wipe of the food preparation surface area. For personal samples, duplicate plates of solid and

liquid foods served to children in the 24-hour sampling period were collected, the child's hand was wiped with 75 percent isopropanol/water before lunch and dinner, child urine samples (overnight diaper or first morning void) were collected, the child's height and weight were recorded, and the child's hand was traced. For the child activity diary and food survey, the parent recorded the following during the 24-hour sampling period: time, duration, and place of activity; type of surface; active play, quiet play, or sleeping; and child behaviors (e.g., playing with pets, sucking thumb). In addition, a food survey was collected detailing the child's usual eating habits over the past year. The next steps in the PEPCOT Study will be to complete selection of eligible participants, continue field-sample collection, and initiate laboratory analysis of collected samples.

Nicole Tulve, EPA's National Exposure Research Laboratory (NERL), asked for further clarification of the recruitment strategy. Dr. Wilson replied that the study initially contacted Comprehensive Employment Training Assistance (CETA) participants who had given consent for further contact; however, only 4 percent could be reached. Next, the study group bought a list of all active telephone numbers in the Durham, Raleigh, and Chapel Hill county regions. Callers spoke with the head of the household for each number on the list, explained the study, and determined whether the household was eligible. To further explain the study, a staff member visited households that agreed to participate. If the household agreed, the adults signed an informed consent form.

Carole Reinisch, Woods Hole Marine Biological Laboratory, expressed concern about the snapshot 24-hour time period of absorbed dose relative to exposure levels. Dr. Wilson replied that the study is looking at the relationship between exposure and dose in a snapshot time period and does not intend to pursue in greater detail other interesting things that might be evaluated.

Estimating Longitudinal Aggregate and Cumulative Exposure and Intake Dose for Young Children

James Leckie

James Leckie, Stanford University's Department of Civil and Environmental Engineering, discussed a two-layer, time-variant numerical model developed to calculate the absorption of chemicals through the skin. The model studies the source, concentration, exposure (dermal, inhalation, ingestion), dose, and health effects of cumulative exposures. The study relies on videotapes to quantify the kind of dermal hand-to-mouth behavior that occurs in children's play. Dr. Leckie emphasized the complexity of dermal exposure. Exposure is dependent on the exposure process, the nature of the chemical agent, the environmental conditions, the vehicle of exposure, the transfer mechanisms, and the part of the body that comes into contact with the chemical.

Valerie Zartarian developed the physical-stochastic Dermal Exposure Reduction Model (DERM) in 1996. DERM uses an algorithm to combine micro-level activity time series (M-LATS), environmental concentrations, and exposure factors. DERM simplifies assumptions for mechanisms of mass transfer; the result is a cumulative mass/area of a chemical on the skin surface. The model makes six simplifying assumptions: (1) every contact event results in mass transfer to the skin, (2) mass transfer is recorded at initiation of the contact event, (3) mass never transfers from the skin to objects, (4) object concentrations remain constant throughout the exposure period, (5) skin is exposed to all of the mass in a soil matrix, and (6) vehicle volume or type is not tracked. To account for the overly conservative assumptions, the default model approach (in 500 simulations per child) lowers all concentrations by one order of magnitude, lowers soil adherence by two orders of magnitude, lowers soil concentration by one order of magnitude, and removes the air-immersion mechanism.

The research team's task was to couple the DERM Model with a percutaneous absorption model to utilize detailed characteristic activity patterns. With respect to exposure, uncertainty in the collection methods for concentration, exposure, and activity data lead to variability across individual concentration data. With respect to dose, uncertainty in the collection methods for chemical, diffusion, and partition data leads to variability across individuals, skin properties, and environment. Dr. Leckie's Percutaneous Absorption Model pays special attention to experiment quality, because the quality of the input data depends on the quality of the experimental work.

The numerical model for dermal exposure shows concentration profiles, responds with diffusion in and out of the skin, maintains compartmental mass balance, shows interfacial resistance, accounts for time-varying surface concentrations, and is suitable for multiple exposure events on the skin surface. In conclusion, Dr. Leckie described the numerical model as suitable for M-LATS, and stated that it allows for more variabilities than does the DERM Model. Future modeling work will refine the dermal exposure model, add inhalation and ingestion routes, incorporate a total body dose in a pharmacokinetic (PBPK) model, analyze long-term cumulative exposure and dose, and further address variability and uncertainty.

A Longitudinal Approach To Assessing Aggregate Exposure to Organophosphorus Pesticides in Children

Alex Lu

Alex Lu, University of Washington's (UW) Department of Environmental Health, noted that the three research objectives of this study are to examine long-term temporal trends of organophosphorus (OP) pesticide exposure among children living in urban and suburban communities, assess children's aggregate exposures to OP pesticides during high residential pesticide use season, and estimate the contribution of dietary exposure to total OP pesticide body burden in children. Three previous studies conducted by STAR-funded researchers at UW included a longitudinal study of children from agricultural communities, a cross-sectional study of urban community children, and a pilot aggregate exposure assessment study designed to assess and compare exposure profiles to help identify common risks and preventative factors among and between children.

Though children who live in agricultural communities may not necessarily have family members involved in agriculture, they may still be exposed to pesticides. In this longitudinal study, biweekly first-morning-void urine samples were collected from 44 agricultural community children over a 21-month period. Analyses revealed that agricultural pesticides are a major risk factor for all children in agricultural communities due to pesticide drift and exposure.

The cross-sectional study of urban children collected first-morning-void urine samples in April/May and July/August from 110 children living in the Seattle metropolitan region. In addition, households were asked to complete a questionnaire that contained three questions: (1) Does the household have a cat or dog? (2) Does the household treat the garden with any pesticides? and (3) Does the household treat the house with any pesticides? Urine sample analysis showed that only 1 out of the 110 children in this study had no detectable level of any of the six dialkylphosphate metabolites (DAPs) measured. This child's parents had provided exclusively organic produce to their child.

Cumulative frequency plots of daily total urinary output of dimethyl DAP in agricultural community children and in urban children found that urban children had dimethyl DAP levels that were seven times higher than those of agricultural community children. This finding was contrary to the study's original hypothesis that agricultural community children would experience higher exposure levels.

To further examine this finding, a pilot aggregate exposure assessment study was performed. This study targeted six agricultural community children and six urban community children with elevated urinary DAP levels. The study measured all possible exposure pathways, including a 24-hour indoor air sample, a 24-hour duplicate food (three meals and snacks) sample, soil samples, hand and toy wipe samples, and a questionnaire and 3-day food diary. The pilot study collected four spot urine samples over the 24-hour period and analyzed household dust. Urine data revealed that pesticide measurement in food tended to be dominant; therefore, the children were exposed to OP pesticides through their diets. This was true when measuring 3,5,6-TCPY (metabolite of chlorpyrifos), IMPY (metabolite of diazinon), MDA (metabolite of malathion), and HMBT (metabolite of azinphosmethyl). Dr. Lu concluded that heightened exposure to OP pesticides in children of agricultural communities might be overstated.

A dietary intervention study currently is underway to examine this finding. Twenty to 30 children ages 2 to 12 years who consume only conventional diets are being recruited for the study. The 15-day study period includes a 5-day organic feeding period. The goal of the study is to see if there is a drop in dimethyl DAP during the organic feeding period. The study will be conducted again in the fall, when no residential pesticide is in use in the region, to show that dietary exposure is the most important exposure pathway.

Shelley Davis, Farmworker Justice Fund, Inc., asked about the children of farm workers whose parents are involved directly in agriculture and pesticide application. Dr. Lu reiterated that the definition of “agriculture children” in his study refers to children who live in agricultural communities; these children more closely represent children of the general population. He cited a 1996 study, which found that children who live in agricultural communities had higher exposure relative to parental occupation.

Melvin Anderson, Center for Health Resources in North Carolina, questioned why the peaks in pesticide exposure also were the points of highest variability. Dr. Lu explained that the peaks in exposure are not uniformly high or low. Different compounds have different half-lives. In a 2-week period, much may occur. The exposure measurement, however, was taken as is, causing much variation. Even for the same individual followed in January and July, the variation was not constant. For the pilot study, with its small sample size, it was hard to make a strong case concerning variation. Dr. Lu commented that it was better to assess his data qualitatively rather than quantitatively.

Environmental Justice

Casa de Salud: A Model for Engaging the Community

**Gretchen Latowsky
Doris Anziani**

Gretchen Latowsky, John Snow Institute (JSI) Center for Environmental Health Studies, described Casa de Salud (Health House) as a research and education model designed to engage residents of highly stressed neighborhoods of Lawrence, Massachusetts, in activities to mitigate the health impacts of environmental toxins. Casa de Salud is a culturally integrated community education and organizing model developed by a collaboration of service providers to address public health problems that affect the city’s largely Hispanic population.

Ms. Latowsky described the organizational structure of the Casa de Salud and the community served by this project. The Casa de Salud Steering Committee serves as the leadership and decisionmaking body for the project. It consists of members of the four partnership agencies and meets on a monthly basis. The purpose of the Steering Committee is to make programmatic decisions, develop training programs, oversee outreach and education efforts, and conduct research. The Advisory Council fosters collaboration between Casa de Salud and other environmental health programs in the city. The Advisory Council is comprised of 11 residents of low-income, Latino neighborhoods and representatives of health care providers and environmental health agencies. The Council meets every quarter to provide guidance and direction to the project, suggest local resources on environmental health issues, provide ideas to help the Casa Leaders take action on identified issues, and help identify health-related environmental concerns in the community. Lawrence is an industrial community that has experienced an influx of immigrants for a long time. Lawrence has drawn immigrants since the 1950s, primarily from Caribbean and South American countries. Currently, there are 70,000 people living in a 10-square-mile area, and the school-age population is more than 80 percent Hispanic. Poverty is widespread and severe, and the city ranks among the 25 poorest in the Nation. Per capita income is less than \$10,000.

The environmental health threats in Lawrence are significant. Nearby freshwater bodies are highly contaminated, and low-income families often consume fish. Lead levels in children are nearly three times the state rate, and pediatric asthma affects nearly 10 percent of all Lawrence public school students. The Casa de Salud program was developed to engage the Latino community. Latino residents were hired to be educators, and residents opened their homes to community health discussions called *charlas*. In this culturally comfortable setting, residents were more inclined to understand the discussion and to ask questions. In addition, Casa de Salud held health fairs, a culturally acceptable way to bring people together to learn about and discuss health concerns. Through *charlas* and fairs, Casa de Salud leaders learned that the residents’ primary concerns included the trash on the streets, the accompanying rodents and roaches, and alternatives to pesticide use in the home. Ms. Latowsky showed slides of Lawrence and the work of Casa de Salud and then introduced Doris Anziani, a Casa de Salud educator.

Ms. Anziani, a Latino resident of Lawrence, further discussed barriers to solving environmental problems in the Lawrence community. The legacy of the town's industrial history and the current density of auto body shops (130 in 6.5 square miles) has left Lawrence with significant environmental hazards and resulting health effects. Residents have come to accept the high incidence of asthma as normal. Hispanic residents are afraid to confront their landlords about concerns of trash, rats, and roaches. They are intimidated by the language barrier or, in many cases, by their nonlegal status. Furthermore, many residents work two or three jobs to survive. Residents often rely on home remedies to avoid or delay a costly doctor visit and have little time to participate in community improvement. Through *charlas*, residents' needs are identified, and Casa de Salud educators develop educational programs tailored to those needs. Since the Casa de Salud program started, other groups, such as citizenship courses, English as a second language courses, and church groups, have invited Casa de Salud educators to hold *charlas*.

Carol Reinisch, Woods Hole Marine Biological Laboratory, asked whether Casa de Salud expects to expand the program in the future. Ms. Latowsky explained the snowball effect in the incremental growth of the project. There were 76 participants during the first year of the project, 409 participants during the second year of the project, and more people keep asking to be involved. In addition, several area pediatricians are involved with Casa de Salud.

North Brooklyn Asthma and Environmental Consortium

Luis Garden Acosta

Luis Garden Acosta, CEO and founder of El Puente, provided an overview of El Puente's North Brooklyn/Williamsburg environmental justice struggles and current research. The overall goal of El Puente's research project is to allow the community to guide scientific research and to investigate environmental exposures related to asthma. Specific objectives of the research project are to: (1) initiate a research program that promotes a partnership between members of an environmentally stressed community, academia, and health practitioners; (2) develop culturally appropriate education and communication activities; (3) design and implement preventive environmental interventions to reduce exposure to key asthma precipitating agents and improve the quality of asthma care; (4) evaluate the process, the partnership, selected health outcomes, and interventions; and (5) disseminate successful outcomes of the program throughout Williamsburg, the North Brooklyn community, and the health care community.

The Williamsburg area is a diverse community composed of Hasidic Jews, Latinos, Polish Americans, Italian Americans, and African Americans. Mr. Garden Acosta described Williamsburg's "new soul" neighborhood as one of the best places to live in America. In April 1991, when New York City planned to site a chemical plant in Williamsburg, the Junior Toxic Avengers of El Puente, which represented the diversity of the neighborhood, united against the environmental threat and succeeded in reducing it. Nonetheless, Williamsburg remains the most toxic community in New York City, with 25 waste transfer stations and a previous oil spill, which was larger than the Exxon-Valdez spill, that still seeps into residents' basements.

In response to Williamsburg's alarming levels of toxicity, El Puente launched a 3-year set of surveys containing 43 questions to ascertain the neighborhood's concerns. Asthma proved to be a major concern, especially among the community's Latino residents. The survey found that asthma affects 5.3 percent of the Dominican population and 13.2 percent of the Puerto Rican population. The environmental justice grant gave the community the capacity to drive research with partners to investigate the high asthma rates among Latinos, primarily Puerto Ricans. New York University School of Medicine is providing strong leadership in the research planning and implementation, and Woodhull Medical and Mental Health Center, one of the largest health care providers in the area, also is participating in the research.

Jan Storm, New York State Department of Health, asked about focus groups and whether the North Brooklyn study interviewed more people with respiratory problems than without respiratory problems. Mr. Garden Acosta explained that the study focused on asthma in the Latino community. The study examined potential variables, such as heavy reliance on medications, that might explain the high prevalence of asthma in Puerto Ricans as compared to Dominicans.

**Bioaccumulative Toxins in Native American Shellfish,
Swinomish Tribal Community**

**Tony Basabe
Jamie Donatuto**

Tony Basabe, Director of the Office of Planning and Community Development of the Swinomish Indian Tribal Community in La Conner, Washington, described the Swinomish Tribe's (ST) dependence on fish, crab, and shellfish for consumption, income, and cultural practices. One thousand enrolled tribal members subsist on 7,344 acres of reservation land and 2,900 acres of tribally owned tideland. In the 1950s, the ST community and surrounding region was a large industrial hub; this resulted in elevated levels of toxins, particularly in local fish, crab, and shellfish.

The EPA environmental justice grant, managed by Jamie Donatuto, will study the low-level, chronic, bioaccumulative toxins that accumulate in the Swinomish people when they gather and consume shellfish. She noted that the shellfish beds are integral to ST subsistence food, income, and cultural ceremonies. Toxic chemical contamination of shellfish poses a serious threat to the ST. Possible sources of contamination include landfills and underground storage tanks, permitted point source releases from adjacent industries (air and water), agricultural runoff (past and present), nonpoint-source suburban runoff, heavy boat traffic, atmospheric deposition from other industries in the air shed (e.g., pulp and paper mills, petrochemical refineries), natural background pollution, and unknown sources.

The project objectives are to: (1) determine whether Swinomish people who eat shellfish harvested from reservation areas are exposed to bioaccumulative toxins, (2) effectively communicate potential health risks in a culturally appropriate manner, (3) develop mitigation measures, and (4) confirm major health problems on the reservation that may be related to eating contaminated shellfish, and develop hypotheses concerning the health problems and toxins that are found. The target chemical groups to be tested for include heavy metals, polychlorinated biphenyl (PCB) aroclors and the World Health Organization's list of congeners, polycyclic aromatic hydrocarbons (PAHs), dioxins/furans, chlorinated pesticides, and butyltins.

Ms. Donatuto emphasized that components of a risk assessment amenable to the tribal lifestyle include health, environmental, cultural, and economic parameters. Communication/education components of the project consist of literature tailored specifically to school groups or community gatherings that introduce principles of toxicology and environmental health. Future educational dissemination outlets may include public service announcements, a 30-minute documentary, or a full-length documentary on the Swinomish Cable Channel.

Ms. Latowski asked whether the fish sampling involved only raw fish, considering that some toxins may be reduced after cooking. Ms. Donatuto replied that sampling occurred on raw fish and shellfish and noted that shellfish are handled by hand before they are eaten, which is another point of exposure. As a mitigation measure, collection sites are ranked in terms of toxicity.

Breakout Session II: Epidemiology and Toxicology

Epidemiology

**Serum Dioxin, P450 Genes, and Birth Defects Among
Offspring of Vietnam Veterans**

Deborah del Junco

Deborah del Junco, Texas A&M University's Health Science Center, detailed the history of herbicide and defoliant use and regulation. Agent Orange, a chemical used in South Vietnam from 1962–1971, was intended to strip the thick jungle canopy that concealed enemy forces, to destroy enemy forces' crops, and to clear perimeters of U.S. base camps. Another chemical, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), is a contaminant in the manufacture of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), a major constituent in four of six herbicides used in Vietnam. Humans are exposed orally, and the toxin is transported via the blood or lymph system and stored in liver and body fat. High

toxicity of TCDD results in reduced fertility and increased fetal death and abnormalities in female animals. The effects in males have not been studied well to date.

The first indication of concern regarding chemical exposure to Agent Orange came after a 1965 industrial accident at Dow Chemical's 2,4,5,-T plant in Midland, Michigan. It caused a chloracne outbreak, and dioxin contamination was discovered. In 1969, the White House began phasing out the defoliation program in South Vietnam; by 1970, domestic use and commercial production of some herbicides and defoliant had been halted.

The Vietnam Veterans and Family Health Study (VVFHS) is testing the hypothesis that cytochrome P450 polymorphisms explain some of the interindividual differences in susceptibility to TCDD toxicity. The study's challenge is to find enough cases to examine complex gene-environment interactions in an epidemiologic study. From the database of Vietnam veterans, the study divided potential matches into Vietnam veteran parents of neural tube defect (NTD)-affected offspring (cases) and normal offspring (controls). Surveys were mailed to 8,000 Vietnam veterans listed in the Department of Defense records. The survey contained questions about military and reproductive history. Those who completed the survey were asked if they were willing to participate in the next phase, which involved collection of a 50-mL blood sample that was tested for the presence of dioxin and for some of the many genes that are involved in the metabolism of dioxin. Results showed toxic equivalency (TEQ) values (g/fat) that were contrary to the hypothesis; controls had more exposure than did cases. In the blood assays for dioxin-like compounds and the CYP1A1 polymorphism, only the log (TCDD) was associated positively with the NTDs in fathers' offspring, although the 2.2 occurrence ratio was not significant.

Dr. del Junco concluded that, based on preliminary data, perceived exposure to Agent Orange, age, ethnicity, branch of service, and military rank were associated with NTDs in fathers' offspring. Continued funding from the EPA will support an evaluation of selection bias (i.e., differential eligibility comparing nonrespondents with respondents).

Future directions for the study will include conducting exploratory analyses using variables in the Vietnam File to examine whether cases and control participants differ from their respective counterparts (provided by the Veterans Administration), considering other gene-dioxin interactions that may be associated with NTD risk in offspring, and conducting experiments in a suitable animal model to evaluate the plausibility of male-mediated developmental toxicity.

Molecular Epidemiology of Hypospadias

Jeanne Manson

Jeanne Manson, Children's Hospital of Philadelphia (CHOP), introduced the preliminary results from an ongoing study, now one-third complete, of epidemiologic risk factors for hypospadias. The study's hypothesis is that allelic variants in genes that control androgen action and metabolism will increase the risk for and severity of hypospadias in male infants, and that exposure to anti-androgenic agents during pregnancy will further increase the risk for hypospadias in infants with a susceptible genotype and result in a gene-by-environment interaction. The specific aims of the study are to: (1) characterize the association between specific allelic variants in genes that control androgen action and metabolism in the infant and the occurrence and severity of hypospadias, (2) characterize the association between familial history of hypospadias and maternal and paternal environmental/occupational exposures and risk for hypospadias, and (3) evaluate the effects of gene-environment interactions between susceptible genotypes in the infant and parental exposures on the risk for hypospadias.

Hypospadias occurs in 5 per 1,000 live male infants; however, it is difficult to get exact birth defect data. There are radical differences in occurrence between races, with African Americans experiencing 3.9 per 1,000 live births and Caucasians experiencing 6.1 per 1,000 live births. The United States experienced a doubling in birth prevalence of mild and severe hypospadias from 1968–1993. Risk factors for hypospadias include low birth weight, shorter length of gestation, growth retardation, paternal subfertility, progestin exposure during the first trimester, genetic predisposition, and endocrine disruptors in the environment.

Dr. Manson explained that the study focus was on three primary susceptibility genes: (1) steroid 5-alpha-reductase type 2, important in the metabolism of testosterone to dihydrotestosterone; (2) 17 beta hydroxysteroid dehydrogenase type 2, important in the testicular metabolism of androstenedione into testosterone; and (3) androgen receptor, important in the mediation of intracellular action of testosterone and dihydrotestosterone.

This hypospadias research project is a case-control study of pediatric urology CHOP male infants less than 1 year of age. Cases are nonsyndromic hypospadias and include those with accompanying chordee, meatal stenosis, microphallus, cryptorchidism, and bifid scrotum. Renal anomalies were excluded, and the total number of included cases was 400. The control population numbered 400 and included those with antenatal hydronephrosis, vesicoureteral reflux, hernias, and hydroceles. Penile abnormalities and cryptorchidism syndromes were excluded. With 400 cases and 400 controls, the study is adequately powered to detect a minimum occurrence ratio of 1.6 to 2.0 for an association between allelic variants in the gene and hypospadias. The study will have 92 percent power to detect an interaction occurrence ratio of 4.0.

In separate questionnaires, mothers and fathers were asked about demographics, reproductive history, familial history of hypospadias, drug exposures during pregnancy, and occupational/environmental exposures during pregnancy. In addition, fathers were asked about any history of subfertility. Children with urogenital anomalies identified at birth were referred to Pediatric Urology at CHOP. Researchers identified eligible patients from the CHOP IDX scheduling system based on age, ICD-9 diagnosis, and personal identifiers. A letter was sent to families of these children 2 weeks before their scheduled CHOP visit, followed by a telephone call 1 week before their scheduled visit. At the doctor's visit, the family was approached to sign an informed consent form, complete the administrative questionnaires, and collect buccal swabs from the mother, father, and baby. Thus far, 254 families have been recruited into the study.

Early results showed that, for maternal occupational and home exposures, those mothers who worked equal to or greater than 6 months of their pregnancy exposed to pesticides, paints, stains, fuels, or solvents had a higher incidence of hypospadias in their children. The same was true for maternal home exposures to pesticides, paints, stains, fuels, and solvents. Dr. Manson stressed that these results were preliminary. The study found that affected control mothers had a higher incidence of gynecologic complication before the index pregnancy (amenorrhea, excessive menstrual bleeding, endometriosis) and of urologic infections during pregnancy. Furthermore, cases showed a trend toward increased occupational and home exposures to environmental chemicals (pesticides, paints, stains, fuels, and solvents) but, until the study goal of 800 participants is reached, these results are not conclusive.

Gene-Environment Interactions and Human Malformations

Gary Shaw

Gary Shaw, a senior epidemiologist at the California Birth Defects Monitoring Program, described the collaborative research on gene-environment interactions and human malformations currently underway. The study aims to make progress in the areas of congenital malformation, specifically impaired biotransformation and detoxification pathogenesis, impaired folate metabolism and transport pathogenesis, and vascular pathogenesis. The population-based, case-control study relies on maternal interview data and DNA for genotyping.

The study's first aim was to look at biotransformation enzymes. Based on interview questions, the study found a 2.4-fold increase in birth defects of isolated cleft lip and cleft palate associated with maternal smoking of greater than or equal to 20 cigarettes per day. The study's second aim focused on multivitamins/folic acid and heart defects. Women with reduced folate carrier 1 (RFC1) have a genetic disposition that affects the transfer of folic acid. RFC1 is a mechanism to transport folate molecules from circulation to peripheral cells. RFC1 protein is capable of generating transmembrane gradients for folate. One study hypothesis is that infants homozygous for RFC1 (i.e., G80/G80 genotype) would be at increased risk for conotruncal heart defects due to an impaired ability to transport folates to the cytoplasm of target cells. Study results thus far suggest that taking folic acid can reduce the risk of having a child with a conotruncal heart defect among mothers who do not process folic acid properly. The third aim of the study is to examine vascular pathogenesis; specifically, gastroschisis, small intestinal atresia, limb deficiencies, *in utero* and neonatal strokes, and porencephaly and other central nervous system disorders.

Dr. Shaw concluded that these analyses contribute to the general scientific understanding of the etiologies of common congenital malformations. Many more genotyping experiments will be conducted, and epidemiologic analyses will be performed that incorporate maternal interview information to detect gene-environment interactions.

Toxicology

Molecular Characterization of a Biological Threshold in Developmental Toxicology

Thomas Knudsen

Thomas Knudsen, Thomas Jefferson University, presented research to characterize in molecular terms the biological basis of a threshold in developmental toxicity and to place this into the context of a quantitative dose-response model for risk assessment. The prototype for the study is 2-chloro-2-deoxyadenosine (2CdA), an ocular teratogen that causes microphthalmia in mice. Susceptibility to 2CdA-induced microphthalmia is dependent on the p53 tumor suppressor genotype of the embryo in that an intact p53 response is necessary to see a developmental effect.

The first step in the study approach focused on the dose-response characteristics at 3 hours post-exposure to 2CdA. EPA's Benchmark Dose Software was used to predict the threshold for microphthalmia. The threshold dose represents the lower 95 percent confidence limit for the modeled dose associated with an increased 5 percent risk for the defect. Dr. Knudsen and his research team identified 182 genes that changed in the 2CdA dose response. The data analysis suggested that there was a transitional response to developmental exposures, implying that a novel cellular regulation was invoked as the toxicant exposure crossed the threshold for developmental toxicity. The second part of the research involved a time-course study of embryo susceptibility. The test period was 3.0 to 6.0 hours post-exposure, covering events on both sides of the induction of the p53 protein at 4.5 hours. The third portion of the research involved an intervention with PK11195, a mitochondrial benzodiazepine receptor ligand that blocks induction of p53, and a subsequent study of the gene expression trajectories. Dr. Knudsen summarized his research results by stating that biological thresholds in developmental toxicity are reflected in the "flicker" of the gene expression phenotype. This was observed through the benchmark dose modeling, the trajectories spanning the exposure phase of the p53 protein induction, and the therapeutic intervention with PK11195.

Mechanisms of Age-Dependent Ozone-Induced Airway Dysfunction

Richard Johnston

Richard Johnston, Harvard School of Public Health, presented research on ozone as an asthma trigger. Ozone causes airway inflammation and airway hyperresponsiveness (AHR). Hospital admissions for asthma increase on days of high ozone concentrations. Relatively few studies have examined age as a factor in responses to ozone. In mice, studies have shown that ozone-induced lung injury is greater in younger animals, yet no studies have investigated age-dependent effects of ozone on airway responsiveness. Children may be particularly susceptible to ozone because they are more active, spend more time outdoors, and have higher metabolic rates than adults. This study investigates whether children are more susceptible to ozone-induced AHR. The model compared adult and juvenile mice ages 2 weeks and 12 weeks.

Results showed that when younger mice inhaled more ozone than did older mice, the younger mice had reduced AHR and ozone-induced airway inflammation as compared to older mice. Dr. Johnston noted that species differences raise a caveat in extending these results to humans. Examples of such differences include: no decreases in ventilation in humans following ozone exposure, potential differences in the postnatal development of systems that are involved in sensing and responding to ozone, and differences in the expression of antioxidant enzymes.

Dr. Johnston reported that tumor necrosis factor (TNF- α) signaling appears to play a role in ozone-induced AHR, but not in ozone-induced airway inflammation following acute ozone exposure. Also, IL-6 deficiency attenuates ozone-induced airway inflammation and injury in mice. IL-6 does not appear to influence ozone-induced AHR, but does promote ozone-induced airway inflammation. In addition, chemokine receptor 2 (CXCR2) appears to contribute to the development of both ozone-induced AHR and ozone-induced airway inflammation.

Preliminary conclusions are that age plays a significant role in airway responses to ozone. TNFR1, TNFR2, and CXCR2 signaling contribute to the development of ozone-induced AHR; and IL-6, macrophage inflammatory protein-2 (MIP-2)/keratinocyte-derived cytokine (KC), and IL-1 contribute to ozone-induced airway inflammation.

Using Human-Derived Cell Systems To Assess the Biochemical Toxicology of Transplacental Carcinogens

Evan Gallagher

Evan Gallagher, University of Florida, discussed why human-derived cell systems might be used to assess the biochemical toxicology of transplacental carcinogens. Reasons include the availability of archived human tissue, freshly isolated cells, cultured human tissue slices, and fetal CD34 cells; donors are anonymous and are typically Institutional Review Board exempt; human liver slices retain architecture and the microenvironment of the liver; and human enzymes that metabolize and detoxify environmental chemicals often differ with respect to gene and protein expression and substrate species. Dr. Gallagher described the role of reactive intermediates generated *in utero* in the etiology of childhood diseases—many drugs and chemicals cross the placenta and undergo *in utero* bioactivation, there is a reduced prenatal detoxification capacity and a high rate of cell proliferation, and there are epidemiological linkages with maternal exposure to alcohol, aflatoxin B₁ (AFB₁), and certain pesticides with childhood diseases.

The goals of this research project are to determine the role of developmental age on the ability of human fetal liver to metabolize the potent transplacental carcinogen aflatoxin B₁ (AFB₁) and to determine the toxicological ramifications of such differences regarding AFBO-DNA injury in sensitive fetal cell targets. The hypothesis is that, during the second trimester of pregnancy, a critical window of susceptibility to aflatoxin DNA damage exists due to high ontogenic expression of AFB₁-activating enzymes and low expression of AFBO-detoxification enzymes.

Dr. Gallagher reported several research findings. Human fetal subcellular fractions were found to convert AFB₁, a known transplacental carcinogen, to DNA-reactive intermediates at rates similar or less than those observed in adults at 100 nM AFB₁. Human prenatal liver slices incubated with AFB₁ did not show gene expression patterns consistent with severe DNA injury. CD34 hematopoietic stem cells may not be sensitive targets of AFB₁ due to low expression of AFB₁ activation enzymes. The research appears to reject the hypothesis that, during the second trimester, a critical window of susceptibility to aflatoxin-DNA damage exists due to high ontogenic expression of AFB₁-activating enzymes and low expression of AFBO-detoxification enzymes. In addition, elevated 4-hydroxynonenal (4HNE) levels were associated with exposure to several environmental chemicals and the pathogenesis of several diseases. 4HNE can be produced at high levels *in utero* during maternal alcohol consumption. Also, it was found that relatively inefficient detoxification of 4HNE is an important factor in fetal cell injury. Glutathione s-transferase (GST) is a key detoxification pathway in protecting against 4HNE injury in the fetal liver. CD34 fetal liver stem cells appear to be targets of 4HNE.

General Forum: Statistics and Panel Session on Molecular Epidemiology

Statistical Models of *Cryptosporidium* Concentrations in Natural Waters

Jery Stedinger

Jery Stedinger, Cornell University's Department of Civil and Environmental Engineering, discussed modeling *Cryptosporidium* concentrations in natural waters. *Cryptosporidium* is a waterborne pathogenic protozoa frequently found in U.S. surface waters. It is difficult to kill because it can survive for more than 100 days in the environment and is resistant to chlorination.

Dr. Stedinger and fellow researchers relied on oocyst recovery using the immunofluorescent antibody assay (IFA) method to discretely count oocysts. One problem was the high coefficient of variation using this method. Using the national immunofluorescence assay method (ICR) *Crypto* data set, Dr. Stedinger used a hierarchical model with regional and site effects. The model incorporated time-site covariates, seasonal effects, and site-specific covariates.

Thus far, the study has modeled covariates and recovery rates. The purpose of modeling covariates of the *Cryptosporidium* model are two-fold: to provide water quality prediction (WQP) and to model health risk analysis (HRA).

Summary results of the generalized linear mixed model (GLMM)-ICR found that significant covariates include log turbidity and carbonate hardness, total organic carbon (reservoir), and log-urban land area (stream). Furthermore, recovery rates are small and highly variable, and the volume analyzed is statistically significant for *Cryptosporidium* and *Giardia*.

Dr. Stedinger concluded that Bayesian statistical analysis of a hierarchical GLMM can represent count data, observed water quality characteristics, recovery rate distributions, natural variability, and persistence of environmental concentrations of pathogens, if the model is carefully formulated. He noted that EPA has used initial analysis of ICR and Information Collection Rule Supplemental Surveys (ICRSS) data to evaluate the benefits of new water treatment standards. Further work will focus on the HRA component of the model.

Panel Session: Molecular Epidemiology

Jeanne Manson
Robin Whyatt
Chris Saint
Deborah del Junco

Jeanne Manson, CHOP, began the panel discussion with a brief presentation on molecular epidemiology and risk assessment. She defined molecular epidemiology as an epidemiological study that examines gene-environment interactions and disease risk. Dr. Manson posed four questions to the panel:

- ❖ What types of translational studies are needed to bring this technology into the risk assessment arena?
- ❖ What is the timing for application of molecular epidemiology to hazard identification/characterization and risk assessment (2, 5, or 10 years)?
- ❖ Will refined risk assessment be possible from increased understanding of interindividual susceptibility to environmental exposures at the genetic level?
- ❖ What ethical issues will arise from screening populations for genes conferring susceptibility to environmental exposures?

Robin Whyatt, Columbia University, first posed a different definition of molecular epidemiology, stating that she thought the term included any epidemiological study that incorporated a biological measure of exposure, dose, effect, or susceptibility. She then noted that the greatest promise for molecular epidemiology is in hazard identification. She stated that biomarkers are being used as part of the dosimetry in large-scale epidemiological studies. Biomarker data provide ranges of susceptibility across populations, but EPA has not used it much in their risk assessments. Biomarkers also could be used to determine the effectiveness of a standard once the standard has been promulgated.

Chris Saint, NCER, commented that the field eventually will incorporate molecular susceptibility into risk assessments. He noted that this will require substantial methods development, especially in the area of genomics and proteomics. He cautioned that, at this juncture, it is critical that ethical questions are raised continually, and it is essential to inform people of research findings that reveal susceptibility. Linda Birnbaum, EPA's National Health and Environmental Effects Research Laboratory (NHEERL), emphasized the utility of understanding the precise risk odds ratios. Researchers must take a great deal of responsibility when they begin telling people they are susceptible to something.

Dr. Deborah del Junco, Texas A&M School of Public Health, agreed with Dr. Whyatt's definition of molecular epidemiology. She then emphasized the importance of an interdisciplinary approach and noted the increasingly

important role that statisticians play in molecular epidemiology and risk assessment. She raised a concern about establishing a cut-point for a p-value. Barbara Glenn, NCER, expressed concern that few researchers look at the environmental reaction of variable polymorphisms. She believes investigators have a responsibility to look at environmental and ethical factors.

Laurence Kaminsky, New York State Department of Health, cautioned against molecular epidemiology's over-reliance on single nucleotide polymorphisms (SNPs), when haplotypes are what is important for biomarkers of exposure. Dr. del Junco responded that epidemiology has worn two hats. The hypothesis-generating capability requires a large sample size, and there will be SNPs that are irrelevant. The objective of research is to exploit every opportunity, and epidemiology is an iterative field. Dr. Manson concluded the panel session with her comments on SNP versus haplotype data. Gene interaction normally is the last study done; not the first study. Usually, the allele frequency is known, along with its functional significance. For large, expensive studies, there must be a rationale to examine SNPs.

Thursday, April 10, 2003

General Forum: Welcome and Regulatory Context for STAR Research

Welcome

Kacee Deener

Kacee Deener, NCER, welcomed participants to the second day of the EPA Human Health Symposium and announced closing logistics for the Symposium.

A Regulatory Context for STAR Research

Elizabeth Mendez

Elizabeth Mendez, EPA's Office of Pesticide Programs (OPP), provided a technical briefing of a draft guidance document on cumulative risk assessment. She provided an organizational overview of the OPP and described the laws that govern pesticide programs—primarily the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA); the Federal Food, Drug, and Cosmetic Act (FFDCA); and their respective amendments in the Food Quality Protection Act (FQPA).

Risk, as defined in cumulative risk assessment, is toxicity multiplied by exposure. To assess risk, the EPA follows the National Academy of Sciences' (NAS) four-step risk assessment paradigm: (1) hazard identification, (2) dose-response assessment, (3) exposure assessment, and (4) risk characterization. Dr. Mendez noted three uncertainty/safety factors that are accounted for in EPA risk assessments: (1) a 10x uncertainty factor for using an animal study in human risk assessment, (2) a 10x uncertainty factor because some people may be more sensitive than others, and (3) a 10x safety factor to account for risk to children as required by the FQPA.

Contaminants reach humans via oral, inhalation, or dermal exposure routes. Toxic effects may vary depending on the route of exposure. Therefore, route-specific data are used for risk assessment whenever possible. For oral exposures, a dietary risk assessment is performed. For dermal and inhalation exposures, an occupational/residential risk assessment is performed. Several factors may influence a person's exposure, including geography, season, climate, age, diet, and activities. In developing a human health risk assessment, EPA conducts a dietary risk assessment, an occupational/residential risk assessment, and an aggregate risk assessment. In a health risk assessment, a common mode of action or common metabolite is identified first to determine whether a cumulative risk assessment is necessary. The evaluations of risk from the different assessments then are integrated into a holistic risk assessment.

Dr. Mendez concluded by identifying several areas of increasing emphasis that require more data. The areas include life-stage sensitivities, mode of action/mechanism of toxicity, PBPK modeling, biomarkers, endocrine disruption, toxicity of degradation products, and genomics and proteomics.

Breakout Session I: Biomarkers

Saliva Biomonitoring for Organophosphate Pesticide Exposure in Children

Alex Lu

Alex Lu, University of Washington, discussed an animal validation and human exposure study that seeks to measure organophosphate (OP) pesticide levels in saliva as an alternative to blood sampling, determine the feasibility of collecting saliva samples from children, and perform pharmacokinetic analysis.

Saliva biomonitoring may prove advantageous because it measures parent compounds (not metabolites), there are fewer practical and ethical difficulties in sampling, it requires a less complex sample matrix, and it potentially may be used as an indicator of tissue availability. Potential limitations of saliva biomonitoring are that it may not be applicable to all chemicals, there may be crosscontamination, and there may be an insufficient sample volume for chemical analysis.

In the animal validation study, male Sprague-Dawley rats were dosed with 1 and 10 mg/kg of diazinon by bolus intravenous injection. Time-matched saliva and arterial blood samples were collected from 10 to 250 minutes after diazinon administration. Concentrations of diazinon were determined using enzyme-linked immunosorbent assay (ELISA) for pesticide analysis.

Dr. Lu and his team have identified a group of children who live in agricultural communities and are known to be exposed to diazinon and chlorpyrifos. The study will enroll children and their parents. Time-matched saliva and blood samples and multiple urine samples will be collected from the parents, and saliva and urine will be collected from the children.

Dr. Lu concluded that results from the animal study found that diazinon is excreted into the saliva, and that this excretion is not affected by the dose administered or by salivary flow rates. There also is a significant correlation between the concentrations of diazinon in saliva and in plasma samples, which suggests that salivary concentration of diazinon can be used to predict plasma levels. The diazinon concentration in saliva represents the protein-unbound fraction of diazinon in plasma. Additional animal experiments and human exposure studies will further validate these findings.

Development of a Physiologically Based Pharmacokinetic and Pharmacodynamic Model To Quantitate Biomarkers of Exposure to Organophosphate Insecticides

Charles Timchalk

Charles Timchalk discussed research performed by the Battelle Pacific Northwest Division Chemical Dosimetry Group. The research project entails developing and validating a physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model for the organophosphate (OP) insecticide chlorpyrifos to quantitate biomarkers of dosimetry and cholinesterase (ChE) inhibition in young rats and children. The hypothesis is that an age-dependent decrement in chlorpyrifos metabolism correlates with the increased sensitivity of young animals, and potentially children, to OP insecticides. Dr. Timchalk explained that algorithms were developed to calculate age-dependent physiological and metabolic parameters, and the study applied them to a PBPK/PD model to adequately describe the blood and tissue time-course of chlorpyrifos as well as the metabolites chlorpyrifos-oxon and trichloropyridinol. The model eventually will be used to quantitate biomarkers of exposure and response during neonatal/juvenile development. Relevant *in vitro* and *in vivo* experiments needed to refine model parameters, validate model responses, and assess the feasibility of using saliva as a biomonitoring matrix for dosimetry and esterase inhibition are being conducted. Analytical methods also have been developed for quantitation of chlorpyrifos and major metabolites to support *in vitro* and *in vivo* experiments. In addition, to evaluate the potential use of saliva for biomonitoring, studies were undertaken to characterize the total salivary ChE activity and estimate the kinetic parameters *in vitro* and *in vivo*.

interaction of chlorpyrifos-oxon with rat salivary ChE. Results suggest that saliva may be a useful biological matrix for monitoring chlorpyrifos exposure and response, either by measuring the metabolite levels or the degree of ChE inhibition.

Dr. Timchalk noted that the PBPK/PD model has been modified to allometrically scale 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. The results suggest that the paraoxonase (PON1) polymorphism had the greatest impact on target tissue dosimetry at dose levels that overwhelmed other detoxification pathways.

Dr. Timchalk made several summary points regarding the neonatal model development and the PBPK/PD model. The neonatal PBPK/PD model response was consistent with the observed age-dependent sensitivity to chlorpyrifos (CPF). Although the neonatal rat is more sensitive to acute effects at a high dose, at low doses the neonate appeared to be no more sensitive than the adult, based on theoretical simulations of brain oxon concentration. Overall, the preliminary model represents an important starting point in the development of an age-dependent PBPK/PD model for CPF. Furthermore, an overall OP PBPK/PD model has been developed for OP insecticides and can be used to address cumulative and aggregate risk. The model is being adapted to incorporate age-dependent metabolic rates and enzyme levels. Research is ongoing to further refine parameter estimates and to validate the model against dosimetry and dynamic response data.

Validation of Biomarkers of Prenatal Exposure to Contemporary-Use Pesticides

Robin Whyatt

Dr. Whyatt presented research on prenatal exposure to pesticides. Residential use of household pesticides is widespread in the United States. For example, 85 percent of urban minority women from New York City use pest control measures during pregnancy, when pesticides can be transferred readily from the mother to the developing fetus. The objective of Dr. Whyatt's study is to determine the extent to which pesticide levels in biologic samples collected during pregnancy and at delivery reflect exposures during the third trimester.

A cohort of 500 African American and Dominican mother/newborn pairs were included in the Columbia Center for Children's Environmental Health (CCCEH) Study. The mothers were nonsmokers, did not use illicit drugs, and had no history of HIV, hypertension, or diabetes. Pre-delivery study components included a prenatal interview and 48-hour indoor personal air monitoring. To validate biomarkers of prenatal exposure to pesticides, a smaller cohort of 100 women from the CCCEH cohort who spend, on average, more than 70 percent of their time in the home was developed. The gold standard used for biomarker validation was pesticide levels in residential air monitored continuously throughout the eighth and ninth months of pregnancy.

Results of the initial range-finding studies found detection limits to be comparable to or lower than in urine, levels to be comparable to those in adult urine, calibration curves to be linear, and metabolites stable at room temperature over 12 hours. Preliminary results showed that pesticide levels in blood and meconium collected from mothers and newborns at delivery were not correlated with levels of chlorpyrifos, diazinon, or propoxur in indoor air samples during the eighth and ninth months of pregnancy. However, a significant correlation was found between levels of chlorpyrifos and diazinon in maternal blood samples and levels of these pesticides in umbilical cord blood samples. Dr. Whyatt found that these diazinon levels were significantly associated with maternal self-reported pesticide use. Further analysis of nonpersistent pesticide levels in biologic samples is ongoing.

Using Biomarkers of Exposure and Neurobehavioral Test Batteries To Assess Children's Vulnerability to Residential Exposure to Tetrachloroethene (Perc)

Jan Storm

Jan Storm, New York State Department of Health, discussed the New York City tetrachloroethene (perc) project. The study's objectives are to assess the vulnerability of children to residential perc exposure and to relate environmental and biological measures of perc exposure to the occurrence of nervous system effects, particularly vision contrast sensitivity (VCS) and color vision.

Buildings with onsite dry cleaners commonly contain elevated levels of perc. Of the 523 buildings in Manhattan with dry cleaners, 190 have onsite dry cleaners, and 90 of those buildings met the study criteria. Residents from these 90 buildings were recruited for the study and were contacted by mail, telephone, or door-to-door recruitment. The participant goal for the study is to have 60 parent/child pairs in buildings with onsite dry cleaners and a control group of 60 parent/child pairs in buildings without dry cleaners.

At each home, parents sign an informed consent form, 24-hour indoor air is monitored, an exhaled breath sample is taken from the child, and a health and residential/occupational exposure history questionnaire is completed. At Mount Sinai Medical Center, another consent form is signed, and a comprehensive eye exam is performed on the child. VCS and color vision are noted, and another exhaled breath sample is taken, along with a blood sample.

The New York Department of Health—Wadsworth Laboratories evaluated the breath samples for perc. The indoor air samples were evaluated for perc and volatile organic compounds (VOCs). The Centers for Disease Control and Prevention (CDC) measured the perc, VOCs, lead, and mercury levels in the blood samples. The New York City Department of Health and Mental Hygiene was informed of buildings with elevated perc levels, and the New York City Department of Environmental Protection was notified of the buildings with elevated perc levels for possible interventions.

Dr. Storm concluded that the preliminary summary findings suggest there are elevated perc levels in buildings with onsite dry cleaners, and that this has some effect on vision. Of the households measured so far, 29 percent contain perc levels greater than $100 \mu\text{m}^3$, and 6 percent contain perc levels greater than $1,000 \mu\text{m}^3$. The elevated perc levels exhibit a clear socioeconomic status effect because they are concentrated in neighborhoods with lower median incomes. The study will continue to examine the relationships between environmental and biological measures of perc exposure and measures of vision function. Recruitment of additional participants will focus on lower income neighborhoods.

Chlorotriazine Protein Binding: Biomarkers of Exposure and Susceptibility

John Tessari
Melvin Anderson

John Tessari and Melvin Anderson, Colorado State University's Department of Environmental and Radiological Health Sciences, jointly presented their research on chlorotriazine protein binding. Their study tests the hypothesis that binding chlorotriazine by hemoglobin and hair proteins can be used to evaluate differences in exposure and in individual sensitivity to chlorotriazines. Their research aims to: (1) refine gas chromatography/mass spectrometry (GC/MS) methods to assess the reactivity of chlorotriazines and metabolites with hemoglobin, (2) determine whether hair binding of sulfhydryl reactive triazines can be used as a noninvasive measure of exposure to these triazines, (3) develop physiologically based pharmacokinetic (PBPK) models for juveniles and adults that include blood protein and hair protein binding, and (4) use these PBPK models with protein binding measurements to recreate exposure characteristics in laboratory animals and in a limited set of human blood and hair samples.

Dr. Tessari presented the findings for binding sites. Radiolabeled atrazine, the most commonly used EPA restricted-use herbicide, and diaminochlorotriazine (DACT) react with rat hemoglobin, suggesting that both compounds form protein adducts with globin that may be used as a biomarker of chlorotriazine exposure. Dr. Tessari noted that human hemoglobin was found to react more slowly with atrazine and DACT than did rat hemoglobin. An analytical

method developed for atrazine and other chlorotriazines was developed in plasma, using GC/MS with selective ion monitoring. Atrazine was found to convert quickly to metabolites, primarily DACT.

Dr. Anderson presented the *in vitro* PBPK metabolite model, a model of the relative ability of hepatocytes to metabolize chlorotriazines into DACT. The *in vitro* study revealed that dose-dependent metabolism clearly is evident. The PBPK model will permit calculation of expected triazine binding in various populations.

Efforts continue to construct and refine PBPK models that link exposure and circulating triazine levels to produce a comprehensive PBPK model of triazine binding to hemoglobin/hair proteins, including: (1) investigating the pre-treatment and digestion/extraction of hair, (2) investigating globin analysis and triazine binding sites in globin, (3) developing *in vivo* techniques with ¹⁴C-atrazine and ¹⁴C-DACT to determine the presence and persistence of Hb adducts and tissue binding, and (4) confirming the present PBPK model by measuring plasmas and Hb binding kinetics *in vitro*.

Biomarkers and Neurobehavioral Effects of Perinatal Exposure to Insecticides

Kathleen Koechlin

Kathleen Koechlin, Ohio State University (OSU) School of Public Health, discussed perinatal exposure to chlorpyrifos and other organophosphate (OP) insecticides. The purpose of the OSU study is to evaluate the putative relationship between prenatal exposure to chlorpyrifos and other OP insecticides and the adverse neurobehavioral effects among infants and young children. The study's goals are to recruit 176 women in their second trimester of a low-risk pregnancy and their healthy full-term newborns and to follow the mother/baby pairs until the child is 24 months of age.

Women in the second trimester of a low-risk pregnancy are recruited, sign a study consent form, and provide blood and overnight urine samples. Blood samples are analyzed for lead and zinc protoporphyrin levels, and red blood cells are separated from plasma to test the mother's paraoxonase (PON1) genotype. Urine samples are analyzed for dialkylphosphates, 3,5,6-trichloro-2-pyridinal (TCP), 2-isopropyl-6-methyl-4-pyrimidinol (IMP), and cotinine. After 35 weeks of gestation, the women provide a second overnight urine sample that is analyzed in the same way as the first sample. Results indicate the degree of perinatal exposure to OPs.

Interviews are an integral part of the study. At 35 weeks gestation, mothers complete a telephone interview. Information is collected on demographics, occupation, health and medication histories, and insecticide and chemical use and exposure. The same project staff member interviews the same women each time to provide consistency.

Once the baby is born, birth data are collected. When the baby is 2 months old, the mother sends an overnight, urine-soaked diaper for analysis of the same compounds analyzed in her urine. This process is repeated at 9, 16, and 23 months of age. At 3 and 24 months of age, the baby undergoes a neurodevelopmental assessment using various standardized exams. The sessions are videotaped for quality assurance. At 12 and 24 months, venous blood is obtained from the baby. One tube is taken to Columbus Children's Hospital and analyzed for lead. The second tube is taken to an OSU laboratory for determination of the infant's paraoxonase (PON1) genotype (at 12 months only).

Because recruitment of pregnant women into this 2-year study has proved to be more challenging than anticipated, recruitment is still in progress. A total of 60 subjects have been recruited to date but, due to complications, only 39 are enrolled actively. The next steps are to continue recruitment and the analysis of blood and urine specimens obtained thus far.

Breakout Session II: Chemical Mixtures

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

Laurence Kaminsky

Laurence Kaminsky, New York State Department of Health, provided an overview of the Wadsworth Center human *in vitro* mutagenicity studies. The overall objective of these studies is to determine the influence of metals in environmental mixtures of PAHs and metals on PAH carcinogenicity. Specific objectives are to: (1) determine the effects of arsenic, cadmium, mercury, and lead (the four most environmentally hazardous metals) on expression levels of human cytochrome P450 (CYP) enzymes, which bioactivate the five most hazardous PAHs to their carcinogenic forms; (2) assess the potential of the metals to affect the function of the human glutathione S-transferase (GST) enzymes in protecting the cells against bioactivated PAHs; and (3) determine the influence of the metals on the mutagenicity of the bioactivated PAHs.

The study approach is to expose human hepatocyte cell cultures, human liver cancer cell lines, and human breast cancer cell lines to five PAHs and the four metals individually and as mixtures to determine the effect on levels of expression of CYP1A1, 1A2, and 1B1. The influences of human CYP bioactivation of PAHs on the mutagenicity of PAHs is tested in *Salmonella typhimurium* bacteria using the umu test. The effects of other PAHs and metals on this mutagenicity also are tested.

Dr. Kaminsky stated that the metals, most noticeably arsenic, mediated a decrease in the extent of PAH induction of the CYPs that catalyze their bioactivation, implying that the carcinogenicity of the PAHs could be diminished by the metals. No indication of the PAH/PAH synergistic enhancement of CYP induction was detected, indicating that mixtures of PAH only will increase their bioactivation to limited extents. The metals individually and in mixtures did not affect the mutagenicity of the PAHs. Further analysis of the five PAHs and four metals will be conducted in environmental samples.

Evaluating the Carcinogenic Potency of Complex Mixtures

David Warshawsky

David Warshawsky, University of Cincinnati, noted that complex mixtures of combustion and related products contain many carcinogens and anticarcinogens of varying potency that interact in theoretically unpredictable ways. He explained that the conventional mouse skin tumor carcinogenesis assay is too time-consuming and expensive to be of more than limited use. As a substitute, his research relied on a new and more rapid approach to assess carcinogenic potency using cancer induction as the endpoint. This involves determining the potency of complex mixtures (e.g., coke oven tar) as tumor initiators and progressors. Benzo[a]pyrene is used as the positive control. The method uses a dominant negative p53 mutant transgenic mouse skin model (Vp53) with the combined topical application of the test mixture and a strong promoter, 12-O-tetradecanoylphorbol-13-acetate (TPA). Fifteen animals are given complex mixtures twice weekly. The co-application of the test agent and TPA saturates the system with promoting action and provides a rapid display of the potency of the test material. Dr. Warshawsky concluded that preliminary findings show that the initiation and progression protocol will be successful.

Comparative *In Vitro* Immunotoxicity of Organochlorine Mixtures

Sylvain De Guise

Sylvain De Guise, University of Connecticut, presented research on mixtures of organochlorines (OC) at relatively low concentrations in mice, humans, and species of marine mammals. The objectives of the project are to: (1) assess the interactions of OCs in mixtures, (2) assess the differences between species, (3) validate the *in vitro* exposure model in mice, and (4) explore the possibility of assessing exposure at the cell level using antibodies. OCs, particularly PCBs, are important to study because they are ubiquitous in the environment, they bioaccumulate, and they are lipophilic. Man-made OC compounds can be found in the tissues of animals, plants, and people.

The immunotoxic potential of all possible mixtures of five individual OC compounds were tested individually and combined: PCB IUPAC #138, 153, 169, and 180, as well as 2,3,7,8-TCDD. These mixtures were used in the *in*

in vitro immunological assays, where functions of exposed cells were compared to those of unexposed cells. Flow cytometry also was conducted to assess exposure to PCBs at the cell level.

Although few toxicology studies have focused on marine mammals, marine mammal models are good indicators of ecosystem health because these mammals are long-lived, outbreed, are at the top of the food chain, and experience chronic environmental exposure. Furthermore, marine mammals embody many characteristics that are similar in humans. Dr. De Guise noted the advantages of evaluating immunotoxicity in marine mammals via *in vitro* analysis because it is difficult to obtain human participants. For marine mammals, blood samples are relatively easy to obtain; assays are validated; reagents are available; and it is easy to include endangered species, measure a function/change in function in exposed cells, and measure direct effects. Marine mammals included in the study were the beluga whale, killer whale, Commerson's dolphin, Northern fur seal, Steller's sea lion, and Harbor seal. The Mouse-B6C3F1 was used to validate the *in vitro* results.

Dr. De Guise noted that, in the studies of laboratory animals, whenever a decrease was exhibited in natural killer (NK) cells, T cells, T cell cytotoxicity, antibody production, B cell differentiation, or lymphoid depletion, this produced increased sensitivity to endotoxins, bacteria, and virus pathogens. He concluded from his research that *in vitro* exposure to PCB mixtures can modulate phagocytosis, NK activity, lymphocyte proliferation, and expression of surface molecules. Research findings also suggest evidence of congener interaction, synergy antagonism, differences among species, and association between contaminant exposure and disease. These results may have important implications for risk assessment evaluation as well as conservation and management strategies.

The development and validation of *in vitro* assays will provide an attractive and economical alternative to *in vivo* assays for regulatory purposes. Data obtained in this study will provide direction for further mechanistic studies. These results also shed light on the inability of the mouse model, which is widely used in risk assessment, to accurately predict the effects of exposure to mixtures of OC in other species.

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

Carol Reinisch

Carol Reinisch, Marine Biological Laboratory, presented research that examined the surf clam (*Spisula solidissima*) embryo as a model system for defining the impact of exposure to a ternary mixture of bromoform/chloroform/tetrachloroethylene (BCPCE). These chemicals are found in high concentrations in the drinking water wells of Brick, New Jersey, where an elevated rate of autism has been found in children. The study's objectives were to study the effects of the chemical mixture on neuronal development in clam embryos, define the structure of p53 gene family members in the surf clam, and identify the regulatory mechanisms that control expression of these neurologically relevant genes.

The study's approach was to: (1) test the major organic contaminants (BCPCE) of the Brick, NJ, water supply in the same ratios as they occur typically; (2) expose embryos to single components, paired components, or all three components after first division and sample at 24-hour intervals thereafter; and (3) use immunofluorescence to quantify neurotransmitters, receptors, and synthetic enzymes of the nervous system.

Using confocal microscopy and Western blot analysis, Dr. Reinisch and colleagues found that exposing surf clam embryos to the triple mixture increased the appearance and relative amount of a subunit of protein kinase (PKA). Treatment of embryos with single, paired, and triple component mixtures had no effect on either neurotransmitters or receptors of the serotonergic-dopaminergic nervous system. Dr. Reinisch concluded that this mixture of chemicals enhance PKA expression. Additionally, p73, a protein known to control neurogenesis, is expressed in *Spisula* neurons.

Dr. Reinisch suggested that regulatory sites that control p73 gene expression represent potential targets for environmental contaminants. Therefore, future studies should focus on molecular mechanisms to regulate the effects of the ternary chemical mixture on expression of the RII subunit of PKA. In addition, the spatial and temporal expression

of p73 variants in *Spisula* embryos, the extent of p53/73 gene family in *Spisula*, determining what p73 variants are expressed in adult nervous systems, and determining whether Brick, NJ, chemicals affect p53/73 expression should be addressed in future studies.

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

Ken Reardon

Ken Reardon, Colorado State University's Chemical Engineering Department, discussed his work on extending lumping modeling to evaluate the fate and transport of organic pollutant mixtures in the environment. Chemical lumping was introduced for oil refining. Two existing lumping methods are discrete and continuous lumping. Discrete lumping focuses on individual chemical selection, and continuous lumping focuses on the values of a property. Dr. Reardon's research is aimed at determining how to group pollutant mixtures for transport and biodegradation. The transport grouping strategy groups according to structural similarities, physical-chemical properties, and experimental properties. The biodegradation grouping strategy groups according to metabolic pathways, structural similarities, physical-chemical properties, and experimental properties.

Dr. Reardon explained that contamination frequently occurs as a mixture. His model's ability to predict whether the set of chemicals at exposure remains unchanged in transport would contribute to more accurate risk assessment because effective risk management requires knowledge of mixture transport and fate. Preliminary groupings based on soil sorption studies found clusters of pseudocompounds. Research has determined that all chemicals are biodegraded in mixture, the mixture effect is apparent, and chlorobenzenes may be co-metabolized. Dr. Reardon now will focus on what happens as these chemicals move through the environment and eventually reach drinking water supplies.

Mechanistic Evaluation of the Toxicity of Chemical Mixtures

Gerald LeBlanc

Gerald LeBlanc, North Carolina State University's Department of Environmental and Molecular Toxicology, presented research on the mechanistic evaluation of the toxicity of chemical mixtures. Dr. LeBlanc's hypothesis is that the toxicity of complex chemical mixtures can effectively be estimated by understanding the mechanisms of toxicity of the individual chemical constituents and by utilizing algorithms that define interactions based on these mechanisms. He described the project's objective to develop algorithms that define toxicity of chemical mixtures that conform to a model of either: (1) concentration addition, (2) independent joint action, (3) antagonism, or (4) synergy. Algorithms are validated by assessing the toxicity of chemical mixtures to daphnids (*Daphnia magna*). Many scenarios of interaction affect the toxicity of chemical mixtures in the environment. In a U.S. Geological Survey analysis of U.S. surface waters, more than 80 percent contained multiple contaminants. Human bodies also contain complex mixtures of metals, pesticides, and phthalates.

Dr. LeBlanc reported that preliminary evidence suggests that algorithms have been highly successful in predicting the toxicity of binary combinations of chemicals. Chemical groupings, called cassettes, have been designed that accurately model concentration addition, independent joint action, antagonism, and synergy. Furthermore, cassette validity and unanticipated interactions can be assessed experimentally using the daphnid model. Mechanism-based cassettes can be assembled to effectively model the toxicity of complex chemical mixtures; however, data in this area are limited. Dr. LeBlanc explained that the next steps of the project are to build models that combine chemical mixtures that can effectively predict the toxicity of complex chemical mixtures.

Wrap-Up and Closing Remarks

Kacee Deener
Nigel Fields

The research areas represented at the STAR Human Health Symposium are part of a larger portfolio of scientific inquiry into the environmental and ecological health supported by the National Center for Environmental Research. This Symposium presented the unique opportunity to review the progress of several overlapping and progressive research areas related to human environmental health, including:

- ❖ Molecular Epidemiology
- ❖ Statistics for Environmental Applications
- ❖ Basic Science of Complex Chemical Mixtures
- ❖ Environmental Justice and Community Participatory Research
- ❖ Environmental Epidemiology and Toxicology.

Keynote speakers Carole Kimmel and Elizabeth Mendez presented two separate landscapes of interdisciplinary common ground—the National Children’s Study and the Office of Pesticide’s Regulatory Framework, respectively—on which these research areas interlock and buttress EPA in achieving the following goals: (1) promoting sound science in decisionmaking, (2) developing more sophisticated tools for assessing complex exposure and risk scenarios, and (3) including affected citizens and communities in research opportunities

These insights were subsequently restated, reviewed, and reincorporated in further discussion throughout the 2 days, and several crosscutting themes emerged:

- ❖ Traditional and molecular epidemiologists often depend on the aid and expertise of motivated community organizations for effective recruitment and retention in their studies. Working closely with the community also improves sampling and supports the use of culturally appropriate language. Researchers also rely on these community groups for dissemination of research results.
- ❖ Communities faced with environmental threats warranting epidemiological study are, more times than not, concerned with a formidable plethora of environmental concerns. In the past decade, these communities have become more vocal in stressing their need for information and research on the complex interactions of multiple toxicants, which may reach their homes and their children through several routes. Many groups are working more closely with scientists and health practitioners specializing in aggregate and cumulative risk.
- ❖ Many of the chemists, hydrologists, and microbiologists attending the Symposium are eager to learn how groups of toxic chemicals migrate and transform in the environment or human body. However, some of the statistical tools and computer modeling needed to quantify and classify complex interactions have not yet been developed.
- ❖ Epidemiologists and toxicologists are aiming to discover how toxicants in various combinations in the environment may act additively, synergistically, or antagonistically. Cutting-edge research on biological markers may provide new tools and technologies that can be used in epidemiological studies to seek answers for environmentally distressed communities. Incorporation of new genomic techniques into traditional epidemiology may help tease out interactions between environmental chemicals and human genetic susceptibility, and possibly determine how these interactions may lead to disease.

There may be several threads one could weave through these disciplines that connect them to the EPA regulatory and programmatic framework outlined by Dr. Mendez or the ambitious design of the National Children’s Study, as

presented by Dr. Kimmel. The STAR Human Health Symposium provided a forum to explore these common threads and to visualize future opportunities and directions for human environmental health research.

The Human Health Research Program at NCER will continue to solicit the highest quality advanced approaches in scientific inquiry to provide evidence and clearer directions concerning our Nation's toughest environmental challenges.

Project Design and Implementation: Bioaccumulation Toxics in Native American Shellfish

Tony Basabe and Jamie Donatuto

*Swinomish Indian Tribal Community, Office of Planning and Community Development,
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The Swinomish Indian Tribal Community is a federally recognized Indian Tribe. The Swinomish Reservation is located 65 miles North of Seattle, Washington, on the southeastern lobe of Fidalgo Island. The Reservation land base is approximately 10,000 acres, which includes 3,000 acres of tidelands (see Figure 1). The Bioaccumulative Toxics in Native American Shellfish Project is a 4-year interdisciplinary project involving sample collection and analysis, human health risk assessment, education and outreach, and mitigation planning. The principal goal of the project is to ascertain whether Swinomish people, who consume subsistence-harvested clams and crabs, are exposed to bioaccumulative toxics that pose chronic and acute health risks.

The project, initiated in April 2002, represents the logical continuation of the Tribe's water quality research on marine water bodies that surround the Reservation. Several publications have reported the presence of chemical contamination in Swinomish tidelands, waters, and usual and accustomed Treaty rights areas. Shellfish found in these areas are a vital subsistence and commercial resource for the Tribe, and their harvest and consumption is an important point of cultural association for Tribal identity.

Two clams and one crab species have been selected based on Tribal shellfish harvesting and consumption preference, abundance of the species, accessibility to sample sites, and proximity to potential contaminant sources. Native littleneck clams (*Prototheca staminea*), butter clams (*Saxidomus giganteus*), and associated sediments were sampled at 15 sites in the early summer of 2002. Dungeness crab samples (*Cancer magister*) was collected in the early summer of 2003. Clam and crab tissues and sediments will be analyzed for heavy metals; polychlorinated biphenyls, aroclors, and congeners; polyaromatic hydrocarbons; dioxins/furans; organotins; and chlorinated pesticides. The health risk assessment will be conducted in the second and third years of the project, and will include cultural risk considerations. Education and community outreach, conducted in a culturally appropriate manner, will be implemented. Development of mitigation options will be explored in the fourth year.

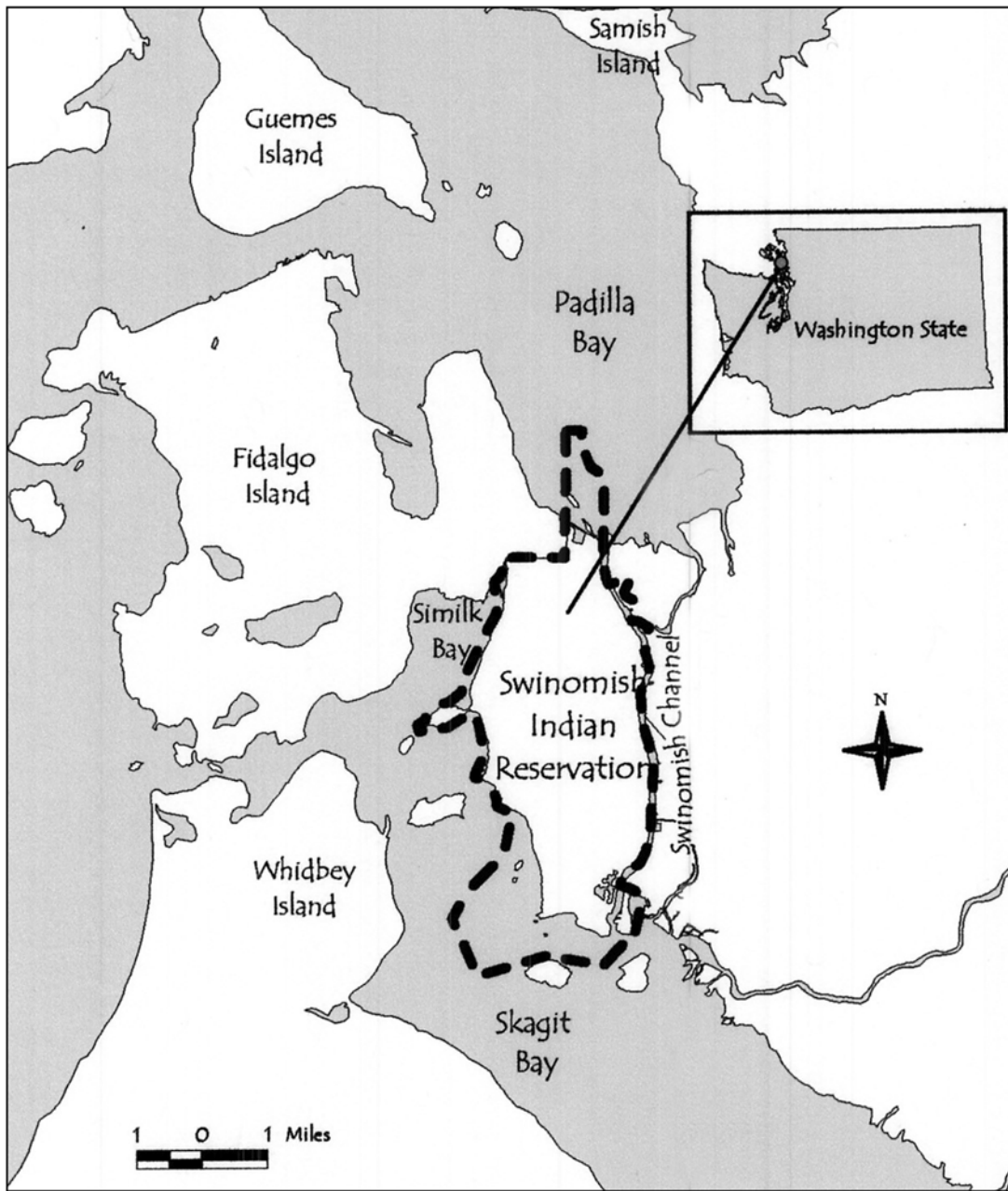


Figure 1. Study site location.

Comparative *In Vitro* Immunotoxicity of Organochlorine Mixtures

Sylvain De Guise

Department of Pathology and Veterinary Science, University of Connecticut, Storrs, CT

The focus of this project is on the immunotoxic potential of simple mixtures of organochlorines at relatively low concentrations in mice, humans, and different species of marine mammals. The objectives of the project are to: (1) assess the interactions of organochlorines (OCs) in mixtures, (2) assess the differences between species, (3) validate the *in vitro* exposure model in mice, and (4) explore the possibility of assessing exposure at the cell level using antibodies.

The mixtures tested represent all the possible combinations using five individual compounds: PCB IUPAC #138, 153, 169, and 180, as well as 2,3,7,8-TCDD. These mixtures are used in the *in vitro* immunological assays where functions of exposed cells (e.g., phagocytosis, respiratory burst, lymphocyte proliferation, NK cell activity, immunophenotyping) are compared to those of unexposed control cells (see Table 1). The significance of *in vitro* exposure to determine immunotoxicity compared to traditional *in vivo* exposures will be validated in mice. A new rapid and economical method of assessing exposure to polychlorinated biphenyls at the cell level using antibodies and flow cytometry.

Using a T-cell mitogen (Con-A), 9 and 2 mixtures significantly reduced lymphocyte proliferation in mouse and killer whale, respectively, while 19, 6, and 4 mixtures significantly increased proliferation in harbor seals, Northern fur seals, and Commerson's dolphin, respectively. No mixtures affected beluga proliferation. Using a B-cell mitogen (LPS), 13 mixtures significantly reduced mouse lymphocyte proliferation. Beluga monocyte phagocytosis was significantly decreased by 16 mixtures. Four and 1 mixtures significantly decreased Northern fur seal and Commerson's dolphin neutrophil phagocytosis, respectively, while 2 mixtures significantly increased neutrophil phagocytosis in killer whales. No mixtures affected mouse phagocytosis. NK activity was significantly increased by 2 mixtures in beluga whales, while 8 mixtures significantly decreased mouse NK activity. Exposure to organochlorines modified the relative proportion of T, B, CD4, and activated T cells when cultured with a T or B cell mitogen or without mitogen. Our results demonstrate both synergistic and antagonistic interactions between congeners. The use of antibodies to assess exposure at the single cell level was not successful.

The development and validation of *in vitro* assays will provide an attractive and economical alternative to the *in vivo* assays for regulatory purposes. Also, it 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 (agonistic/antagonistic properties) between individual compounds in immunotoxicity of mixtures of OCs will be important. The data obtained in this study will provide directions for further mechanistic studies. The results shed light on the inability of the mouse model, which has been widely used in risk assessment, to accurately predict the effects of exposure to mixtures of organochlorines in other species. This model will be useful for future conservation as well as population and habitat management and risk assessment for wildlife as well as humans.

Table 1. Monocyte phagocytosis.

	Mouse	Beluga
PCB 138	No Effect	No Effect
PCB 153	No Effect	No Effect
PCB 180	No Effect	No Effect
PCB 138 + 153	No Effect	Suppression
PCB 138 + 180	No Effect	Suppression
PCB 153 + 180	No Effect	Suppression

Increased Vulnerability of Neonates to Naphthalene and Its Derivatives

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University of California, Davis, CA

The overall objective of this project was to identify the factors critical to establishing whether infants and/or children are more vulnerable to environmental contaminants that produce lung toxicity in adults. This project addressed whether: (1) the increased pulmonary susceptibility of neonates is chemical- and/or species-specific, (2) an increase in injury in the neonates is due to a difference in the balance of activation and deactivation enzymes in favor of the activation pathway, and (3) an increase in neonatal pulmonary injury is due to a decreased ability to regulate glutathione pools.

First, the acute pulmonary cytotoxicity of three polyaromatic hydrocarbons (naphthalene, 1-nitronaphthalene, and 2-methylnaphthalene) was examined in neonatal and adult mice and rats at 24 hours post-treatment. Second, the bioactivation potential of the differentiating Clara cells in the neonates and adults was compared by evaluating *in vitro* metabolism. Finally, the neonates were evaluated for their ability to regulate their glutathione pools in response to the above environmental toxicants.

The neonatal rats and mice were found to be more vulnerable to naphthalene, 1-nitronaphthalene, or 2-methylnaphthalene exposure than the adult animals. Injury progressed in a dose-dependent fashion; however, the dose-response curve for neonatal animals was shifted to the left. The one exception was that there was no detectable injury following any dose of naphthalene in either adult or 7-day-old rats. For a summary of results, see Table 1.

The total metabolic potential of 7-day-old and adult mouse and rat airways *in vitro* was measured by incubating the airways with naphthalene or 1-nitronaphthalene. No neonates of either species were found to produce more metabolites than adults. In addition, covalent binding levels were similar for both neonates and adults of both species.

Airway epithelium also was evaluated for glutathione content using high performance liquid chromatography (HPLC)-electrochemical detection. Neonatal mice and rats had slightly higher baseline levels of glutathione than corresponding adult animals. Treatment with naphthalene or 1-nitronaphthalene resulted in a decrease in the amount of glutathione present in both ages of both species in the first 6 hours following exposure, but levels had rebounded to baseline levels in adults by 24 hours. Neonatal glutathione levels were statistically higher than baseline levels at 24 hours.

The neonatal susceptibility to environmental pollutants was found to be species- and chemical-specific, but could not be reliably predicted based on adult susceptibility. Neonatal susceptibility also cannot be reliably predicated based on any one facet of xenobiotic metabolism. The evaluation of neonatal susceptibility to bioactivated toxicants must utilize an integrative approach. The temporal boundaries of the neonatal “critical window of susceptibility” to pulmonary injury will be defined, and the long-term effects of this injury on further lung development will be examined.

Table 1. Summary of acute cytotoxicity in neonatal mice and rats 24 hrs post-treatment.¹

Compound: Naphthalene			Dose (mg/kg)			
			12.5	25	50	100
Mice	Neonate	Bronchi Bronchioles	- +	- +	- ++	+ +++
	Adult	Bronchi Bronchioles	- -	- -	- +/-	- +
Rats	Neonate	Bronchi Bronchioles	- -	- -	- -	- -
	Adult	Bronchi Bronchioles	- -	- -	- -	- -

Compound: 1-Nitronaphthalene			Dose (mg/kg)			
			12.5	25	50	100
Mice	Neonate	Bronchi Bronchioles	- ++	++ +++	+++ +++	NA ² NA ²
	Adult	Bronchi Bronchioles	- -	- -	+ +	++ ++
Rats	Neonate	Bronchi Bronchioles	- ++	+++ +++	++++ ++++	++++ ++++
	Adult	Bronchi Bronchioles	- -	+ +/-	++ ++	++++ +++

Compound: 2-methylnaphthalene			Dose (mg/kg)			
			20	35	70	140
Mice	Neonate	Bronchi Bronchioles	? ?	? ?	? ?	? ?
	Adult	Bronchi Bronchioles	? ?	? ?	- +	- ++
Rats	Neonate	Bronchi Bronchioles	- -	+/- +	++ ++	++ ++
	Adult	Bronchi Bronchioles	+/- -	+/- +/-	+ ++	++ ++

¹ Degree of toxicity represented as follows: (-) no toxicity, (+) few vacuolated cells present, (++) 50 percent of cells vacuolated, (+++) majority of cells vacuolated or exfoliated, (++++) majority of cells exfoliated.

² No data available; no animals survived to the 24 hr timepoint.

Biological Monitoring of Diazinon Exposure Using Saliva in an Animal Model

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Alternative biological monitoring methods have recently been pursued for better quantifying pesticide exposures. In this study, the feasibility of using saliva for biomonitoring of diazinon exposure was determined in an animal model. Male Sprague-Dawley rats were dosed with 1 and 10 mg/kg of diazinon by bolus intravenous injection (i.v.). Time-matched saliva and arterial blood samples were collected from 10 to 250 minutes post diazinon administration. The concentrations of diazinon in the saliva and arterial plasma samples were determined by using an enzyme-linked immunosorbent assay (ELISA). Diazinon was distributed and eliminated quite rapidly in rats once it was introduced by i.v. bolus injection, according to a two-compartmental pharmacokinetic analysis.

The pharmacokinetics for the plasma and saliva samples are comparable to each other, indicating diazinon behaves similarly in the arterial plasma and saliva compartments. Salivary concentration of diazinon showed a strong correlation with plasma concentration of diazinon ($r^2=0.82$, $p<.01$). The S/P concentration ratio of diazinon was not affected by administered dose, sampling time, or salivary flow rate, suggesting that salivary excretion of diazinon in rats is fairly constant. Diazinon concentrations in saliva were consistently lower than those in arterial plasma. The mean S/P concentration ratios of diazinon were 0.16 and 0.13 for 1 and 10 mg/kg i.v. bolus doses, respectively. It is most likely that the incomplete transfer of diazinon from plasma to saliva is due to protein binding of diazinon in plasma. If the protein-unbound fraction of diazinon in plasma is used to calculate the S/P ratio, the S/P concentration ratio of diazinon would be close to unity.

The results from this study support the conclusion that diazinon salivary concentrations can be used to predict the plasma levels of diazinon in rats, and also reflect the unbound fraction of diazinon in plasma that is available to cause toxicity in tissues. This animal model is being applied for study of the feasibility of saliva biomonitoring of chlorpyrifos and permethrin. A group of children have been identified who live in agricultural communities and are known to be exposed to diazinon and chlorpyrifos. We will be enrolling children and their parents in the study in which time-match saliva and blood samples and multiple urine samples will be collected from the parents, whereas saliva and urine samples will be collected from children.

Casa de Salud: A Model for Engaging Community in Lawrence, Massachusetts

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¹Family Service, Inc., Lawrence, MA; ²JSI Center for Environmental Health Studies, Boston, MA; ³Greater Lawrence Family Health Center, Lawrence, MA; ⁴Lawrence Teen Coalition, Lawrence, MA

Lawrence, Massachusetts is the oldest planned industrial community in the United States. Rapid growth during the industrial revolution was responsible for an influx of immigrants. As factories moved away and closed, Lawrence continued to draw immigrants, and the current population is now more than 70 percent Hispanic. Poverty is widespread and severe, ranking the city among the 25 poorest in the Nation, with an average per capita income of less than \$10,000. Environmental health threats are significant. Lead levels in children are nearly three times the state rate, and pediatric asthma rates are the highest in Massachusetts.

Casa de Salud (Health House) (see Figure 1) is a community research and education effort designed to engage Latino residents in raising awareness and mitigating health impacts of exposure to environmental toxins. Its primary objectives are to develop and demonstrate the effectiveness of training residents to conduct neighborhood meetings (*charlas*) as a means to: (1) conduct mutual education and planning among residents, health care providers, and researchers; (2) increase scientific knowledge and community understanding of specific environmental health threats and change strategies; (3) develop and implement effective, neighborhood-based education tools to help families address known health concerns, especially lead exposure and respiratory illness; (4) gather data regarding residents' health concerns and knowledge of environmental health threats; and (5) develop culturally appropriate community-based interventions that build on increased scientific and medical knowledge and awareness.

Using an integrated community education and organizing model, project partners develop culturally appropriate environmental health educational materials written and translated for a lay audience, train residents to become neighborhood leaders and educators, and conduct mutual education and intervention planning among health care providers, scientists, and community members. Casa Leaders opened their homes for monthly *charlas* that serve as the primary sites for interaction in a highly accessible setting, where residents outnumber outside "experts" and neighborhood culture is dominant.

In the first 2 years of the program, Casa Leaders held *charlas* for 409 residents, organized and conducted neighborhood health fairs reaching an estimated 500 residents, and worked with project partners to make presentations to community organizations such as the Mayor's Health Task Force, the Hispano Network, and the Area Health Education Council. The project's success can be attributed to a mutual respect and understanding that has developed among Casa Leaders and project partners and the project's ability to focus on issues identified by the community that validate their concerns and respect their level of knowledge. In response to requests by Casa Leaders, project partners developed educationally appropriate Spanish-language training programs on recycling, trash, asthma, household hazards, pesticides, ritual use of mercury, fish consumption, and identifying small sources of pollution in neighborhoods. In addition to neighborhood meetings, Casa Leaders are sharing this information with in-home day care providers, church groups, parent organizations, and English as a Second Language classes.



Figure 1. Casa de Salud logo.

Using Human-Derived Cell Systems To Assess the Biochemical Toxicology of Transplacental Carcinogens

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Environmental chemicals such as alcohol, benzo[a]pyrene, and the dietary carcinogen aflatoxin B₁ (AFB₁) may be transferred from the mother to the developing fetus during pregnancy and elicit toxicological effects that may not be manifested until childhood. For some of these agents, key enzyme pathways involved in detoxification (protective) reactions are a critical determinant of sensitivity to toxic injury. These enzymes may be qualitatively and quantitatively different in rodents than in humans, thus providing a degree of uncertainty when extrapolations are drawn from rodent studies to humans during the risk assessment process. The goal of this project is to incorporate human-derived cell models to study these protective pathways and also the toxic sensitivity to chemicals of transplacental concern to humans. These studies have involved two toxicologically important agents, AFB₁ and 4-hydroxynonenal (4HNE), the latter representing an extremely mutagenic toxic metabolite of alcohol that can be produced *in utero*. Studies to date indicate that AFB₁ does not appear to be highly injurious to cultured human prenatal liver tissue. Furthermore, exposure of cultured prenatal liver slices to tissue levels of AFB₁ that are relevant to transplacental exposure does not cause a pattern of gene expression changes that mimic those associated with damage from some other carcinogens.

Studies also are being conducted using human fetal liver hematopoietic stem cells, which comprise a significant percentage of human prenatal cell populations and appear to be important in the effects of transplacental exposures on blood-borne diseases in the offspring. These studies indicate that relevant doses of AFB₁ do not readily affect prenatal liver hematopoietic stem cell survival or DNA injury. In contrast, hematopoietic stem cells are very sensitive to the toxic effects of 4HNE, as evidenced by a significant loss of cell viability and the formation of one or more high molecular weight 4HNE-protein adduct(s) (see Figure 1). The susceptibility to 4HNE was directly correlated to a decreased ability of the human fetal liver tissue and hematopoietic stem cells to detoxify 4HNE through important biochemical pathways.

Other studies using phenytoin, an anti-epileptic drug and known human developmental toxicant, indicate that the level of protective detoxification genes in human prenatal liver tissue can be increased in cell culture by chemical exposure. However, the functional relevance of gene induction needs to be elucidated. Collectively, the results indicate that the use of human-derived cell systems can increase our understanding on how toxic chemicals of transplacental significance may interact with prenatal cell sites and cause injury. These studies also indicate that the inefficient detoxification of 4HNE, a primary metabolite of alcohol, may underlie a susceptibility to cell injury. However, human fetal liver hematopoietic stem cells may not be a sensitive target of the known rodent transplacental carcinogen AFB₁. Ongoing and future studies are directed toward elucidating if the aforementioned chemicals, and others linked to childhood leukemias, specifically altered gene expression and differentiation pathways of fetal liver hematopoietic stem cells that may increase their leukogenic potential.

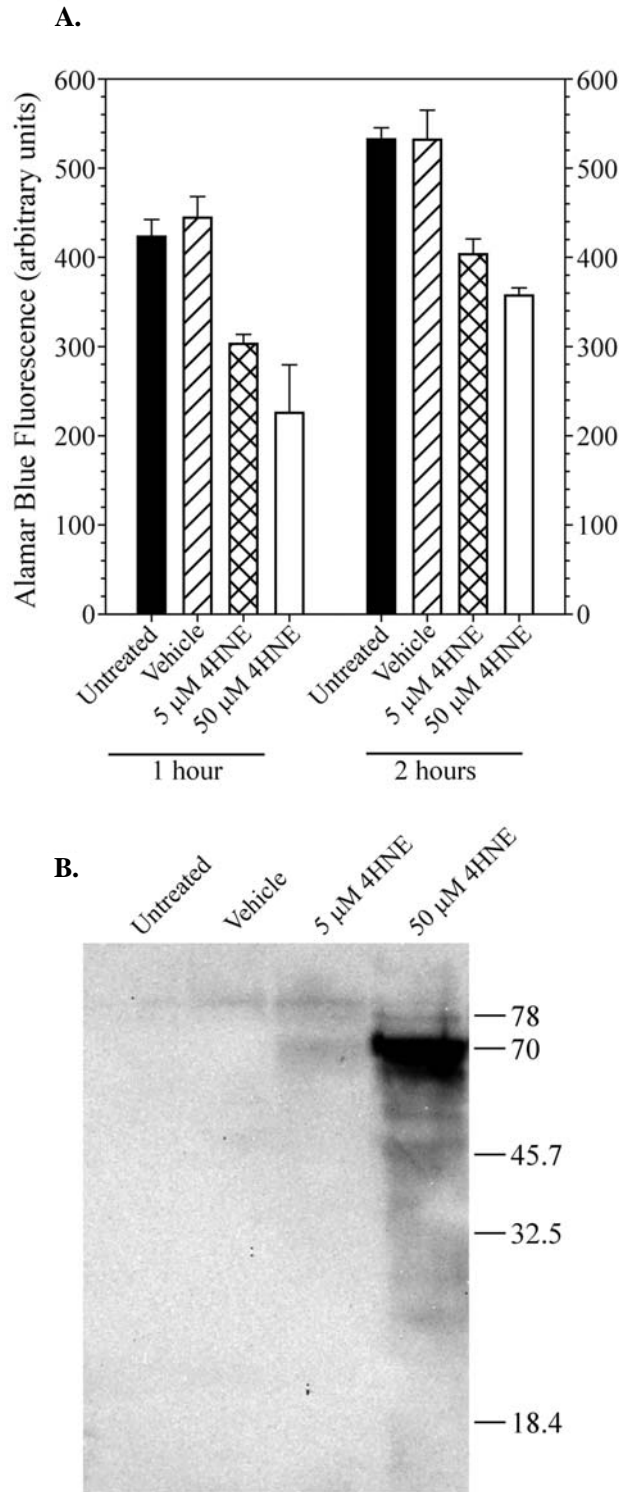


Figure 1. Sensitivity of cultured fetal liver hematopoietic stem cells to 4HNE toxicity and 4HNE-protein adduct formation. **A.** Viability falls sharply on treatment with low concentrations (5 μ M) of 4HNE. Error bars indicate standard error of three experiments. **B.** SDS-PAGE followed by Western analysis using a 4HNE-protein adduct antibody reveals formation of high molecular weight 4HNE-protein adducts with discrete banding. Electrophoretic standards were used to discern the noted molecular weights (kDa); molecular weight of the primary band was subsequently interpolated.

Empowering the Community To Address Their Own Concerns About Asthma and Environmental Concerns in North Brooklyn

Luis Garden Acosta

North Brooklyn Asthma and Environment Consortium: El Puente, Brooklyn, NY; New York University School of Medicine, New York, NY; and Woodhull Medical and Mental Health Center, Brooklyn, NY

The emerging epidemic of asthma in the United States has manifested itself in urban environments over the past 20 years, particularly in areas where poverty levels are lower than surrounding areas, housing conditions are substandard, and communities consist of traditionally underserved populations. One such area where asthma prevalence is disproportionately high is North Brooklyn, particularly among the Latino population that the community-based organization El Puente serves.

El Puente's EPA-funded environmental justice research is rooted in the belief that the struggle for health promotion must be rooted in the community, if public health is going to actively confront the myriad of environmental and health risks facing the mosaic of neighborhoods in today's America. The overall goal of El Puente's research project is for the community to drive scientific research, in this case, investigating the environmental exposures related to asthma. The objectives of this research project are to: (1) initiate a research program that promotes a partnership between members of an environmentally stressed community, academia, and health practitioners; (2) develop culturally appropriate education and communication activities; (3) design and implement preventive environmental interventions to reduce exposure to key asthma precipitating agents, and interventions to improve quality of asthma care; (4) evaluate the process, the partnership, selected health outcomes, and interventions; and (5) disseminate the successful outcomes of the program throughout Williamsburg and the North Brooklyn community, the health care community, and other communities. The goal of this project is to conduct scientific research investigating the environmental exposures related to asthma that is truly driven by the community.

The Consortium is comprised of a partnership between El Puente (a community-based organization with a long history of empowering the community to address its health concerns, particularly asthma management); New York University School of Medicine (providing a strong leadership in the research planning and implementation); and Woodhull Medical and Mental Health Center (one of the largest health care providers in the area that serves a diverse population).

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

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The overall goal of this project is to determine the influence of metals in environmental mixtures of polycyclic aromatic hydrocarbons (PAHs) and metals on PAH carcinogenicity. The specific objectives are to: (1) determine the effects of the four most environmentally hazardous metals (arsenic, cadmium, lead, and mercury) on expression levels of human cytochrome P450 (CYP) enzymes, which bioactivate the five most hazardous PAHs (benzo[a]pyrene (BAP); dibenz[a,h] anthracene (DBAHA); benzo[a]anthracene (BAA), benzo(k)-fluoranthene (BKF), and benzo[b] fluoranthene (BBF)) to their carcinogenic forms; (2) assess the potential of the metals to affect the function of the human glutathione S-transferase (GST) enzymes in protecting the cells against bioactivated PAHs; and (3) determine the influence of the metals on the mutagenicity of the bioactivated PAHs.

The general approach is to expose fresh human hepatocyte cell cultures and the human liver and breast cell lines, HepG2 and T-47D, to the five PAHs and the four metals individually and as mixtures to determine the effect on levels of expression of human CYP1A1, 1A2, and 1B1, the PAH bioactivating enzymes. The influences of human CYP bioactivation of PAHs on PAH mutagenicity and the effects of other PAHs and the metals on this mutagenicity are tested in *Salmonella Typhimurium* bacteria using the induction of the umuC gene.

In T-47D cells, arsenite (10 μ M) produced 86 and 92 percent decreases in BAP (3 μ M) induction of CYP1A1 and 1B1, respectively. The levels of CYP1A1 and 1B1 mRNA were not significantly altered by the arsenite, implying post-transcriptional mechanisms. In hepatocytes, the PAH induction efficiency for CYP1A1 and 1A2 was in the order BKF >DBAHA>BAP>BBF>BAA. All four metals (1-5 μ M) decreased CYP1A1/1A2 induction by some of the PAHs. Arsenite (5 μ M) decreased induction by 47 percent for BAP, 68 percent for BAA, 45 percent for BBF, 79 percent for BKF, and 53 percent for DBAHA. None of the four metals (1-5 μ M) or non-bioactivated PAH-diols induced mutations in the umu assay. Mutagenic potencies of human CYP1A1 bioactivated PAH-diols were in the order BAP-7,8-diol>BBF-9,0-diol>BAA-3,4-diol >>>BKF-8,9-diol>BKF-2,3-diol = DBAHA-3,4-diol = DBAHA-5,6-diol (see Figure 1). None of these mutagenicities were altered by any of the metals (1 μ M) or by mixtures of the metals (total of 4 μ M). Both the PAHs and the metals induced GST-A1 in HepG2 cells. Mixtures of the PAHs and metals did not yield synergistic or even additive induction of GST and of CYPs.

The metal (most markedly arsenic) mediated decrease in the extent of PAH induction of the CYPs that catalyze their bioactivation implies that in PAH/metal mixtures, carcinogenicity of the PAHs could be diminished by the metals. No indication of PAH/PAH synergistic enhancement of CYP induction was detected, indicating that mixtures of PAH will only increase their bioactivation to limited extents. The metals individually and in mixtures did not affect the mutagenicity of the PAHs.

Environmental samples, selected for their contamination by PAHs and metals, will be analyzed for content of the five PAHs and four metals. The extracts will be tested for effects on CYP1A1, 1A2, and 1B1 induction and for CYP-potentiated mutagenicity, and the results will be compared to predicted results from our studies based on the PAH/metal contents.

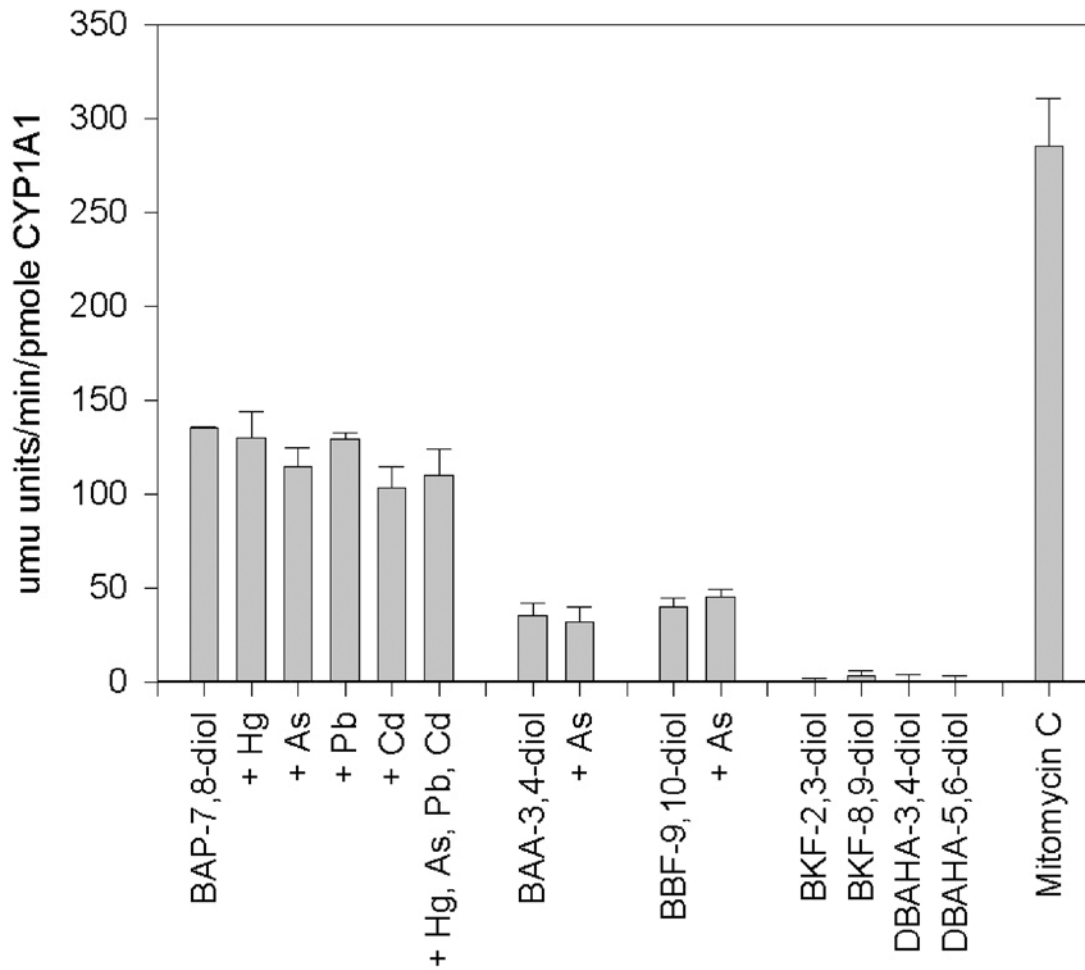


Figure 1. Mutagenic potencies of human CYP1A1 bioactivated PAH-diols.

Molecular Characterization of a Biological Threshold in Developmental Toxicology

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The goal of this research project is to characterize, in molecular terms, the biological basis of a threshold in developmental toxicity and place this into the context of a quantitative dose-response model for risk assessment. The test agent, 2-chloro-2'-deoxyadenosine (2CdA), is a prototype requiring an intact p53 tumor suppressor response for developmental effects. The research objectives are to profile gene expression in early mouse embryos at dose levels flanking the threshold for eye malformations, across the time of p53 protein induction, and in therapeutic intervention with Bzrp ligands (see Figure 1).

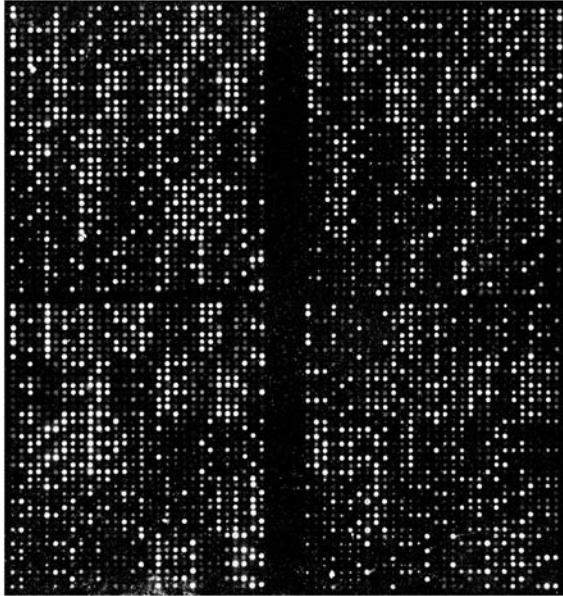
BMDS software predicted the threshold for microphthalmia at 2.0 mg/kg 2CdA, a dose intermediate between the no effect level (NOAEL = 1.5 mg/kg) and benchmark dose for a 5 percent increased risk of malformation ($BMD_5 = 2.5$ mg/kg). Trajectories of gene expression were studied at three time points after exposure to 2.5 mg/kg 2CdA flanking p53 protein induction at 4.5 hours. Trajectories also were studied in embryos where p53 was tempered with a potent Bzrp ligand (PK11195) and a less active one (Ro5-4864). Data analysis was conducted using Gene Spring software applied to 50 replicate conditions and variance components analysis (t-test and the Benjamini and Hochberg correction, $\alpha = 0.05$).

Data analysis returned 182 genes changing across the 2CdA dose response and clustered them as those altered at the threshold dose (2.5 mg/kg) and those altered at the teratogenic dose (5.0 mg/kg). The 180 genes sensitive to 2.5 mg/kg 2CdA over time fell into three principal behaviors of phasing with respect to p53 protein induction, with therapeutic intervention with PK11195 having its greatest impact 3 hours post-exposure (e.g., before p53 protein induction). A significant component of the "PK11195-operon" was insensitive to Ro5-4864; hence, the embryo reacts strongly and distinctively with respect to its biological threshold for developmental toxicity.

These data suggest a transitional response to developmental toxicant exposure. The system moves from State 1 (baseline) reversibly into State 2 (transitional) at the biological threshold for disease, and in the absence of adaptation moves irreversibly into State 3 (disease). Checkpoints between States 1 and 2 are sensitive to mitochondrial benzodiazepine receptor ligands (e.g., PK11195) and between States 2 and 3 are at least partly sensitive to p53 protein induction.

A comprehensive gene expression matrix that captures the biological complexity of the embryonic transcriptome and its regulation provides a computational resource for deducing the physiological state of the embryo during development and disease. With this information, can we ask what factors and signals move the embryo from one physiological state to the next? Will these multigenic responses be captured into a biologically based dose response model to improve our understanding of key changes at the low end of the dose response curve?

Early mouse embryo



Early rat embryo

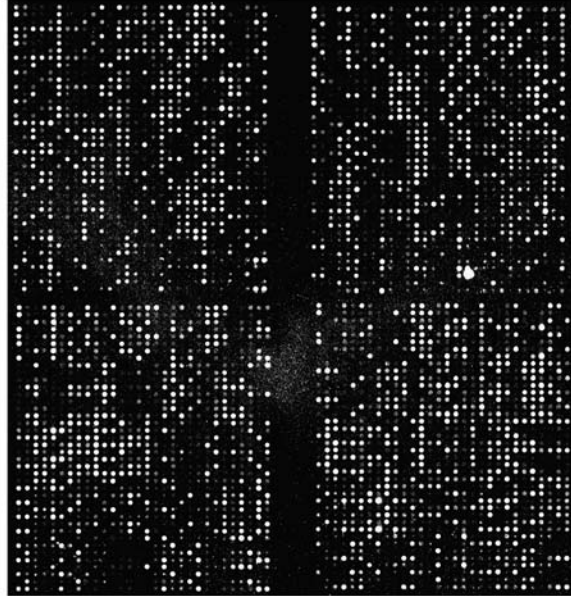


Figure 1. Microarray hybridization of embryonic RNA from the mouse vs. rat at the early headfold stage of development.

Mechanistic Evaluation of the Toxicity of Chemical Mixtures

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Evaluating the toxicity of chemical mixtures is one of the most pertinent toxicological issues to face modern society and one of the most daunting challenges to modern toxicologists. The virtually infinite number of chemical combinations that constitute environmentally relevant mixtures renders toxicological characterization via standard descriptive approaches obsolete. The general hypothesis being tested in this research program is that the toxicity of complex chemical mixtures can be satisfactorily estimated by understanding the mechanisms of toxicity of the individual constituents and utilizing algorithms that define interactions based on these mechanisms. The first aim of the program is to develop algorithms that define toxicity of chemical mixtures that conform to a model of either: (1) concentration addition, (2) independent joint action, (3) antagonism, or (4) synergy. The algorithms then are validated by assessing the toxicity of chemical mixtures to daphnids (*Daphnia magna*). Daphnids are used as the whole organism model in the toxicity assessments because of their small size and amenability to laboratory culture. Also, more than 100,000 organisms will be required to complete this project, placing considerable time, cost, and ethical considerations into the use of vertebrate animal models.

The algorithms thus far have been highly successful in predicting the toxicity of binary combinations of chemicals (see Figure 1). The next goal will be to evaluate the ability of integrated algorithms to predict toxicity of more complex chemical mixtures that exhibit multiple modes of toxicity and interaction. In this assessment, chemicals are assigned to groups (cassettes) based on common modes of action. The cassettes then are integrated into algorithms that consider the independent joint action of the compounds, along with any antagonistic or synergistic interactions. Currently, quaternary mixtures are being evaluated in which the four compounds conform to one of two possible modes of action (i.e., two cassettes), and then the independent joint action of the cassettes are predicted. Validation experiments thus far demonstrate that these integrated algorithms can successfully predict the toxicity of complex mixtures.

The final aim of this program will be to expand the approach to incorporate multiple sublethal indices of toxicity (reproductive toxicity, developmental toxicity, etc.) into the algorithms and validate the models experimentally. It is anticipated that results from this research program will provide a solid research framework to structure evaluations of the toxicity of complex chemical mixtures. The framework will be highly adaptable for use in human health and environmental health assessments of the toxicity of chemical mixtures. Further, the framework will be applicable for use in both retrospective and prospective assessments of the hazards of chemical mixtures.

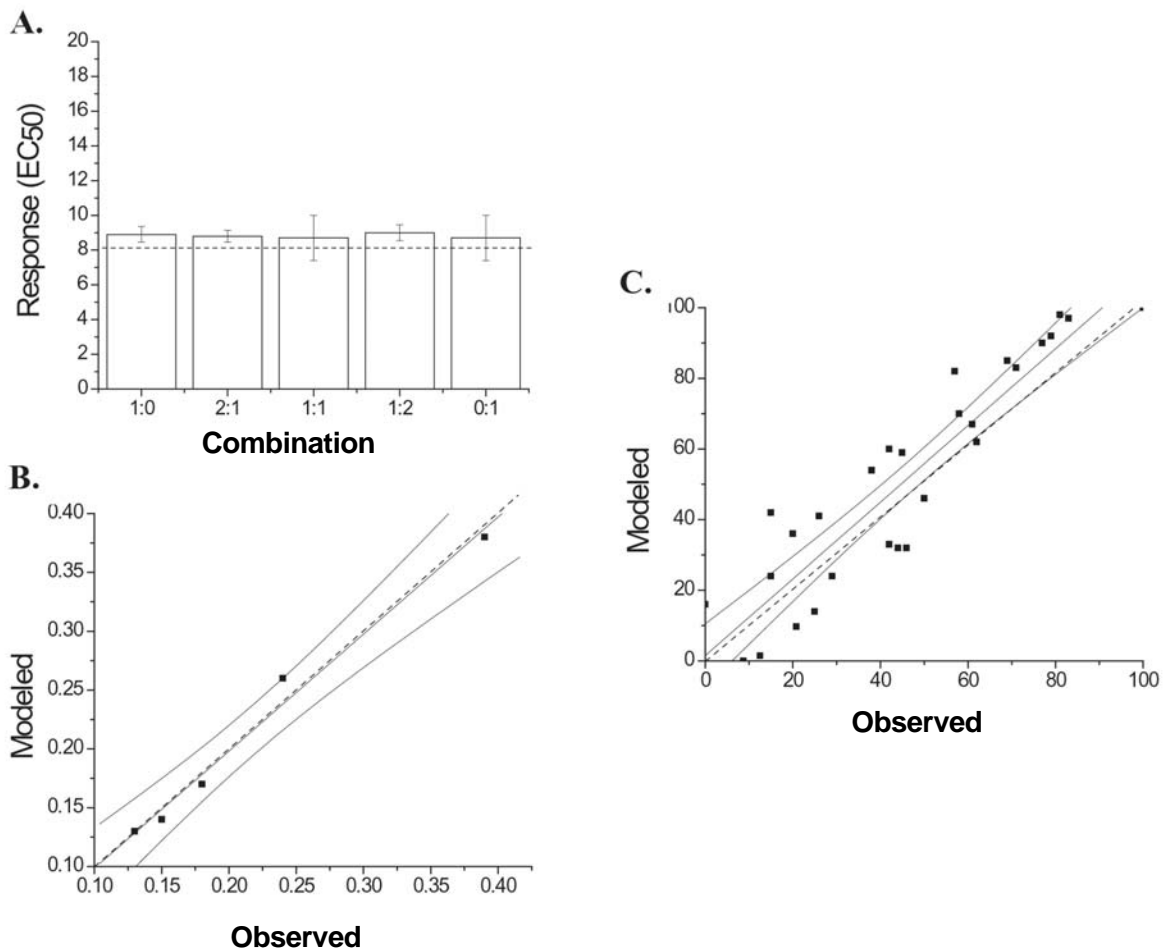


Figure 1. Examples of modeled and observed toxicity of binary combinations of chemicals. **A.** Measured toxicity (presented as EC50 values (nM)) of five combinations of malathion and parathion that were predicted to cause 50 percent effect based on the model for concentration addition (dashed line). **B.** Measured toxicity of five combinations of piperonyl butoxide and malathion (concentration response line and 95 percent confidence interval) compared to the predicted concentration-response as modeled using the algorithm for antagonism (dashed line). **C.** Measured toxicity of 30 combinations of fenarimol and tes-tosterone (concentration response line and 95 percent confidence interval) compared to the predicted concentration response as modeled using the algorithm for synergy (dashed line).

Coupled Physical-Stochastic Dermal Exposure/Two-Layer, Time-Variant Percutaneous Dose Model

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A two-layer, time-varying, numerical model has been developed to calculate the absorption of chemicals through the human skin, and has been coupled to a previously developed physical-stochastic exposure model (i.e., Dermal Exposure Reduction Model-DERM). The coupled model is unique in its use of micro-level activity time series data (MLATS), collected through videotaping and video-translation methodologies, that describe in detail the contact events leading to the loading of chemical mass on the skin surface and the boundary and time conditions for diffusion through the skin. The coupled dose and exposure model are available in the S-Plus programming platform, with a user-friendly interface for managing the routine calculations and generating quantitative and graphical output. Care has been taken to ensure that the two interfaced models function smoothly (i.e., that dermal exposure data from DERM is correctly handled by the routines of the numerical model). Simulations, using the MLATS data for 20 children, ages 1 to 6 years, have been conducted with the coupled exposure and numerical dose model to demonstrate their suitability for use with multiple exposure events (i.e., skin contact events defined by MLATS). Figure 1 illustrates how DERM and the numerical dose model use the information from a MLATS file, and also the additional data requirements of the models.

The equations of the percutaneous numerical model fully describe contaminant diffusion, out of and into the vehicle, through the *stratum corneum* and the viable epidermis into the bloodstream. In particular, the numerical model accounts for time varying surface boundary conditions, such as the depletion of the chemical agent out of the vehicle due to absorption through the skin, or increase due to multiple chemical loadings, or even diffusion out of the skin following surface removal events. In addition, the boundary conditions of the model permit more appropriate modeling of multiple exposure events by accounting for previous skin concentrations from one exposure event to the next. Therefore, simulations will show that solutions to the coupled exposure and dose equations provide the concentration at the surface of the skin and within the skin as a function of both time and space. Thus, we know not only how much contaminant mass has entered the bloodstream, but also the distribution of the chemical concentration remaining in the skin and at the surface of the skin.

The development of a two-layer, time-variant, numerical model and its coupling with an existing physical, stochastic exposure model for the dermal route is part of a larger effort by the Exposure Research Group at Stanford University to develop a model for estimating cumulative (i.e., more than one chemical having the same toxicological endpoint) and aggregate (i.e., all three routes) exposure and dose to environmental contaminants, using micro-level activity time series data. Major improvement of risk estimation can be achieved by development of more robustness in the exposure and dose estimates. Therefore, the work so far has produced a much-needed coupled exposure and dose model for the dermal pathway, which is considered the most difficult pathway to model appropriately.

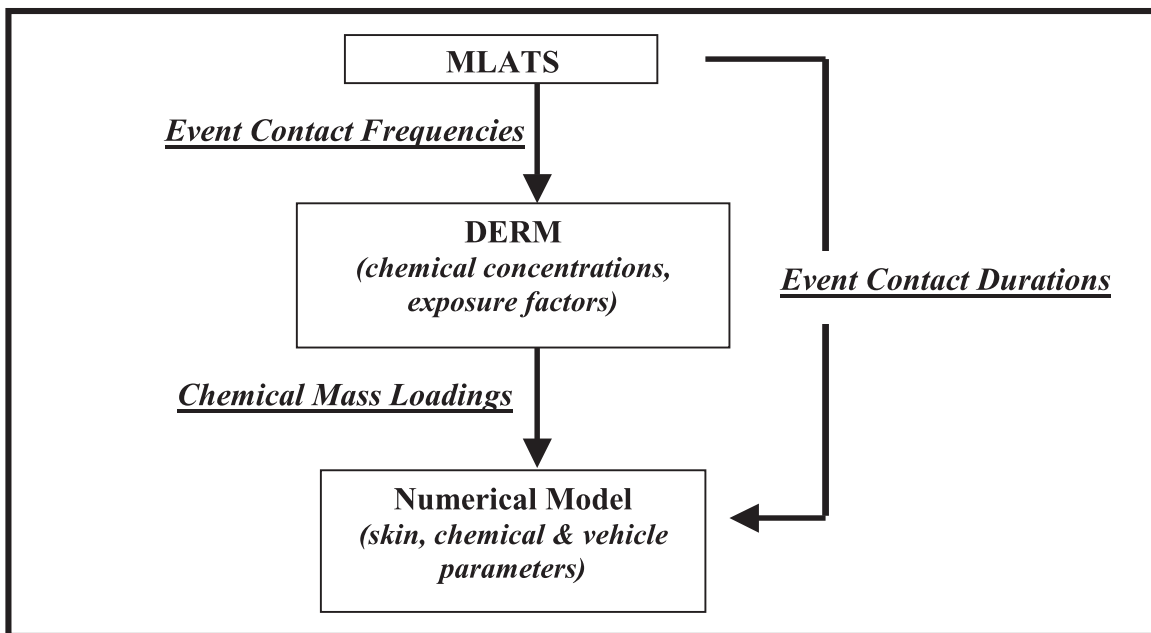


Figure 1. MLATS, DERM, and the Percutaneous Dose Model working together.

A Longitudinal Approach To Assessing Aggregate Exposure to Organophosphorus Pesticides in Children

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This study has three primary objectives. First, it is designed to characterize the temporal and inter-individual variability of total organophosphorus (OP) pesticide exposures in children in relation to both residential pesticide use and dietary intake. Second, 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 body burden. Third, this study will establish a baseline level of OP pesticide exposures in children, and will determine the contribution of OP pesticide residues in children's diets to this baseline.

A combination of biological monitoring and multi-pathway sampling techniques are proposed as complementary approaches to exposure characterization in which not only children's exposure to OP pesticides can be more effectively and accurately assessed, but also it is likely to generate meaningful results as well. The study design for this project has three components. First, a longitudinal biomonitoring study will be conducted in which spot urine samples will be collected from a cohort of children ages 2-12 residing in the Seattle metropolitan area for a 12-month period. The second component of this study focuses on assessing aggregate OP pesticide exposures to children with elevated OP pesticide exposures, as measured in the previous longitudinal biomonitoring study. Environmental and biological media, including soil, indoor air, dust, duplicate diet and urine, will be sampled before and after a known exposure event occurred. The third component of this study is to establish a baseline level of OP pesticide exposure in this cohort. The contribution of dietary OP pesticide ingestion to this baseline level will be estimated by providing exclusive organic produce to children for 1 week during the low OP exposure period. A drop of the baseline is anticipated, followed by a gradual return to the normal levels when conventional produce is reintroduced.

The work outlined in this study is likely to expand knowledge regarding children's aggregate exposures to pesticides. This work will establish a temporal profile of OP pesticide exposures to young children and will evaluate the relationship between this profile and both residential pesticide use and children's diets. This valuable database will have the implication of implementing the requirements under the Food Quality Protection Act of 1996. Second, the outcome from the longitudinal study will assist us in designing a cost-effective multi-pathway sampling study in which key exposure pathways are evaluated at the time of occurrence. Also, it is likely that this study will result in the establishment of a baseline level of OP pesticide exposure in children residing in metropolitan communities. This information will have the implications for cumulative risk assessment and for regulations regarding a health-based pesticide exposure standard for children.

Molecular and Epidemiologic Risk Factors for Hypospadias: Preliminary Results From an Ongoing Study

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The goals of this project are to understand the genetic and environmental risk factors for hypospadias to determine if a gene-environment interaction is the cause of this developmental defect. The hypothesis is that allelic variants in genes controlling androgen action and metabolism (steroid 5-alpha reductase [SRD5A2], androgen receptor [AR] and 17-beta hydroxysteroid dehydrogenase type 3 [HSD17B3]) will be highly associated with the risk for hypospadias in male infants. Parental exposure to environmental agents with anti-androgenic activity during pregnancy may further increase the risk for hypospadias in male infants with a susceptible genotype, resulting in a gene-by-environment interaction. The association between allelic variants in these genes and hypospadias only has been evaluated to date in consanguineous families or small case series. This will be the first study in a large, outbred population to investigate gene-environment interactions on the risk of this rapidly increasing developmental defect.

Families of case infants less than 1 year of age presenting for diagnosis/surgical repair of hypospadias in a pediatric urology clinic are recruited into the study. Families of control infants with renal anomalies, primarily hydro-nephrosis, are recruited from the same population. Parents are administered questionnaires to obtain information on reproductive and obstetrical history and exposures to drugs and environmental chemicals. Buccal swabs are collected from the mother, father, and infant at the same time, and DNA is extracted for evaluation of candidate genes for hypospadias.

Results to date on approximately 250 families indicate that traditional epidemiologic risk factors for hypospadias have been found in the case group. These include race, decreased birth weight, preterm birth, and primiparity. Risks associated with the affected control group include maternal gynecologic conditions such as amenorrhea and endometriosis, as well as maternal complications during the index pregnancy of urinary tract infections. Cases show a significant trend toward increased occupational and home exposures to environmental chemicals (e.g., pesticides, paints, stains, fuels, solvents). Cases also have a higher incidence of mutations in Exon 1 of the SRD5A2 gene (V89L) that produces approximately a 60 percent reduction in enzyme activity. These results are preliminary and reflect results from the first 250 families (out of 800) to be recruited.

The findings of increased exposure to specific classes of environmental chemicals in the case group are notable, as the study has been designed to minimize recall bias with the selection of an affected control group. The V89L mutation found in Exon 1 of the SRD5A2 gene also is of significance, as this mutation reduces but does not abolish enzyme activity. If the mutation inactivated the enzyme, a gene-environment interaction would not be feasible. Given the increased environmental exposures and occurrence of the V89L mutation in the case group, evaluation of gene-environment interactions appears to be a feasible goal for this project. Efforts are underway to improve recruitment and to develop more efficient methods for genotyping to meet the project goals of enrolling 800 families.

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

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The surf clam (*Spisula solidissima*) embryo is used as a model system for defining the impact of exposure to a ternary mixture of bromoform/chloroform/tetrachloroethylene (BCPCE). These chemicals are found in high concentrations at the EPA superfund site in Brick Township, New Jersey, where an elevated rate of autism has been found in children. Initially, the effects of this chemical mixture were studied on neuronal development in clam embryos. The second objective was to define the structure of p53 gene family members in surf clam, and to identify the regulatory mechanisms controlling expression of these neurologically relevant genes. By confocal microscopy and Western blot analyses, it was demonstrated that surf clam embryos exposed to an environmentally relevant combination of BCPCE have an increased amount of a developmentally expressed isoform of the regulatory subunit (RII) of protein kinase A (PKA). Furthermore, in embryos exposed to the chemical mixture, RII reactivity was increased in the innervated regions of the primordial gill and ciliated velar epithelium (see Figure 1). This heightened RII reactivity directly correlated with elevated ciliary activity. These effects were observed only with the ternary mixture. When components were tested individually and in pairs, only the chloroform/PCE combination led to an increase in RII. This was not statistically significant. There was no statistically significant effect of exposure to the ternary mixture on either the neurotransmitters or receptors of the serotonergic-dopaminergic nervous system. This is the first report that bromoform, chloroform, and tetrachloroethylene act synergistically to alter a key regulator of neuronal development, which may have implications for autism.

Polymerase chain reaction (PCR) was used to identify two p73 variants, members of the p53 gene family with demonstrated relevance to the developing nervous system. The nucleotide sequences of the two variants are identical up to their stop codons, but diverge in their 3'-untranslated regions (UTRs). The two transcripts represent alternate polyadenylation sites for the *Spisula* p73 gene. This is the first identification of multiple p73 variants in any nonmammalian species. In addition, although alternative splicing has been well documented in the p73 gene family, this is the first report of alternate polyadenylation site choice as a control point for p73 gene expression. To identify specific post-transcriptional and post-translational signals controlling regulation of p73 expression, *Spisula* p73 nucleotide and deduced amino acid sequences were compared to corresponding regions of mammalian and nonmammalian p73s. Within the *Spisula* 3' UTRs, multiple AU-rich elements were identified that may control translation activation. In the deduced amino acid sequence, potential sites were identified for sumoylation, a post-translational process characteristic of p73 proteins. These results provide information about signaling mechanisms that control expression of p73 isoforms during neuronal development. Most importantly, these data define potential genetic regulatory sites targeted by chemical exposure. In year 2 of this project, the effects of chemical exposure on p73 gene expression will be examined in developing embryos. This will be accomplished using *Spisula* p73 gene sequence information to develop specific molecular probes for use in the *in situ* hybridizations and Northern blot analyses.

Labeling of the Gill Region With an RII Antibody

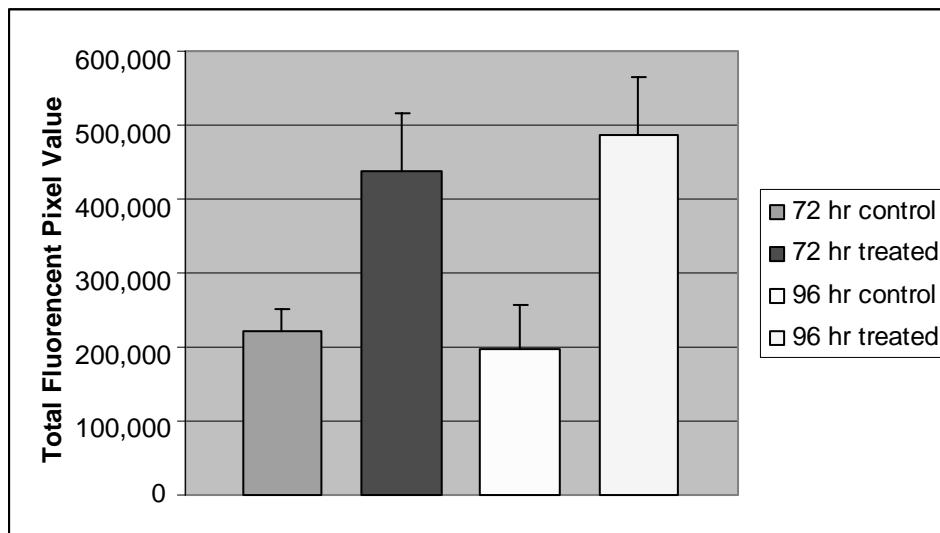
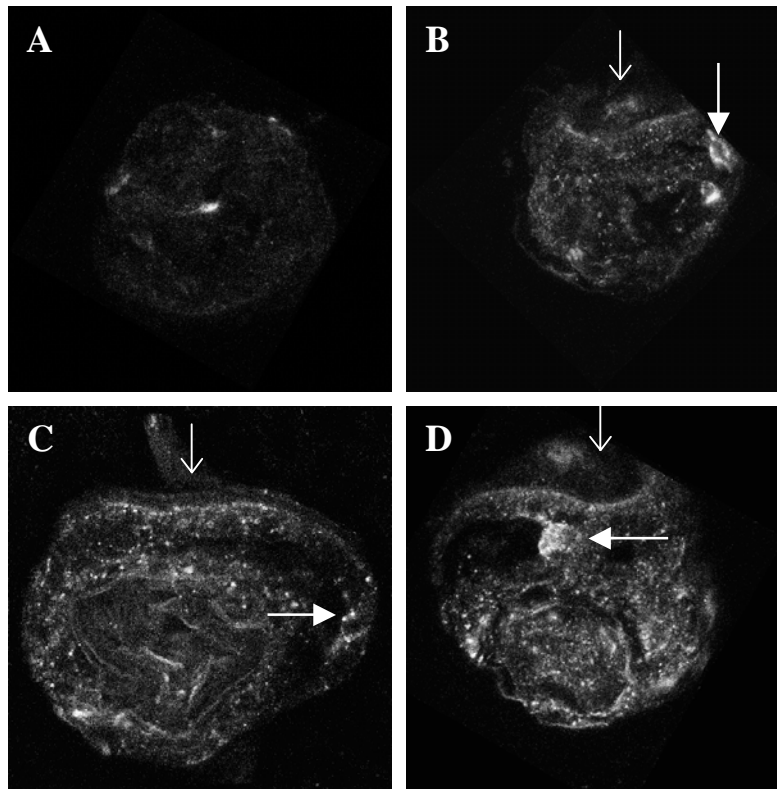


Figure 1. Confocal micrographs of *Spisula* embryos labeled with an RII antibody. A and B—72 hour control and 1,000x treated embryos, respectively. C and D—96 hour control and 1,000x treated embryos, respectively. The open arrow shows the position of the velum, which is oriented up. The solid arrow indicates the developing gill region. The graph shows the mean of the total integrated fluorescence intensity of the gill region for each group. Note that the RII labeling in the gill region increases with treatment.

Gene-Environment Interactions and Human Malformations

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This funded research program has been developed to investigate: (1) if genetic variation of infant and maternal genes involved in biotransformation and detoxification modify risks of malformations, in the presence or absence of selected maternal exposures to toxicants; (2) if genetic variation of infant and maternal genes involved in folate metabolism and transport modify risks of malformations, in the presence of variations in maternal folate intakes; and (3) if genetic variation of infant and maternal genes involved in vascular development and function modify risks of malformations, in the presence or absence of maternal exposures to vasoactive chemicals.

The population-based case-control research design includes approximately 5,000 cases and controls and focuses on several malformations: neural tube defects, selected heart malformations, orofacial clefts, limb defects, gastroschisis, and intestinal atresias. The analytic plan combines maternal interview data with multiplex polymerase chain reaction-based genotyping for nearly 40 candidate genes on more than 7,200 samples. Preliminary findings associated with two of the above aims, namely folate transport/metabolism and vascular development, will be presented. With respect to both the folate and the vascular development aims, the use of an exciting new multilocus genotyping assay, developed by Roche Molecular Systems, is being perfected. This assay detects 32 polymorphisms from 23 candidate genes. These genes are: MTHFR, CBS, Factor II, Factor V, Factor VII, fibrinogen, glycoprotein 1a, glycoprotein IIIa, plasminogen activator inhibitor-1, endothelial leukocyte adhesion molecule, intracellular adhesion molecule 1, tumor necrosis factor-alpha, tumor necrosis factor-beta, stromelysin, angiotensin converting enzyme, angiotensinogen, angiotensin II receptor type I, alpha-adducin, atrial natriuretic peptide, Beta2 adrenergic receptor, epithelial NA channel alpha subunit, and G3 B3 subunit.

With respect to folate, a candidate gene that is involved in folate transport—the reduced folate carrier-1 gene (RFC1)—is being investigated. In addition, this study investigated whether risk of orofacial clefts or conotruncal heart defects were influenced by a polymorphism of infant RFC1 at nucleotide 80 (A80G), or by an interaction between the RFC1 polymorphism and maternal periconceptional use of vitamins containing folic acid. An increased risk was observed for infants who were either heterozygous or homozygous for RFC1 A80G, but only for conotruncal defects. The study also revealed modest evidence for a gene-nutrient interaction between infant homozygosity for the RFC1 G80/G80 genotype, and maternal periconceptional intake of vitamins containing folic acid on the risk of conotruncal heart defects. To date, the Roche multilocus assay was performed on more than 700 subject samples. Preliminary findings specific to nonmalformed controls will be presented on many of these genotype experiments.

The analyses contribute to the general scientific understanding of the etiologies of common congenital malformations. Many more genotyping experiments on thousands of DNA samples will be conducted, and epidemiologic analyses will be performed, with the incorporation of maternal interview information, to detect gene-environment interactions on malformation.

Mechanisms of Age-Dependent Ozone-Induced Airway Dysfunction

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Acute exposure to ozone (O_3) causes airway hyperresponsiveness (AHR), a characteristic feature of asthma. O_3 may be a particularly important respiratory hazard for children because they spend more time outdoors, where O_3 levels are higher. The purpose of this study was to determine whether O_3 -induced AHR was greater in immature mice than adult mice, and to examine the mechanistic basis for O_3 -induced AHR. The hypothesis was that differences in metabolism and physical activity between adult and immature mice would lead to differences in the response to O_3 . A/J mice ages 2, 4, 8, or 12 weeks, were exposed to O_3 (0.3 - 3.0 ppm for 3 hours) in nose-only exposure plethysmographs. Baseline minute ventilation normalized for body weight (V_E/g) decreased with age. O_3 caused a concentration-related decrease in V_E in mice of all ages, but the response was significantly less in 2-week-old mice than in older mice. The net effect of the increased V_E/g and the reduced decrements in V_E during O_3 exposure was an inhaled dose of O_3 normalized for body weight that was 3-4 times higher in 2-week-old mice than in adult mice. O_3 exposure caused a concentration-related increase in airway responsiveness in 8- and 12-week-old mice, but did not cause AHR at any concentration in either 2- or 4-week-old mice, despite the higher inhaled dose. Bronchoalveolar lavage (BAL) cytokines were increased in 8-week old mice compared to 2-week-old mice exposed to O_3 . Similar results were obtained during more chronic O_3 exposure (0.2 - 0.5 ppm O_3 for 48 hours). The results suggest that immature mice are less sensitive than adult mice to O_3 , at least in terms of the ability of O_3 to induce AHR and promote release of certain cytokines. The mechanistic basis for O_3 induced AHR appears to be inflammation arising from oxidant injury to the lungs and airways. However, the precise aspect of the inflammatory cascade required for AHR is still not firmly established. Mice genetically deficient in the TNF receptors (TNFR1 and TNFR2), in IL-6, or in the CXCR2 receptor, for which the neutrophil chemotactic factors MIP-2 and KC are ligands, were used to characterize the role of these cytokines and chemokines in O_3 -induced AHR and O_3 -induced inflammation. Results indicate that activation of TNFR2 contributes to O_3 -induced AHR but not polymorphonuclear leukocyte migration into the lungs, whereas IL-6, MIP-2, and KC are important for polymorphonuclear leukocyte migration (see Figure 1) but not AHR.

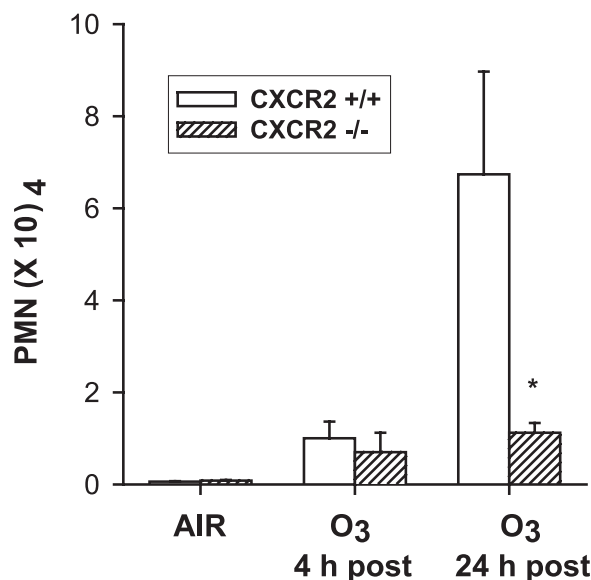


Figure 1. Bronchoalveolar lavage polymorphonuclear leukocytes (PMN) in CXCR2 sufficient (+/+) and deficient (-/-) exposed to O_3 (1 ppm for 3 h) or filtered air. Mice were killed 4 or 24 hrs after cessation of O_3 exposure. Results are mean \pm SE of data from 6 mice per group. * $P < 0.05$ compared to CXCR2 +/+ mice.

Statistical Modeling of Waterborne Pathogen Concentration

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One of EPA's current efforts in maintaining high-quality drinking water involves setting treatment standards for the removal of pathogens (especially *Cryptosporidium* and *Giardia*) from raw waters. This research focuses on development of statistical methods to describe environmental distributions of microorganisms, such as *Giardia lamblia* and *Cryptosporidium parvum* protozoa, to support health risk analyses. Ideally, the statistical analysis will characterize what is known about the distribution of such pathogens at individual sites and within a region; understanding such uncertainties is important to any characterization of health risks, and also aids in the development of monitoring strategies to reduce uncertainties.

The research addresses model formulation, parameter estimation, and the precision of estimated pathogen concentrations. Counts are often zero. Some sites may have all zeros. Furthermore, the Information Collection Rule (ICR) method for estimation of *Cryptosporidium* concentrations has a highly variable recovery rate, which further complicates the analysis. Models with a hierarchical structure that includes sites and regions, as well as hydrologic and watershed effects, were developed to summarize the information provided by EPA's ICR and their Supplemental Survey data. Time-dependent covariates include sampling date, flow rate, pH, and water turbidity.

Zero counts are part of the sampling variation of count data and were modeled as zero counts to allow correct inferences concerning environmental concentrations. Figure 1 displays the whole ICR *Cryptosporidium* data set. The research developed models that describe the variation of *Cryptosporidium* as a function of water quality and hydrologic covariates, as well as time, site, and regional random effects. Modeling the variation of pathogen concentration using observed count data is a significant challenge, especially in the ICR study where more than 90 percent of the observed counts are zero; the large variation in laboratory recovery rates further complicates the analysis. From the laboratory spiking study, volume analysis helped to explain *Cryptosporidium* and *Giardia* recovery rates, and for *Giardia* there were significant laboratory effects. Including volume-analysis in the recovery rate model caused a large reduction in the coefficients for turbidity and pH in the *Cryptosporidium* stream model, but not in the reservoir-lake model. Despite the large percentage of zeros, the analysis demonstrated that *Cryptosporidium* concentrations were on average larger in streams than in reservoirs and lakes, several covariates were statistically significant, and there were important differences among regions.

Even though the recovery rate and *Cryptosporidium* models are relatively complex, they were easily analyzed with WinBugs, a standard package for the numerical evaluation of the posterior distribution of a Bayesian model, using Markov Chain Monte Carlo simulation. Reformulation of the Generalized Linear Mixed Model into a hierarchical structure resulted in significant computational improvements. Overall, the study shows that hierarchical Bayesian models are an incredibly flexible and numerically feasible general statistical methodology to describe environmental concentrations of pathogen and microbiological organisms.

The next step is to effectively integrate the hierarchical Bayesian statistical analysis with a risk analysis for public exposure to *C. parvum*. Generally, the data analysis is conducted separately from the risk analysis. The separation becomes less natural when the posterior distribution of events in a Bayesian framework is employed. The use of numerical Bayesian procedures to conduct the Bayesian analysis provides new opportunities because the posterior distributions are described by generated samples, which can then become the basis of a Monte Carlo risk analysis.

Publications/Presentations:

Crainiceanu CM, Ruppert D, Stedinger JR, and Behr CT. Improving MCMC mixing for a GLMM describing pathogen concentrations in water supplies. In: Gatsonis C, Kass RE, Carlin B, Carriquiry A, Gelman A, Verdinelli I, and West M, eds. *Case Studies in Bayesian Statistics*. Volume 6. New York: Springer Verlag, 2002, pp. 207-222.

Crainiceanu C, Stedinger J, and Ruppert D. Bayesian recovery rates modeling for waterborne pathogens (Applications to ICR spiking lab data). Technical Report, Cornell University, Ithaca, New York, February 13, 2002.

Crainiceanu CM, Stedinger JR, Ruppert D, and Behr CT. Modeling the national distribution of waterborne pathogen concentrations with application to *Cryptosporidium parvum*. *Water Resources Research* February 2003 (accepted).

Relevant Web Site: <http://www.orie.cornell.edu/~davidr/epa-risk>

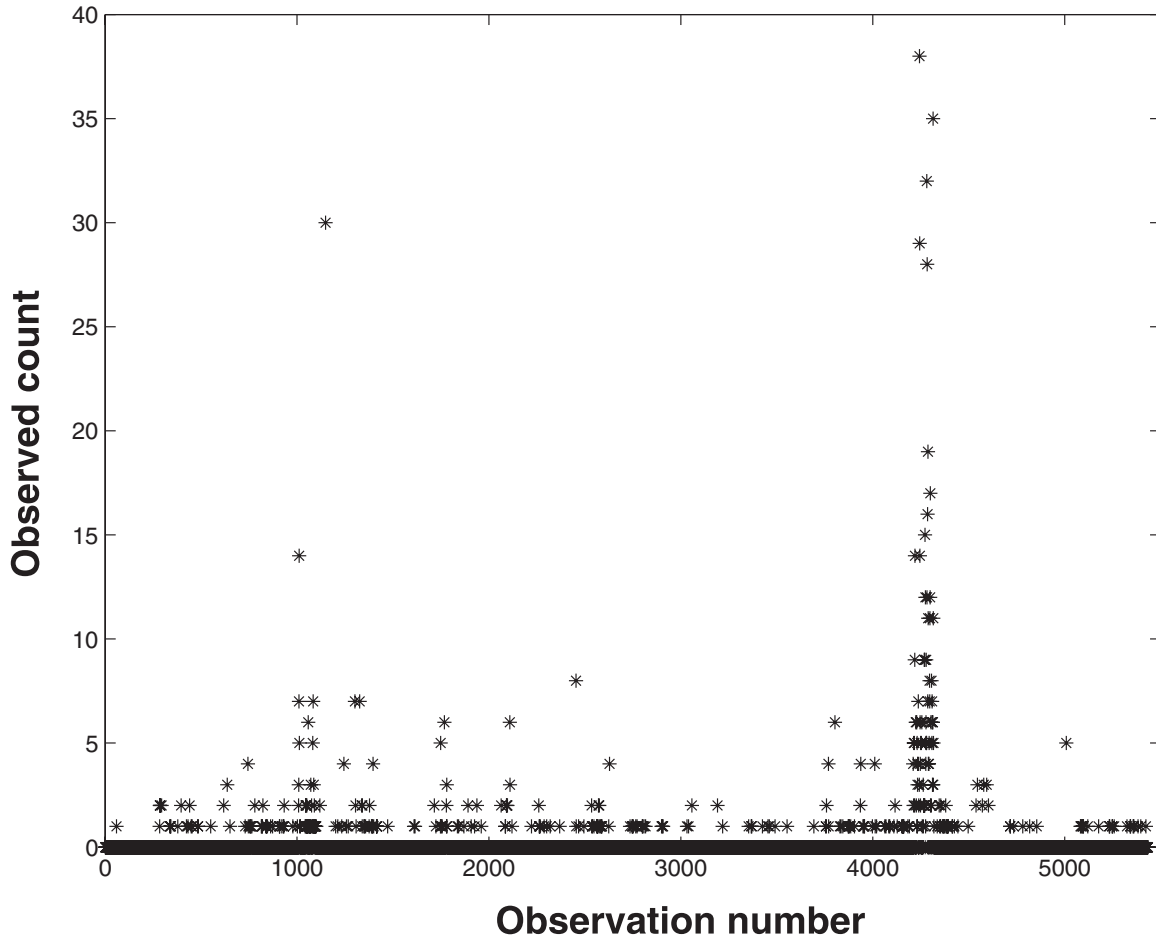


Figure 1. Oocyst counts reported for different months at different sites in the National ICR Survey. A large fraction of these observations are zero counts (93%). Among the remaining non-zero observations, most are just one or two, but there are several large counts (of 38, 35, 30) indicating strong overdispersion with respect to the Poisson distribution.

Using Biomarkers of Exposure and Neurobehavioral Test Batteries To Assess Children's Vulnerability to Residential Exposure to Tetrachloroethene (Perc)—NYCity Perc Project

Jan Storm

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The objectives of this project are to assess vulnerability of children to residential perc exposure and to relate environmental and biological measures of perc exposure to occurrence of nervous system effects. Environmental (24-hour TWA indoor air perc levels) and biological (exhaled breath, blood) measures of perc exposure are being determined for adult-child pairs residing in buildings with dry cleaners using perc (exposed) and for adult-child pairs residing in buildings without dry cleaners (controls). All buildings are in New York City. Nervous system function is being assessed using measures of visual function (visual contrast sensitivity [VCS], color vision) previously shown to be adversely affected by solvent exposure. Perc exposures and visual function are being compared across groups by age (exposed vs. control) and within the exposed group (adult vs. child; perc exposure vs. visual function endpoints).

Fifty-one exposed and 40 control parent-child pairs have been recruited and have completed at least some aspect of this study. These numbers represent 3 percent of nearly 6,000 households contacted. Only 20 percent (n=10) of exposed residences have perc levels greater than the New York State Department of Health residential guideline of 100 $\mu\text{g}/\text{m}^3$; 31 percent (n=16) of exposed residences have perc < 5 $\mu\text{g}/\text{m}^3$. Preliminary analyses indicate median perc levels are higher in exposed residences (15 $\mu\text{g}/\text{m}^3$) than in control residences (< 5 $\mu\text{g}/\text{m}^3$), and that median breath and blood perc levels of exposed residents (20 $\mu\text{g}/\text{m}^3$ breath; 0.048 ppb blood) are higher than control residents (6 $\mu\text{g}/\text{m}^3$ breath; 0.004 ppb blood). Within the exposed group, average ratios of child to adult breath and child to adult blood perc levels are 0.8 and 1.4, respectively. For both adults and children, breath perc level is significantly and directly related to indoor air level, whereas blood perc level is not. Preliminary group analyses so far suggest no difference in VCS or color vision between exposed and control adults or children. However, there is some suggestive evidence that adults with the highest exposures to perc (median 2250 $\mu\text{g}/\text{m}^3$; range 760-5000 $\mu\text{g}/\text{m}^3$) may have a small but statistically significant deficit in color vision as compared to control adults.

Overall, perc levels in exposed residences are markedly lower than levels found in 1995-1996; however, they are still elevated, especially in neighborhoods with lower median incomes. Based on current data, where median income is less than \$25,000, median perc level is 225 $\mu\text{g}/\text{m}^3$ (range 2.5-5000 $\mu\text{g}/\text{m}^3$); whereas, where median income is greater than \$50,000, median perc level is 28 $\mu\text{g}/\text{m}^3$ (range 2.5-372 $\mu\text{g}/\text{m}^3$) (see Figure 1).

Although incomplete, these findings suggest that residential perc exposures in buildings with dry cleaners are still elevated (especially in lower income neighborhoods), are associated with increased perc in blood and breath, and may adversely impact vision. Additionally, higher blood perc level in exposed children compared to exposed adults suggests that children might experience greater exposures to perc than adults residing in the same household.

Relationships between environmental and biological measures of perc exposure and measures of visual function will continue to be explored. Additionally, recruitment of additional participants will be focused in lower income neighborhoods.

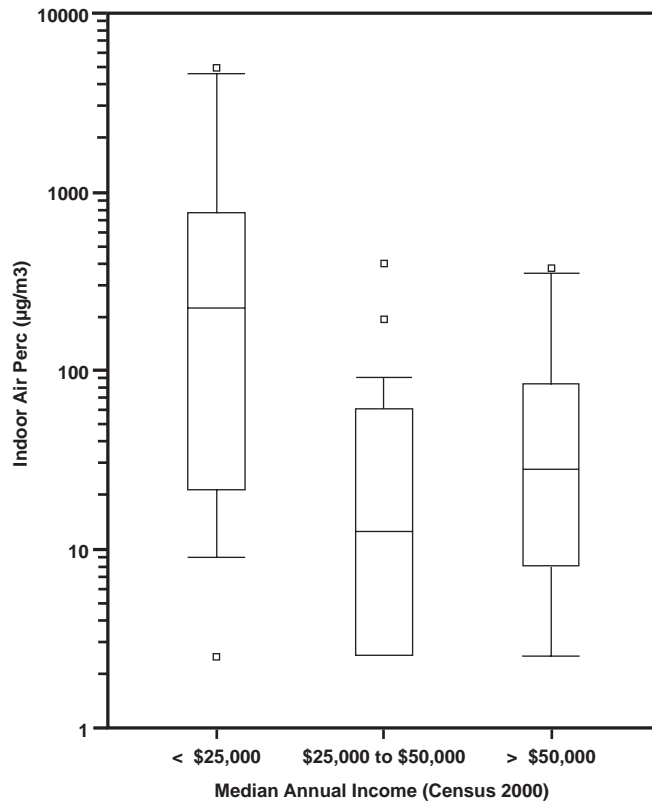


Figure 1. Neighborhood income and indoor air perc levels in residential buildings with dry cleaners.

Reproductive Health, Serum Dioxin, and P450 Genes in Vietnam Veterans

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Several epidemiological investigations examining the association between paternal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and neural tube defects (NTDs) among their offspring have yielded varying results. Despite the controversy, the Agent Orange Benefits Act of 1996 allowed for the Department of Veterans Affairs to compensate the offspring of Vietnam veterans for expenses associated with spina bifida. Most of the previous research examining this relationship utilized service in Vietnam as the measure of exposure and did not include an assessment of genetic susceptibility. This study was a nested case-control study of the relationship between serum TCDD levels, P450 polymorphisms, and NTDs among a national sample of Vietnam veterans.

Cases (Vietnam veteran parents of an NTD-affected pregnancy) and controls (Vietnam veteran parents of unaffected pregnancies) were identified through linkage of parental identifiers, obtained from birth, death, and fetal death certificates noting the occurrence of an NTD between 1965-1995, with the Department of Defense's registry of Vietnam veterans. Current addresses were obtained through an interagency agreement with the Internal Revenue Service. A total of 7,721 potential matches were identified, and a screening survey was mailed to them to determine eligibility, defined as having served in Vietnam and having had a pregnancy after their Vietnam tour. Of these, 1,314 (17%) had incorrect addresses; 619 (8%) were deceased; 1 percent (n=85) were ineligible, mainly due to non-veteran status; 4 percent (n=317) refused to participate; and 1 percent (n=61) indicated they would enroll but never returned a completed survey. There was no response from 45 percent (n=3,485); followup is underway to determine the reason for the nonresponse, which may be due to non-veteran status. From the 1,840 who returned completed screening surveys, 143 cases were identified; indepth questionnaires were obtained from 80 cases and 226 controls. Due to budgetary restrictions, a subset of 55 cases and 55 controls provided serum samples for determination of CYP1A1 polymorphism status, as well as analysis by the CALUX assay for dioxin-like activity. A subset of 25 samples also was analyzed by high-resolution gas chromatography/mass spectrometry (GC/MS) for correlation with the CALUX assay results. Overall, controls had higher TEQs than cases, with values at the 75th percentile of 42 pg/g and 29 pg/g, respectively (background levels being 20-30 pg TEQ/g fat).

Preliminary results from a logistic regression model assessing the interaction between presence of the variant allele (20% of cases and 25% of controls) and a TEQ greater than 25 pg TEQ/g fat and NTD-affected pregnancy indicated the following odds ratios: TEQ > 25 pg TEQ/g fat (OR=0.68, 95% CI 0.27-1.7); presence of variant allele (OR=0.45, 95% CI 0.15-1.35); and TEQ > 25 with variant allele present (OR=1.89, 95% CI 0.26-13.79) (see Table 1). Additional analyses, including the examination of the dioxin congeners identified in the GC/MS analysis and their role in the occurrence of an NTD-affected pregnancy, also will be presented.

Table 1. Preliminary results of the Logistic Regression Model for NTDs and TEQs and risk allele, VVHFS.

Factor	OR	95% CI
TEQ >25	0.68	.27-1.7
Risk gene	0.45	.15-1.35
TEQ >25* gene	1.89	.26-13.79

Total cases/controls in TEQ >25* gene = 10/16.

Chlorotriazine Protein Binding: Biomarkers of Exposure and Susceptibility

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This study tests the hypothesis that binding of chlorotriazines by hemoglobin and hair proteins can be used to evaluate differences in exposure and in individual sensitivity toward chlorotriazines. The studies address four specific aims: (1) refine gas chromatography/mass spectroscopy (GC/MS) methods to assess the reactivity of chlorotriazines and metabolites with hemoglobin; (2) determine whether hair binding of sulfhydryl reactive triazines can be used as noninvasive measures of exposure to these triazines; (3) develop physiologically based pharmacokinetic (PBPK) models for juveniles and adults that include blood protein and hair protein binding; and (4) use these PBPK models with protein binding measurements to recreate exposure characteristics in laboratory animals and in a limited set of human blood and hair samples.

Using GC techniques and/or GC/MS, the reactivity of chlorotriazines and metabolites with hemoglobin and hair proteins are measured. PBPK models that utilize blood protein and hair adduct levels will be developed to assess tissue exposure to total chlorotriazines in relation to ambient exposure.

Hemoglobin isolation for analysis of binding sites. Radiolabeled atrazine and DACT react with rat globin, suggesting that both compounds form protein adducts with globin that may be used as a biomarker of chlorotriazine exposure. The adduct formation by high performance liquid chromatography (HPLC)/MS and matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF) is being identified.

Analytical methods for chlorotriazines in biological matrices. An analytical method for Atrazine, De-ethylatrazine (DeEt-atraz), De-isopropylatrazine (DeIp-atraz), and Di-aminochloroatrazine (DACT) was developed in plasma, using GC/MS with selective ion monitoring (GC/MS/SIM). Recoveries ranged from 84 percent to 97 percent with a limit of detection of 100 ng/mL (PPB).

Blood kinetics of chlorotriazines. After a single oral gavage of 90 mg Atrazine/kg, the majority of total area under the curve for chlorotriazines in plasma was DACT (>95% total AUC) followed in order by DeIp-atraz, DeEt-atraz, and Atrazine. Maximum absorption of DACT was observed 8 hours after dosing, followed by first-order elimination with a half-life of 12 hours.

In vitro metabolism in isolated primary hepatocytes (see Figure 1). An analytical method (GC/MS/SIM) was developed for determination of Atrazine and major metabolites in a primary rat hepatocyte matrix. Recoveries of the triazines from these suspensions ranged from 50 percent with a LOD of 15 ng/mL (ppb). An *in vitro* kinetic enzyme model has been constructed that includes mono-dealkylated and di-dealkylated metabolite formation. These data will allow interspecies extrapolation of our PBPK model after similar studies are conducted with human hepatocytes.

The PBPK model has blood, body, and brain compartments. It is designed to support biomonitoring/exposure assessment studies. An example of this model is shown in Figure 2.

This study will improve our understanding of risks to children from these herbicides. Its value though has to be measured in relation to two phases: (1) development of accurate tools to assess both exposure and potential susceptibility to triazine herbicides in children, and (2) use of these tools with specific populations of children who may be at higher risks.

The PBPK model will permit calculation of expected triazine binding in various populations. Study design criteria for biomonitoring in children and workers can be established partially at least on the basis of this research.

Work will continue to construct and refine PBPK models that will link exposure and circulating triazine levels to produce a comprehensive PBPK model of triazine binding to hemoglobin/hair proteins, including: (1) investigating the pretreatment and digestion/extraction of hair; (2) investigating globin analysis and triazine binding sites in globin; (3) developing *in vivo* techniques with ¹⁴C-ATRA and ¹⁴C-DACT to determine presence and persistence of Hb adducts and tissue binding; and (4) confirming the present PBPK model by measuring plasma and Hb binding kinetics *in vitro*.

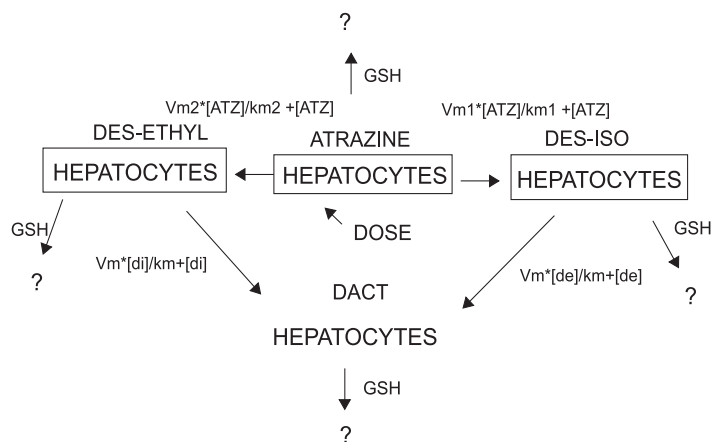


Figure 1. *In vitro* PBPK Metabolite Model.

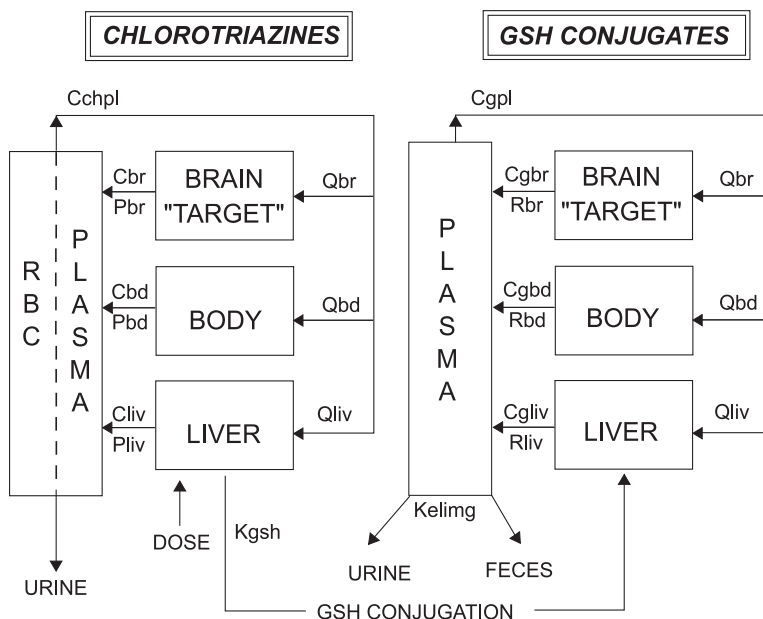


Figure 2. PBPK Model for total chlorotriazines.

US EPA ARCHIVE DOCUMENT

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

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This research project entails development and validation of a PBPK/PD model for the organophosphate 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 apply them to a PBPK/PD model to adequately describe the blood and tissue time-course of chlorpyrifos, as well as the metabolites chlorpyrifos-oxon and trichloropyridinol. Once fully developed, the model will be utilized 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 are being 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 role 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 will be used for further validation of the PBPK/PD model for chlorpyrifos.

The PBPK/PD model has been modified to allometrically scale (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.

Future studies are planned to quantitate age-dependent changes in dosimetry and ChE inhibition kinetics in neonatal rats following exposure to a range of chlorpyrifos doses and will extend the model to incorporate comparable age-dependent parameter changes in children.

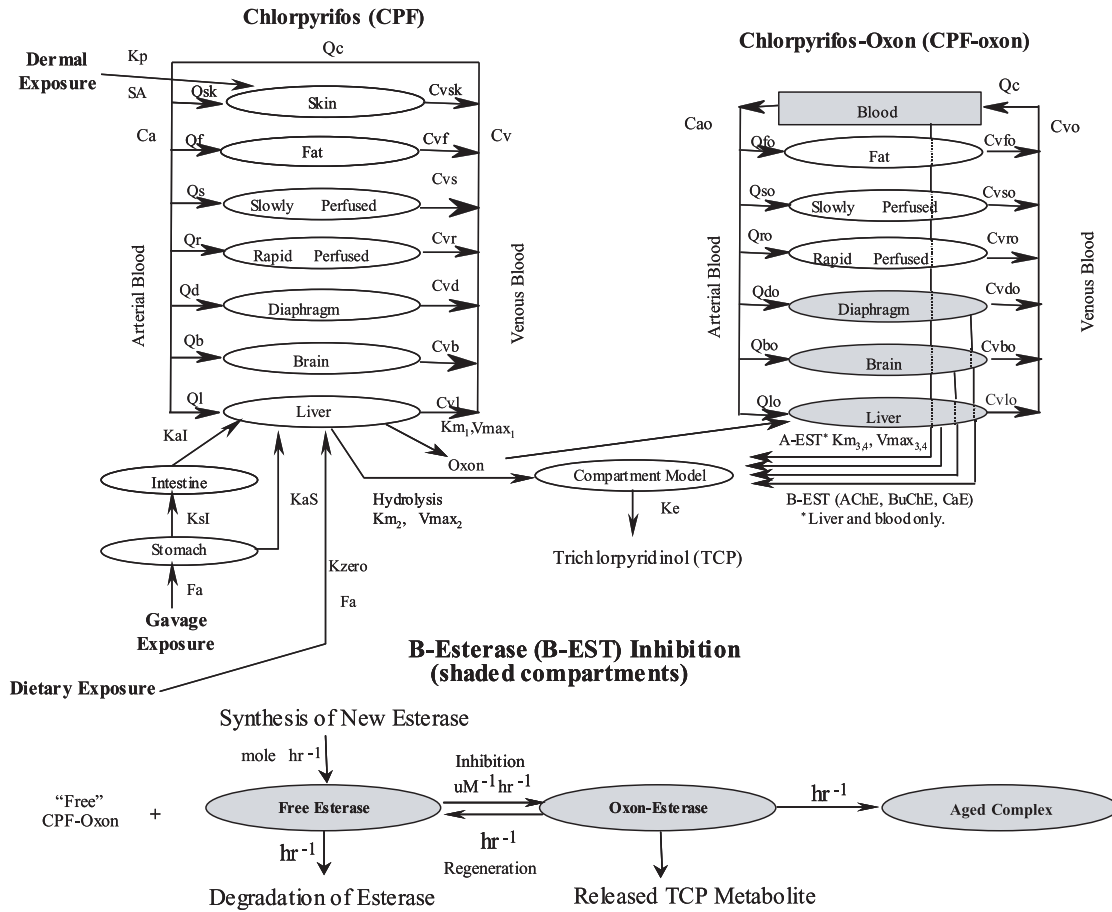


Figure 1. Physiologically Based Pharmacokinetic and Pharmacodynamic (PBPK/PD) Model for the organophosphate insecticide chlorpyrifos.

US EPA ARCHIVE DOCUMENT

Evaluating the Carcinogenic Potency of Complex Mixtures

David Warshawsky, Roy Albert (Deceased, March 25, 2002), Marshall Anderson, Kathy LaDow, Weiling Xue, Sharon Spalding, and Paul Succop

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Complex mixtures of combustion and related products contain many carcinogens and anti-carcinogens of varying potency that interact in theoretically unpredictable ways. The conventional mouse skin tumor carcinogenesis assay is too time-consuming and expensive to be of more than limited use. If based on tumor initiation alone, skin tumor assays are of uncertain accuracy in the prediction of cancer outcomes and also are not rapid. A new and more rapid approach will be evaluated to assess carcinogenic potency using cancer induction as the endpoint. This involves determining the potency of complex mixtures (e.g., coke oven tar) as tumor initiators and progressors. Benzo[a]pyrene will be used as the positive control. The method uses a dominant negative p53 mutant transgenic mouse skin model (Vp53) with the combined topical application of the test mixture and a strong promoter, 12-O-tetradecanoylphorbol-13-acetate (TPA). The co-application of the test agent and TPA saturates the system with promoting action and provides a rapid display of the potency of the test material.

Measurement of Non-Persistent Pesticides in Postpartum Meconium

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The research goal of this project is to validate a battery of biomarkers of prenatal exposure to non-persistent pesticides (NPP). Biomarkers are NPP levels in: (1) maternal prenatal urine samples, (2) maternal and umbilical cord blood samples, and (3) postpartum meconium. Biomarker validation is needed to facilitate evaluation of health effects associated with NPP exposures during pregnancy. The cohort consists of 100 urban minority 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, provide the gold standard for biomarker validation. To date, 130 women have been enrolled; indoor air monitoring has been completed for 59 women and 58 have delivered with biologic samples collected at delivery. Data analysis for 10 NPP has been completed for 152 2-week integrated indoor air samples collected from 38 women. Twenty-seven (71%) women reported using pest control during the air monitoring: 15 (39%) reported using lower toxicity pest control methods only (i.e., gels, baits, and traps) and 12 (32%) reported using can sprays, pest bombs, and/or exterminator sprays. Three NPP were detected in all (100%) of the air samples: chlorpyrifos, diazinon, and propoxur (see Figure 1). The correlation between the 2-week integrated indoor air levels for all three pesticides was highly significant (see Table 1). Of the remaining 7 NPP, 3 were not detected (malathion, methyl parathion, and carbofuran), and 4 (permethrin, piperonyl butoxide, carbaryl, and bendiocarb) were detected in less than 50 percent of air samples (range 11%-43%). Diazinon levels were significantly associated with maternal self-reported pesticide use (see Figure 2). Analysis of NPP levels in biologic samples is ongoing and will be reported.

Table 1. Correlation between 2-week integrated indoor air pesticide levels during 32nd to 40th week gestation.

	Chlorpyrifos			Propoxur			Diazinon		
	32nd-34th	34th-36th	36th-38th	32nd-34th	34th-36th	36th-38th	32nd-34th	34th-36th	36th-38th
38th-40th	t = 0.77*	t = 0.81*	t = 0.83*	t = 0.71*	t = 0.71*	t = 0.95*	t = 0.88	t = 0.92*	t = 0.94*
36th-38th	t = 0.82*	t = 0.80*		t = 0.86*	t = 0.83*	t = 0.89*	t = 0.93*		
34th-36th	t = 0.95*			t = 0.95*			t = 0.96*		

*p < 0.001, Spearman's rank.

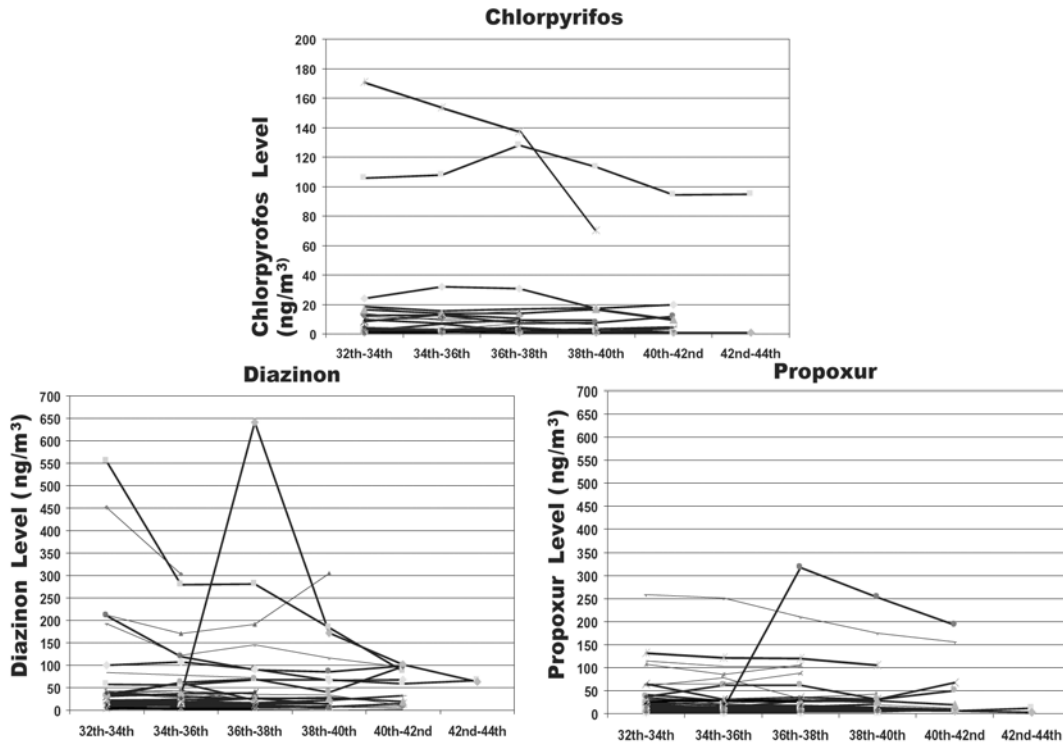
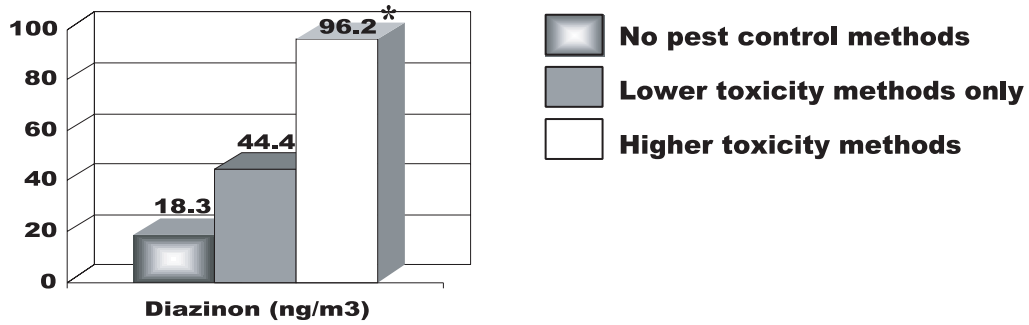


Figure 1. Two-week integrated indoor air pesticide levels for 38 women between 32nd-40th week of pregnancy.



*p=0.03 compared to no pest control (Mann-Whitney U).

Figure 2. Average diazinon levels in 2-week integrated indoor air samples by maternal self-reported use of pest control.

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

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Employing biomarkers of exposure and susceptibility in a longitudinal design, the research 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. These compounds have been demonstrated to be neurodevelopmental toxicants in animal studies.

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., Pb); (3) maternal demographics and other potentially confounding family-based factors (e.g., SES); and (4) relevant clinical information pertaining to the pregnancy and birth event (e.g., APGAR 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 postnatal. 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 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 (TCP), the urinary metabolite specific for CP, and 2-isopropyl-6-methyl-4-pyrimidinol (IMP), the urinary metabolite specific for diazinon. In addition, two postnatal blood samples will be obtained from the child (one at 12 months, one at 24 months). Blood Pb will be determined, as will the infant's PON1 genotype.

Because recruitment of pregnant women into this 2-year study has proved to be more challenging than initially anticipated, recruitment is still in progress. A total of 60 subjects have been recruited to date, but due to fetal complications and dropouts, 39 are actively enrolled. The next step is to continue recruitment and analysis of blood and urine specimens obtained thus far in the study.

Pesticide Exposures of Preschool Children Over Time: Design and Recruiting

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and ²Battelle Memorial Institute, Columbus, OH

The effects on children's exposures of recent changes in pesticide uses, such as those resulting from the federally mandated phaseouts of residential use of chlorpyrifos (CPF) and diazinon (DZN) and the increased use of alternative pesticides, are not established. Thus, the main objective of this research is to estimate the longitudinal changes in aggregate exposures to targeted pesticides for selected preschool children in the same age group over 4 years. The aggregate exposures are those from all sources in the children's everyday environments such as air, food, beverages, and house dust. The exposures can occur through routes such as breathing, eating, and drinking, and touching contaminated surfaces, as Thomas is doing in Figure 1. The question to be asked is, "Whether the aggregate exposures of the children to the targeted pesticides change or remain the same over the 3 to 4-year period?" The variability among preschool children's exposures in similar everyday environments and among those in different everyday environments also is not well known. Thus, a second objective is to estimate the variability of these exposures among preschool children living in the same homes and among children living in different homes.

The aggregate exposures of 50 children 3 years of age and their younger siblings (2 children in 50 households) to selected organophosphorus and pyrethroid insecticides and to acid herbicides will be obtained yearly in one of three separate sampling periods: spring, summer, and fall. Approximately equal numbers of households will be assigned to each sampling period. The children's exposures will be estimated from the concentrations of the pesticides in their home air, house dust, and play area soil, and from personal samples of food and beverages, hand wipes, and urine, as well as children's time-activity diaries.

Recruiting was done through listed telephone screening and accepting referrals from respondents. To increase the chances of reaching households with children 3 years of age and younger, the telephone sample was restricted to households whose head of household is between ages 18 and 35. Of the households contacted, approximately 4 percent had two children under age 3, and were thus eligible for the study. Assuming the eligibility of non-contacted households is the same as that of contacted households, the final response rate will be above 70 percent. As of the end of March, 66 eligible households were identified; recruiting was still in progress. Field measurements began in mid-March 2003.

The next steps are to complete recruiting, continue field measurements, and initiate laboratory chemical analysis of the collected samples.



Figure 1. Children's exposures to pollutants can occur from touching contaminated surfaces.

U.S. Environmental Protection Agency

Human Health Symposium—A STAR Progress Review Workshop

Academy for Educational Development (AED)

Universal South Building - 8th Floor

1825 Connecticut Avenue, NW

Washington, DC

April 9-10, 2003

Agenda

Wednesday, April 9, 2003

- 8:00 – 9:00 a.m. **Registration**
- 9:00 – 9:10 a.m. **Welcome and Introduction—Goals of Symposium**
(Location: Academy Hall)
Jack Puzak, Acting Director, National Center for Environmental Research
- 9:10 – 9:15 a.m. **General Symposium Information**
(Location: Academy Hall)
Kacee Deener, National Center for Environmental Research
- 9:15 – 9:45 a.m. **Research Needs Related to the National Children’s Study**
(Location: Academy Hall)
Carole Kimmel, National Center for Environmental Assessment
- 9:45 – 10:00 a.m. **Break**
- Note: Location of All Session I Presentations: Academy Hall*
Location of All Session II Presentations: Vista Room
- 10:00 – 10:30 a.m. **Session I – Exposure Assessment**
Pesticide Exposure of Preschool Children Over Time – Nancy Wilson, Battelle
- Session II – Epidemiology**
Reproductive Health, Serum Dioxin, and P450 Genes in Vietnam Veterans –
Deborah del Junco, Rural School of Public Health, Texas A&M University
- 10:30 – 11:00 a.m. **Session I – Exposure Assessment**
Estimating Longitudinal Aggregate and Cumulative Exposure and Intake Dose for
Young Children – James Leckie, Stanford University
- Session II – Epidemiology**
Molecular Epidemiology of Hypospadias – Jeanne Manson, Children’s Hospital
of Philadelphia

- 11:00 – 11:30 a.m.** **Session I – Exposure Assessment**
A Longitudinal Approach of Assessing Aggregate Exposure to Organophosphorous Pesticides in Children – Alex Lu, University of Washington
- Session II – Epidemiology**
Gene-Environment Interactions and Human Malformations – Gary Shaw, March of Dimes
- 11:30 – 12:30 p.m.** **Lunch**
- 12:30 – 1:00 p.m.** **Session I – Environmental Justice**
Casa de Salud: A Model for Engaging the Community – Gretchen Latowsky and Doris Anziani, Family Services, Inc.
- Session II – Toxicology**
Molecular Characterization of a Biological Threshold in Developmental Toxicology – Thomas Knudsen, Thomas Jefferson University
- 1:00 – 1:30 p.m.** **Session I – Environmental Justice**
North Brooklyn Asthma and Environmental Consortium – Luis Garden Acosta, El Puente
- Session II – Toxicology**
Mechanisms of Age-Dependent Ozone Induced Airway Dysfunction – Richard Johnston, Harvard School of Public Health
- 1:30 – 2:00 p.m.** **Session I – Environmental Justice**
Bioaccumulative Toxins in Native America Shellfish – Tony Basabe, Swinomish Tribal Community
- Session II – Toxicology**
Fetal Metabolism of Aflatoxin B1 and Susceptibility to Childhood Cancer – Evan Gallagher, University of Florida
- 2:00 – 2:15 p.m.** **Break**
- 2:15 – 2:45 p.m.** **Session I – Statistics**
Statistical Modeling of Waterborne Pathogen Concentrations – Jerry Stedinger, Cornell University
- 2:45 – 3:30 p.m.** **Panel Session: Molecular Epidemiology (Location: Academy Hall)**
Jeanne Manson, Chair, Children’s Hospital of Philadelphia
Robin Whyatt, Columbia University
Chris Saint, National Center for Environmental Research
- 3:30 – 3:45 p.m.** **Wrap-Up (Location: Academy Hall)**
- 3:45 p.m.** **Adjournment**
- 6:15 p.m.** **Optional Dinner at the Daily Grill**
(Meet in the hotel lobby at 6:15 p.m.)

Thursday, April 10, 2003

7:30 – 8:30 a.m. Registration

8:30 – 8:35 a.m. Welcome (Location: Academy Hall)
Kacee Deener, National Center for Environmental Research

8:35 – 9:00 a.m. A Regulatory Context for STAR Research (Location: Academy Hall)
Elizabeth Mendez, Office of Pesticide Programs

*Note: Location of All Session I Presentations: Academy Hall
Location of All Session II Presentations: Vista Room*

9:00 – 9:30 a.m. Session I – Biomarkers
Saliva Biomonitoring for OP Pesticide Exposure in Children – Alex Lu, University of Washington

Session II – Chemical Mixtures
PAH/Metal Mixtures – Human In Vitro Mutagenicity Studies – Laurence Kaminsky, New York State Department of Health

9:30 – 10:00 a.m. Session I – Biomarkers
Development of a PBPK/PD Model To Quantitate Biomarkers of Exposure to OP Insecticides – Charles Timchalk, Battelle, Pacific Northwest Division

Session II – Chemical Mixtures
Evaluating the Carcinogenic Potency of Complex Mixtures – David Warshawsky, University of Cincinnati

10:00 – 10:30 a.m. Session I – Biomarkers
Measurement of Non-Persistent Pesticides in Postpartum Meconium as a Biomarker of Prenatal Exposure: A Validation Study – Robin Whyatt, Columbia University

Session II – Chemical Mixtures
Comparative In Vitro Immunotoxicity of Organochlorine Mixtures – Sylvain DeGuise, University of Connecticut

10:30 – 10:45 a.m. Break

10:45 – 11:15 a.m. Session I – Biomarkers
Using Biomarkers of Exposure and Neurobehavioral Test Batteries To Assess Children's Vulnerability to Residential Exposure to Tetrachloroethene – NYCity Perc Project – Jan Storm, New York State Department of Health

Session II – Chemical Mixtures
Regulation of Embryonic Neuronal Development by Chemical Mixtures From Brick – Carol Reinisch, Woods Hole Marine Biological Laboratory

- 11:15 – 11:45 a.m.** **Session I – Biomarkers**
Chlorotriazene Protein Binding: Biomarkers of Exposure and Susceptibility –
John Tessari and Melvin Andersen, Colorado State University
- Session II – Chemical Mixtures**
*Lumped Chemical Approach for Fate and Transport Modeling of Organic Pollutant
Mixtures* – Ken Reardon, Colorado State University
- 11:45 – 1:00 p.m.** **Lunch**
- 1:00 – 1:30 p.m.** **Session I – Biomarkers**
Biomarkers and Neurobehavioral Effects of Perinatal Exposure to Insecticides –
Kathleen Koechlin, Ohio State University
- Session II – Chemical Mixtures**
Mechanistic Evaluation of the Toxicity of Chemical Mixtures – Gerald LeBlanc,
North Carolina State University
- 1:30 – 1:45 p.m.** **Wrap-Up (Location: Academy Hall)**
- 1:45 p.m.** **Adjournment**

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