EPA STAR Grants Summary on Exposure, Genetic Susceptibility and Tools to Assess Gene-Environment Interactions, With Implications for Health Disparities

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Updated March 2008

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Introduction

The Science To Achieve Results (STAR) Program in the Office of Research and Development's National Center for Environmental Research (NCER) at the U.S. Environmental Protection Agency (EPA) manages a portfolio of extramural research grants investigating novel exposure assessment methods, gene-environment interactions in human populations, and environmental health disparities. This document presents summaries of a subset of grants involving gene-environment interactions with implications for health disparities, originally prepared for a conference sponsored by the Harvard/MGH Center on Genomics, Vulnerable Populations and Health, and is being published to make it available to a wider audience.

In recent years, the STAR Program has focused attention on examining exposure and susceptibility factors in at-risk populations, particularly in urban, agricultural, and tribal communities, where disproportionate burdens of toxicant exposure have been documented. Consistent with EPA's emphasis on children's health protection, the STAR Program has stimulated cutting-edge, multidisciplinary research on life stage susceptibility, community-based intervention, and children's health promotion. To date, research results have been: (a) translated into local and state policy; (b) incorporated into deterministic, probabilistic, and cumulative risk frameworks; and (c) used as guidance for clinicians, community advocates, and parents in creating safer, healthier environments.

The grant summaries presented here fall into three main categories as they relate to health disparities:

(I) Differential exposure and effects (health disparities related to exposure)
(II) Life stage and genetic susceptibility to adverse health outcomes (health disparities related to life stage and genetic susceptibility)
(III) Novel exposure tools and methods.

Also included is a short summary of some other investigations that may be of interest, including ongoing work with tribal populations.

A brief summary of some of the research projects within each category, condensed or quoted verbatim from published abstracts, is provided along with a hyperlink to a key paper for each study (if available). Details such as study design, marginally significant results and study limitations are not included here and will only be present in the original paper. The STAR grant number is hyperlinked to the NCER Web page containing more information about each research project. Entries in the Table of Contents are hyperlinked to each summary – to use these, hold down the "Ctrl" key and click on the title.

Thanks to NCER and other ORD technical staff; namely, Elaine Francis, Nigel Fields, Kacee Deener, Chris Saint, Montira Pongsiri, Stacey Katz, Gail Robarge, and Barbara Glenn for assistance in preparing this summary.

I. Differential Exposure and Effects

Traffic Patterns Related to Higher Risk of Childhood Asthma
University of Southern California/University of California at Los Angeles (USC/UCLA) Children's Center
STAR Grants R826708, R831861

The EPA/National Institute of Environmental Health Sciences (NIEHS) Center for Children's Environmental Health and Disease Prevention Research (Children's Center) at USC and UCLA was established in 1998 to investigate effects of the environment on children's respiratory health, with a focus on asthma and allergic airway disease. The Center has made substantial progress in understanding the effects of ambient air pollutants and environmental tobacco smoke (ETS) on children's respiratory health, and researchers have identified important details about the susceptibility of children. This contributes to a growing consensus that current levels of combustion-related air pollutants are more detrimental to children's airways than previously thought. Exhaust emissions from local traffic and regionally transported secondary pollutants are implicated in asthma pathogenesis, allergic airway disease, and reduced lung function and growth. The Center also partners with two local community organizations in the Community Based Participatory Research (CBPR) Project and has established a community outreach and education program to translate research findings for use in education and in public health policy.

Disadvantaged communities often are located close to major traffic hubs and roadways. The USC/UCLA Children's Center has examined the relationship of local traffic-related exposure and asthma and wheeze in southern California school-age children (ages 5-7). They found that residence within 75 m (250 feet) of a major roadway was associated with an increased risk of lifetime asthma (odds ratio [OR] = 1.29, 95% CI, 1.01-1.86); prevalent asthma (OR=1.50, 95% CI, 1.16-1.95); and wheeze (OR=1.40, 95% CI, 1.09-1.78). Susceptibility increased for long-term residents with no parental history of asthma for lifetime asthma (OR=1.85; 95% CI, 1.11-3.09); prevalent asthma (OR=2.46; 95% CI, 0.48-4.09); and recent wheeze (OR=2.74; 95% CI, 1.71-4.39). The higher risk of asthma near a major road decreased to background rates at 150-200 m from the road. However, in children with a parental history of asthma and in children moving to their residence after 2 years of age, there was no increased risk associated with exposure. The absence of any effect of proximity to a major road among children moving to their residence after 2 years of age may indicate vulnerability during the prenatal period of infancy. This is consistent with other studies that provide evidence of early exposures increasing the risk of asthma, particularly *in utero* exposure. The effect of residential proximity to roadways was larger for girls. A similar pattern of effects was observed with traffic-modeled exposure. These results indicate that residence near a major road is associated with asthma.

McConnell, et al., 2006. Traffic, susceptibility, and childhood asthma. *Environ Health Perspect* 2006;114(5):766-72.

Early-Life Environmental Risk Factors for Asthma Include Exposure to Soot, Herbicides, Pesticides, Cockroach Allergens, and Farm Crops
USC/UCLA Children's Center
STAR Grants R826708, R831861

Early-life experiences and environmental exposures have been associated with childhood asthma. To investigate further whether the timing of such experiences and exposures is associated with the occurrence of asthma by 5 years of age, researchers at the USC/UCLA Children's Center conducted a prevalence case-control study nested within the Children's Health Study, a population-based study of more than 4,000 school-aged children in 12 southern California communities. Cases were defined as physician-diagnosed asthma by age 5, and controls were asthma-free at study entry, frequency-matched on age, sex, and community of residence, and countermatched on *in utero* exposure to maternal smoking. Telephone interviews were conducted with mothers to collect additional exposure and asthma histories. Conditional logistic regression models were fitted to estimate odds ratios (ORs) and 95 percent confidence intervals (CIs). Asthma diagnosis before 5 years of age was associated with exposures in the first year of life to wood or oil smoke, soot, or exhaust (OR = 1.74; 95% CI, 1.02-2.96); cockroaches (OR = 2.03; 95% CI, 1.03-4.02); herbicides (OR = 4.58; 95% CI, 1.36-15.43); pesticides (OR = 2.39; 95% CI, 1.17-4.89); and farm crops, farm dust, or farm animals (OR = 1.88; 95% CI, 1.07-3.28). The ORs for herbicide, pesticide, farm animal, and crops were largest among children with early-onset persistent asthma. The risk of asthma decreased with an increasing number of siblings (ptrend = 0.01). Day care attendance within the first 4 months of life was positively associated with early-onset transient wheezing (OR = 2.42; 95% CI, 1.28-4.59). In conclusion, environmental exposures during the first year of life are associated with childhood asthma risk.

Salam, Li, et al. 2004. Early-life environmental risk factors for asthma: findings from the Children's Health Study. *Environ Health Perspect* 2004;112(6):760-5.

Association Between Ozone, Carbon Monoxide, and Adverse Birth Outcomes
USC/UCLA Children's Center
STAR Grants R826708, R831861

The USC/UCLA Children's Center investigated the effects of air pollutants on birth weight among term infants who were born in California during 1975-1987 and who participated in the Children's Health Study. Monthly average air pollutant levels were interpolated from monitors to the ZIP code of maternal residence at childbirth. Results from linear mixed-effects regression models showed that a 12 parts per billion (ppb) increase in 24-hr ozone averaged over the entire pregnancy was associated with 47.2 g lower birth weight [95% CI = 27.4-67.0 g], and this association was most robust for exposures during the second and third trimesters. A 1.4 ppm difference in first-trimester carbon monoxide exposure was associated with 21.7 g lower birth weight (95% CI, 1.1-42.3 g) and 20% increased risk of intrauterine growth retardation (95% CI, 1.0-1.4). First-trimester carbon monoxide (CO) and third-trimester ozone exposures were associated with 20 percent increased risk of intrauterine growth retardation. A 20 $\mu g/m^3$ difference in levels of particulate matter $\leq$ 10 µm in aerodynamic diameter (PM_{10}) during the third trimester was associated with a 21.7 g lower birth weight (95% CI, 1.1-42.2 g), but this association was reduced and not significant after adjusting for ozone. In summary, ozone exposure during the second and third trimesters and CO exposure during the first trimester were associated with reduced birth weight. There is significant evidence that CO crosses the placental barrier binding to fetal hemoglobin, which has a greater affinity for binding CO than does adult hemoglobin, outcompeting oxygen at the binding site. Thus, fetal growth may be significantly compromised by fetal tissue hypoxia, resulting in reduced fetal growth. Furthermore, ozone may play a role in reduced birth weight through modulation of maternal inflammatory processes, particularly mid- to late-gestational exposures. Because exposures to the levels of ambient air pollutants observed in this study are common, and fetal growth is an important determinant for childhood and adult morbidity and mortality, these findings are likely to have important public health and regulatory implications.

Salam, Millstein, et al., 2005. Birth outcomes and prenatal exposure to ozone, carbon monoxide, and particulate matter: results from the Children's Health Study. *Environ Health Perspect* 2005;113(11):1638-1644.

Adverse Birth Outcomes Linked to Exposure to ETS, PAHs, Pesticides, and Maternal Hardship
Columbia Children's Center
STAR Grants R827027, R832141

The Columbia Center for Children's Environmental Health conducts community-based research in New York City, Poland, and China to examine the health effects of prenatal and early postnatal exposures to common urban pollutants, with the aim of preventing environmentally related disease in children. Participants in the prospective New York City Mothers and Children Study are drawn from a largely African-American and Dominican population residing in Washington Heights, Central Harlem, and the South Bronx. Inner-city disadvantaged populations are at high risk for adverse birth outcomes and more likely to be exposed to environmental contaminants.

The Mothers and Children Study is following more than 700 mother-child pairs through the children's eighth birthdays and examining respiratory health, cognitive development, and cancer risk in children prenatally exposed to urban air pollutants such as polycyclic aromatic hydrocarbons (PAHs) and fine particulate matter ($PM_{2.5}$), including diesel exhaust particulates (DEP), environmental tobacco smoke (ETS), pesticides, and home allergens from cockroaches, mice, and dust mites. The Study also is looking at the effects on fetal growth, early neurodevelopment, and respiratory health. Exposure measurement techniques include using personal air monitors to assess levels of airborne pollutants and biomarkers in blood, urine, and meconium.

A recent publication summarized the results of three published studies demonstrating the effects of prenatal ETS, PAH, and pesticides on birth outcomes and/or neurocognitive development. Researchers analyzed the questionnaire data, cord blood plasma (including biomarkers of ETS and pesticide exposure), and Benzo[a]Pyrene-DNA adducts (a molecular indicator of PAH exposure and dose).

Self-reported ETS level was associated with decreased head circumference, and there was a significant interaction between ETS and DNA adducts such that combined exposure had a significant multiplicative effect on birth weight and head circumference after adjusting for confounders. A second analysis examined the neurotoxic effects of prenatal ETS exposure and postpartum material hardship (unmet basic needs in the areas of food, housing, and clothing) on 2-year cognitive development, as measured by the Bayley Scales of Infant Intelligence-II (BSID-II). Both exposures depressed cognitive development and there was a significant interaction such that children with exposure to both ETS and material hardship exhibited the greatest cognitive deficit (7.1 points). A third analysis found that cord blood levels of the pesticides chlorpyrifos, diazinon and propoxur-metabolite, were inversely associated with birth weight and/or length. These results underscore the importance of policies that reduce children's exposure to ETS, air pollution, and pesticides, all of which have potentially adverse effects on fetal growth and neurodevelopment.

Perera, Rauh, et al., 2005. A summary of recent findings on birth outcomes and developmental effects of prenatal ETS, PAH, and pesticide exposures. *Neurotoxicology* 2005;26(4):573-87.

Additional work by the Columbia Children's Center demonstrated that prenatal exposure to ETS combined with chronic material hardship (unmet basic needs of food, clothing, and adequate housing) was associated with significantly higher rates of developmental delay at 2 years of age compared to children who were prenatally exposed to ETS but whose families did not experience material hardship. This suggests that the harmful effects of ETS are exacerbated under conditions of deprivation, with cognitive outcomes similar to those associated with prenatal low-level lead exposure. (Rauh, Whyatt, et al., 2004).

The Center also found that prenatal exposure to the pesticides chlorpyrifos and diazinon was associated with reduced birth weight; and improved birth outcomes were evident in the cohort after EPA's ban of these pesticides for home use in 2000/2001. The findings on birth weight reduction support EPA regulatory action to phase out residential uses of these insecticides (Whyatt, Rauh, et al., 2004).

Also, the Center has also shown that prenatal exposure to PAHs can be associated with an increase in chromosomal alterations. This is the first study to show that environmental exposure during pregnancy to PAHs can cause a low level of chromosomal abnormalities in fetal white blood cells (WBCs). Such genetic alterations have been linked in other studies to increased risk of cancer in children and adults. (Bocskay, Tang, et al., 2005).

Rauh, Whyatt et al., 2004. Developmental effects of exposure to environmental tobacco smoke and material hardship among inner-city children. *Neurotoxicol Teratol* 26(3): 373-385.

Whyatt, Rauh, et al., 2004. Prenatal insecticide exposures and birth weight and birth length among an urban minority cohort. *Environ Health Perspect* 2004;112(10):1125-32.

Bocskay, Tang et al., 2005. Chromosomal aberrations in cord blood are associated with prenatal exposure to carcinogenic polycyclic aromatic hydrocarbons. *Cancer Epidemiol Biomarkers Prev* 14(2): 506-511.

Columbia Children's Center Web Site: http://www.ccceh.org/

New Evidence That *In Utero* Exposure to Urban Air Pollutants May Increase Risk of Developmental Delay in Children
Columbia Children's Center
STAR Grants R827027, R832141

Results from the Columbia Children's Center suggest that prenatal exposure to air pollutants in New York City can adversely affect child development. Previous studies showed that air pollutants can reduce fetal growth (both weight and head circumference at birth). This study, which examined a sub-group of the same children at 3 years of age, is considered to be the first to find that those pollutants also can affect cognitive development.

Investigators studied a sample of 183 children who were 3 years of age and of non-smoking African-American and Dominican women residing in Washington Heights, Central Harlem, and the South Bronx. They found that exposure to PAHs during pregnancy was linked to significantly lower scores on mental development tests (including the Bayley BSID-II test) and more than double the risk of developmental delay at age 3, which is indicative of higher risk for performance deficits in language, reading, and math in the early school years.

The mothers' exposure during pregnancy to air pollutants, specifically polycyclic aromatic hydrocarbons (PAHs), was measured by personal air monitoring. PAHs are emitted from combustion, including car, truck, or bus engines; residential heating; power generation; or tobacco smoking. These pollutants can cross the placenta to reach the fetus.

Children who were exposed in the womb to the highest levels of PAHs scored on average 5.7 points (6.3%) lower on cognitive tests than the less-exposed children; and their risk of being developmentally delayed was 2.9 times greater than that of children who had lower prenatal exposure; both results were statistically significant. Investigators controlled for other exposures that might have contributed to developmental problems such as socioeconomic factors, exposure to tobacco smoke, lead, and other environmental contaminants.

This is the largest study to date characterizing personal exposures to PAHs and is unique in its focus on pregnant minority women.

Tonne, Whyatt, et al., 2004. Predictors of personal polycyclic aromatic hydrocarbon exposures among pregnant minority women in New York City. *Environ Health Perspect* 2004;112:754-59.

Perera, Rauh, et al., 2006. Effect of prenatal exposure to airborne polycyclic aromatic hydrocarbons on neurodevelopment in the first three years of life among inner-city children. *Environ Health Perspect* 2006 Aug;114(8):1287-92, .

Exposure to PCBs Leads to Auditory Deficits in Rodents
University of Illinois Children's Center
STAR Grant R829390

The FRIENDS Children's Environmental Health Center at the University of Illinois, Urbana-Champaign, was established in 2001 to investigate the interactive effects of polychlorinated biphenyls (PCBs) and methyl mercury (MeHg) on neurodevelopment. Research included longitudinal assessment of a birth cohort exposed to these chemicals through maternal consumption of contaminated fish, and complementary laboratory-based projects that include animal and *in vitro* models to determine the mechanisms through which these contaminants induce neurological deficits in children. The population being studied was comprised of Hmong and Laotian refugees who settled in northeastern Wisconsin after the Vietnam War and who regularly consume fish from the Fox River, much of which is contaminated with PCBs and MeHg. A priority of the Center was to develop effective educational strategies to reduce exposure of this population to these fish-borne contaminants. These fish contain a specific congener profile of PCBs that the Center has successfully replicated in the laboratory.

Previous studies have indicated that developmental exposure to PCBs may result in hearing impairment in rats. The cochlea is the suggested site of action, based on one study demonstrating a loss of outer hair cells on the basilar membrane, and another demonstrating deficits in distortion product otoacoustic emissions (DPOAEs). The current study was conducted to assess the possible ototoxic effects of a unique PCB mixture formulated to model the congener profile of PCBs found in the fish consumed by a human population in northeastern Wisconsin. Female Long-Evans rats were dosed orally with the PCB mixture beginning 28 days prior to breeding and continuing until the pups were weaned. Dams were fed one-half of a cookie onto which was pipetted 0, 1, 3, or 6 mg/kg of the PCB mixture dissolved in a corn oil vehicle. On postnatal day (PND) 21, pups were weaned, and one male and one female from each litter were randomly selected for auditory assessment. DPOAEs were measured to assess cochlear function, and auditory brainstem responses (ABRs) were measured to determine the effects on central nervous system auditory pathways. DPOAE amplitudes were decreased, and DPOAE and ABR thresholds were elevated across a range of frequencies in PCB-exposed rats. These results support and extend previous reports of auditory impairment in PCB-exposed rats. Developmental exposure to PCBs also may result in subtle auditory impairments in humans, and may contribute to some of the cognitive deficits that have been observed in epidemiological studies.

Powers, Lasky, et al., 2006. Auditory deficits in rats exposed to an environmental PCB mixture during development. *Toxicological Sciences* 2006;89(2):415-22.

Pesticide Exposure Levels and Urinary Metabolite Concentrations Significantly Higher for Children in Agricultural Families

University of Washington Center for Child Environmental Health and Risks Research
STAR Grants R826886, R831709, EPA Cooperative Agreement R819186

The Center for Child Environmental Health and Risks Research (CCEHRR) at the University of Washington aims to understand the biochemical, molecular, and exposure mechanisms that define children's susceptibility to pesticides and the implications for assessing the risks of childhood pesticide exposure on normal development and learning. CCEHRR participants include members from multiple institutions, schools, and varied departments and clinics. The Center maintains partnerships with Yakima Valley communities situated within the agricultural center of Washington State to jointly investigate and promote sustainable, effective intervention methods, which reduce childhood pesticide exposures via the take-home pathway.

This study examined children's exposure to organophosphate (OP) pesticides in an agricultural community in central Washington State. Spot urine and hand wipe samples were collected from 109 children, 9 months to 6 years of age, as were house dust samples and wipe samples from various surfaces. Children were categorized based on parental occupation (agricultural vs. nonagricultural) and on household proximity to pesticide-treated orchards. Median house dust concentrations of dimethyl OP pesticides in homes of agricultural families were seven times higher than those of reference families (1.92 vs 0.27 lg/g). Median pesticide metabolite concentrations in agricultural children were five times higher than those in reference children (0.05 vs 0.01 lg/mL). Median pesticide concentrations in house dust and metabolite concentrations in urine from agricultural families were significantly higher in the children living near treated orchards (within 200 ft or 60 m) than those living more distant. Ten of 61 agricultural children had detectable OP pesticide levels on their hands, whereas none of the reference children had detectable levels. This indicates that children living with parents who work with agricultural pesticides, or who live in proximity to pesticide-treated farmland, have higher exposures than do other children living in the same community.

Lu, Fenske, et al., 2000. Pesticide exposure of children in an agricultural community: evidence of household proximity to farmland and take home exposure pathways. *Environmental Research* 2000;4(3):290-302.

Children's IQ at Ages 3 and 5 Is Inversely Associated With Blood Lead Concentration Below the CDC/WHO Level of Concern
Cincinnati Children's Center
STAR Grant R829389

The Cincinnati Children's Center focused on reducing disease and disability in children from environmental hazards, including lead, tobacco and pesticides, unsafe housing, and indoor allergens that are risk factors for asthma. Research focused on neurobehavioral effects of environmental toxicants that can affect a child's physical and mental health. The research program included examining the effect of children's blood lead levels on IQ and other tests, and research on whether children's behavioral problems can be linked to exposure to pesticides, environmental tobacco smoke, and lead before and after birth. This project used magnetic resonance imaging to evaluate brain metabolism and function in specific regions that may be damaged as a result of early exposure to lead.

Many U.S. housing units, especially those in low-income urban areas, are likely to contain lead paint and children can be significantly exposed through inhaling or ingesting the dust. The Cincinnati Children's Center participated in a study of the effects of blood lead concentrations below the Centers for Disease Control and Prevention/World Health Organization (CDC/WHO) Level of Concern of 10 micrograms/deciliter (µg/dL) and found significant effects on IQ.

Researchers measured blood lead concentrations in 172 children at 6, 12, 18, 24, 36, 48, and 60 months of age and administered the Stanford-Binet Intelligence Scale at the ages of 3 and 5 years. The relation between IQ and blood lead concentration was estimated with the use of linear and nonlinear mixed models, with adjustment for maternal IQ, quality of the home environment, and other potential confounders.

Blood lead concentration was inversely and significantly associated with IQ. In the linear model, each increase of 10 µg/dL in the lifetime average blood lead concentration was associated with a 4.6-point decrease in IQ, whereas for the subsample of 101 children whose maximum lead concentrations remained below 10 µg/dL, the change in IQ associated with a given change in lead concentration was greater, and the relation between children's IQ score and their blood lead concentration was nonlinear. For a nonlinear model with the full sample, IQ declined by 7.4 points as lifetime average blood lead concentrations increased from 1 to 10 µg/dL. This could potentially lower test results enough to result in a designation of mild mental retardation and placement in a special education program.

Blood lead concentrations, even those below 10 µg/dL, are inversely associated with children's IQ scores at 3 and 5 years of age, and associated declines in IQ are greater at these concentrations than at higher concentrations. This suggests that more U.S. children may be adversely affected by environmental lead than previously estimated.

Canfield, Henderson, et al., 2003. Intellectual impairment in children with blood lead concentrations below 10 µg per deciliter. *N Engl J Med* 2003;348(16):1517-26.

II. Lifestage and Genetic Susceptibility to Adverse Health Outcomes

PON1 (paraoxonase) Status and Pesticide Sensitivity Shows Wide Range of Variability
University of California (UC) at Berkeley, University of Washington (UW), Mt. Sinai Children's Centers
STAR Grants R826709, R831710, R826886, R831709, R827039, R831711

Although residential use of organophosphate (OP) pesticides is restricted by EPA, mainly because of risk to children, they are still widely used in agriculture. Several of the EPA/National Institute of Environmental Health Sciences Children's Centers have been investigating the effects of OP pesticides, how they are metabolized, and how gene-environment interactions may modify health risks from these compounds. The primary mechanism of OP toxicity is associated with inhibition of acetylcholinesterase. Recent studies have focused on paraoxonase 1 (PON1), a liver and serum enzyme that breaks down the toxic metabolites of a number of OP compounds, including diazinon and chlorpyrifos, and its role in modulating the toxicity of OPs.

Mechanisms of Pesticide Toxicity

The UC Berkeley Children's Center is conducting a study of farmworkers in California's Salinas Valley (the CHAMACOS Study) and has shown that susceptibility to pesticides is highly variable among Latina women and children. Working together, investigators at the UC Berkeley and University of Washington Children's Centers have shown that PON1 OP detoxifying ability varies greatly among individuals. PON1 status (as explained below) predicts a 65-fold to 160-fold difference in sensitivity to some OPs in the study population, with an average of 6- to 10-fold variability in sensitivity between groups of mothers and their newborns. Researchers used levels of PON1 activity measured in blood samples as a marker for pesticide susceptibility, and the research demonstrates that both the quality and quantity of the PON1 enzyme is important for its detoxification capacity.

The ability of the PON1 enzyme to detoxify OP pesticides is determined by whether a person has the Q or R form of the PON1 gene at position 192 on the chromosome. People with the QQ genotype have two copies of the Q variant of the PON1 gene, producing a PON1 enzyme that is significantly less efficient at detoxifying the chlorpyrifos oxon. People with the RR genotype have two copies of the R variant of the PON1 gene, producing a PON1 enzyme that is more efficient at detoxifying chlorpyrifos oxon. Inheriting one type of gene from each parent leads to a QR genotype with intermediate detoxifying ability. Additional genetic variants also affect the serum levels of enzyme available and it is mainly the serum level of PON1 enzyme that determines detoxifying ability for diazinon, another OP pesticide. For all groups, infants are at particular risk because the level of PON1 enzyme in newborns averages one-third or less than that of adults and it can take 6 months to 2 years for a baby to develop mature levels of PON1 (see below). Of particular concern are exposures of pregnant mothers and newborns with low PON1 status. Also, the frequency of the PON1 genotype varies by ethnicity. Approximately 10 to 20 percent of African Americans have the less efficient QQ genotype, compared with 50 percent of whites. Approximately 25 to 35 percent of the Latino population has the less efficient QQ genotype.

Furlong, Holland, et al., 2006. PON1 status of farmworker mothers and children as a predictor of organophosphate sensitivity. *Pharmacogenetics & Genomics* 2006;16(3):183-90.

Holland, Furlong, et al., 2006. Paraoxonase polymorphisms, haplotypes, and enzyme activity in Latino mothers and newborns. *Environ Health Perspect* 2006;114:985–91.

Genetic Susceptibility to Pesticides

The University of Washington (UW) Children's Center is examining the role of genetic variability in PON1 in protecting against developmental neurotoxicity associated with pesticides. Recent studies have shown that developmental onset of PON1 is highly variable among children. It can appear at ages as early as 6 months and as late as 2 years. Inter-individual variability in PON1 192 genotype, PON-1 enzyme levels and developmental time course means that children can have different susceptibilities to adverse effects of pesticides. The UW Children's Center research also is focusing on molecular mechanisms of pesticide-induced developmental neurotoxicity. The Center's community-based participatory research project has evaluated the impacts of the occupational take-home pathway on pesticide exposure in children of farmworkers in Washington State.

Four recent papers from the UW Children's Center provide details of PON1 genomics and enzyme activity. Investigators have coined the term "PON1 status" to enable comparisons between individuals. This encompasses the PON1(192)Q/R polymorphism (which affects catalytic ability toward different substrates) and PON1 levels (which are modulated in part by a C-108T polymorphism) rather than straight genotyping.

(1) In the first paper noted here (*Furlong, Cole, et al., 2005*), investigators provide evidence that children less than 2 years of age represent a particularly susceptible population for OP exposure, due to low abundance of PON1 and variable onset of plasma PON1 activity. They describe studies examining the neurotoxic effects of chronic, low-level OP pesticide exposure in mice, showing perinuclear vacuolization of cells in a discrete area of the neocortex and irregular distribution of neurons in the cortical plate. They describe a transgenic mouse model used to assess the importance of the Q192R polymorphism during development; adult mice expressing hPON1R192 were significantly more resistant than hPON1Q192 mice to chlorpyrifos oxon (CPO) toxicity. The studies indicate that children less than 2 years old, especially those homozygous for PON1Q192, would be predicted to be particularly susceptible to CPO toxicity.

(2) A second paper (*Costa, Cole et al., 2005*) discusses the polymorphisms of PON1 and determination of PON1 status.

(3) A third paper (*Costa, Vitalone et al., 2005*) examines the major factors (environmental chemicals, drugs, smoking, alcohol, diet, gender, age, and disease conditions) that have been shown to modulate PON1 activity in either direction. The investigators point out that as PON1 plays a protective role in OP toxicity, and because of its antioxidant capacity, in cardiovascular disease, a better understanding of how PON1 can be modulated by environmental factors has potential toxicological and clinical consequences.

(4) A subsequent paper (*Costa, Cole et al., 2006*) explains the genetic polymorphisms of PON1, and why a functional genomic analysis through a 2-substrate enzyme assay utilizing paraoxonase and diazoxonase (which metabolize parathion and diazinon, respectively) is the most informative approach in determining an individual's PON1 function.

(1) *Furlong, Cole et al., 2005.* Role of paraoxonase (PON1) status in pesticide sensitivity: genetic and temporal determinants. *Neurotoxicology* 2005;26(4):651-9.

(2) Costa, Cole et al., 2005. Measurement of paraoxonase (PON1) status as a potential biomarker of susceptibility to organophosphate toxicity. *Clin Chim Acta* 2005;352(1-2):37-47.

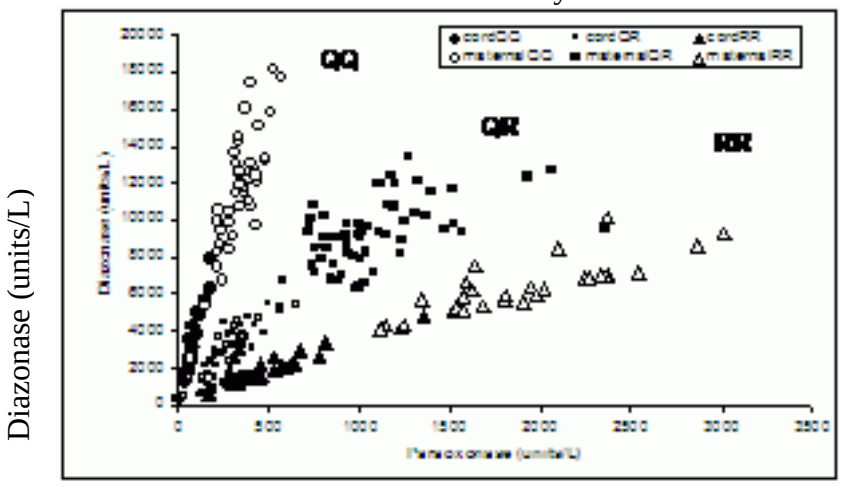
(3) *Costa, Vitalone et al.,2005.* Modulation of paraoxonase (PON1) activity. *Biochem Pharmacol* 2005;69(4):541-50.

(4) Costa, Cole et al., 2006. Gene-environment interactions: Paraoxonase (PON1) and Sensitivity to Organophosphate Toxicity. *Lab Med* 2006;37(2):109-14.
Alternate access: http://www.medscape.com/viewarticle/522383_print

PON1 Status

$PON1_{192}$ genotype can be predicted with high accuracy from looking at the two-dimensional plot of paraoxon and diazoxon hydrolysis rates, although the two-substrate assay also provides the level of plasma PON1 activity (see figure below) . The combination of functional $PON1_{192}$ genotype and plasma level is termed "PON1 status." (Note the large variability in PON1 levels, even among individuals of the same Q192R genotype.) –*Furlong, Holland, et al., 2006* and *Costa, Cole et al., 2006*.

PON1 status plot for mothers and newborns for a subset of the CHAMACOS cohort from the UC Berkeley Children's Center



Open circles: Data points for $PON1_{Q192}$ homozygous mothers (QQ);
Closed circles: Data points for $PON1_{Q192}$ newborns (QQ);
Closed squares: Data points for heterozygous mothers (QR);
Open squares: Data points for heterozygous newborns (QR);
Open triangles: Data points for $PON1_{R192}$ mothers (RR);
Closed triangles: Data points for $PON1_{R192}$ newborns (RR).
Furlong, Holland, et al., 2006. PON1 status of farmworker mothers and children as a predictor of organophosphate sensitivity. *Pharmacogenetics & Genomics* 2006; 16(3):183-90 (Reprinted with permission.)

PON1 Variability: Neonates Have Lower PON1 Activity Than Adults, Differences Between Ethnic Groups
Mt. Sinai Children's Center
STAR Grants R827039, R831711

The Mt. Sinai Children's Center studies have enrolled participants from a largely low-income population in East Harlem, New York City. The Center has developed new, high-throughput techniques for geno- and phenotyping of PON1 and other pesticide-metabolizing enzymes and found that decreased maternal PON1 levels are associated with a small reduction in newborn head circumference (see below). Mt. Sinai Center research also shows that neonates can have a lower PON1 activity level than adults, and research has shown differences in PON1 activity between ethnic groups.

PON1 (paraoxonase-1) detoxifies organophosphates (OPs) by cleavage of active oxons before they have a chance to inhibit cholinesterases. The corresponding PON1 gene has common polymorphisms in both the promoter (-909, -162, -108) and the coding region (L55M, Q192R). The five *PON1* genotypes were determined for maternal blood (n = 402) and cord blood (n = 229) as part of a study of the effects of OP pesticide exposure on infant growth and neurodevelopment. Participants in this study are primarily of Caucasian, Caribbean-Hispanic, or African American ancestry. PON1 enzymatic activity was strongly dependent on the promoter alleles in both maternal and cord blood. For example, PON1 activities for position -108*CC*, *CT*, and *TT* mothers were 146, 128, and 109 arylesterase U/mL (analysis of variance, $p < 0.0001$) , whereas the same PON1 activities for the respective cord bloods were 49.0, 32.4, and 23.2 U/mL ($p < 0.0001$). Compared with adults, neonates had lower PON1 activity, implying reduced capacity to detoxify OPs. In addition, there was a larger difference in activity between genotype groups in neonates than in adults. Because the five polymorphisms in *PON1* occur in a short stretch of DNA, they were tested for linkage disequilibrium (LD). Significant LD was found among all three promoter polymorphisms as well as between promoter polymorphisms and L55M, with the strongest LD for Caucasians and the weakest for African Americans. The Caribbean Hispanics fall between these two groups. Surprisingly, significant LD also was observed between the promoter polymorphisms and C311S in *PON2*. LD between the promoter polymorphisms and Q192R was not significant.

Genotype-phenotype associations of PON1 vary by allele, are independent of race/ethnicity, and are stronger for infants than mothers. Results suggest that infants may be more susceptible to toxic effects of pesticides detoxified by PON1. Mt. Sinai Investigators have developed a new robust single molecule-based haplotyping technology that was verified by showing haplotype-based variation in PON1 activity in mothers heterozygous at two loci, a result that could not have been determined by genotyping or by haplotype inference. High-throughput assays for UGT2B7 and lingual lipase are under development to investigate these new endocrine disruptor-related susceptibility factors over the next several years. One goal of this research is to establish an inexpensive biomarker assay to permit large population studies of human variation in metabolism of phthalates (used in soft plastics).

Chen, Kumar et al., 2003. Increased influence of genetic variation on PON1 activity in neonates. *Env Health Perspect* 2003;111(11):1403-10.

Low Maternal Levels of PON1 Associated With Smaller Head Circumference in Newborns
Mt. Sinai Children's Center
STAR Grants R827039, R831711

Although the use of pesticides in inner-city U.S. homes is considerable, little is known about the potentially adverse health effects of such exposure. Recent animal data suggest that exposure to pesticides during pregnancy and early life may impair growth and neurodevelopment in the offspring. To investigate the relationship among prenatal pesticide exposure, *PON1* polymorphisms and enzyme activity, and infant growth and neurodevelopment, the Mt. Sinai Children's Center researchers conducted a prospective, multiethnic cohort study of mothers and infants delivered at Mt. Sinai Hospital in New York City. They evaluated the effects of pesticide exposure on birth weight, length, head circumference, and gestational age among 404 births between May 1998 and May 2002. Pesticide exposure was assessed by a prenatal questionnaire administered to the mothers during the early third trimester as well as by analysis of maternal urinary pentachlorophenol levels and maternal metabolites of chlorpyrifos and pyrethroids. Neither the questionnaire data nor the pesticide metabolite levels were associated with any of the fetal growth indices or gestational age. However, when the level of maternal PON1 activity was taken into account, maternal levels of the organophosphate pesticide chlorpyrifos above the limit of detection coupled with low maternal PON1 activity were associated with a significant but small reduction in head circumference. In addition, maternal PON1 levels alone, but not PON1 genetic polymorphisms, were associated with reduced head size. Because small head size has been found to be predictive of subsequent cognitive ability, these data suggest that chlorpyrifos may have a detrimental effect on fetal neurodevelopment among mothers who exhibit low PON1 activity.

Berkowitz, Wetmur, et al., 2004. In utero pesticide exposure, maternal paraoxonase activity, and head circumference. *Environ Health Perspect* 2004;112(3):388-91.

Associations of Tumor Necrosis Factor G-308A With Childhood Asthma and Wheezing
University of Southern California (USC)/University of California at Los Angeles (UCLA) Children's Center
STAR Grants R826708, R831861

Tumor necrosis factor (TNF-α) plays a central role in initiation of airway inflammation and the generation of airway hyperreactivity, and has a recognized role in asthma pathophysiology. It mediates a spectrum of airway inflammatory responses, including those to air pollutants, and is an asthma candidate gene. One TNF promoter variant (G-308A) affects expression of TNF and has been associated with inflammatory diseases; however, studies of asthma have been inconsistent. Because ozone produces oxidative stress, increased airway TNF, and inflammation, the associations of the -308 TNF polymorphism with asthma may vary by ozone exposure and variants of the oxidant defense genes, glutathione-S-transferase (GST) M1 and GSTP1.

The objectives of this research were to investigate the association of TNF G-308A with asthma and wheezing and to determine whether these associations vary with ozone exposure and GSTM1 and GSTP1 genotype.

Researchers studied associations of TNF-308 genotype with lifetime and current wheezing and asthma among 3,699 children in the Children's Health Study. They examined differences in associations with community ozone and by GSTM1 null and GSTP1 105 Ile/Val (A105G) genotype.

Children with TNF-308 GG had decreased risk of asthma (odds ratio OR = 0.8; 95% confidence interval (CI) 0.7-0.9) and lifetime wheezing (OR=0.8; 95% CI = 0.7-0.9). The protective effects of GG genotype on wheezing outcomes were of greater magnitude in lower compared with higher ozone communities. These findings were replicated in the two cohorts of fourth grade children recruited in 1993 and 1996. The reduction of the protective effect from the -308 GG genotype with higher ozone exposure was most marked in the GSTM1 null and GSTP1 Ile/Ile groups.

The protective effect of the TNF-308 GG genotype in children with low ozone exposures or protective GSTM1 or GSTP1 genotype suggests that this relatively common genetic polymorphism, or haplotype marked by this polymorphism, plays a protective role in asthma pathogenesis in children, depending on airway oxidative stress levels. Earlier work (additional reference) found that the *GSTP1* genotype was associated with risk for respiratory illness severe enough to result in a school absence in school-aged children.

Li, Gauderman, et al., 2006. Associations of tumor necrosis factor G-308A with childhood asthma and wheezing. *Am J Respir Crit Care Med* 2006;173(9):970-6.

Gilliland, Rappaport, et al., 2002. Effects of glutathione *S*-transferase *P1*, *M1*, and *T1* on acute respiratory illness in school children. *Am J Respir Crit Care Med* 2002;166:346-51.

Infants With Higher Prenatal Exposure to Urban Air Pollutants Are Born With Genetic Damage Associated With Increased Cancer Risk
Columbia Children's Center
STAR Grants R827027, R832141

The Columbia Children's Center has found that prenatal exposure to combustion-related urban air pollutants can alter the structure of chromosomes of babies in the womb. This is the first study to show that environmental exposure during pregnancy to such pollutants can cause a modest but significant increase in chromosomal abnormalities in fetal tissues. Such genetic alterations have been linked in other studies to increased risk of cancer in children and adults.

The research involved a sample of 60 newborns and their nonsmoking mothers in low-income neighborhoods of New York City (Harlem, Washington Heights, and the South Bronx). The mothers' exposure during pregnancy to varying levels of airborne polycyclic aromatic hydrocarbons (PAHs) was measured by personal air monitoring of the mothers during pregnancy. PAHs are carcinogenic air pollutants that cross the placenta and enter the environment when combustion occurs such as from a car, truck, or bus engines, residential heating, power generation, or tobacco smoking.

Prenatal exposure was assessed by the use of questionnaires and the personal air monitors worn by the mothers during the third trimester of their pregnancies. Chromosomal aberrations were measured in cord blood lymphocytes by fluorescence *in situ* hybridization, a method that allows visualization of such abnormalities. In a subset of newborns, PAH-related DNA damage also was measured in the umbilical cord blood.

About 40 percent of the babies in the study were born with genetic damage associated with air pollution; airborne PAHs were significantly associated with stable aberration frequencies in cord blood. Although the frequency of these aberrations was quite low, aberrations that are stable, and therefore persistent, are of particular concern for cancer risk. In a subset of 22 newborns with detectable levels of the biomarker, PAH-DNA adducts were not associated with chromosomal aberrations, possibly because of the limited size of the subset. Of particular concern was that newborns had higher levels of carcinogen-DNA adducts than mothers per unit of estimated exposure, indicating greater fetal susceptibility and potential risk from these pollutants.

Bocskay, Tang, et al., 2005. Chromosomal aberrations in cord blood are associated with prenatal exposure to carcinogenic polycyclic aromatic hydrocarbons. *Cancer Epidemiol Biomarkers Prev* 2005;14(2):506-11.

Gene-Environment Interaction and Human Malformation
Gary M. Shaw, California Birth Defects Monitoring Program
STAR Grant R828292

The goal of this research project was to elucidate gene-environment interactions underlying etiologies of human malformations. This multidisciplinary research program investigated the hypotheses that genetic variation of candidate genes involved in impaired detoxification pathways, folate metabolism and transport, and vascular integrity modify risks of human malformations. This included an analysis of whether genetic variations in infant or maternal folate-pathway genes modify risks of malformations, with regard to variations in maternal folate intake, and to see whether genetic variations in genes associated with vascular development and function modify risks of malformations in the presence or absence of maternal exposures to vasoactive chemicals. This group developed new methods for multiplex genotyping for *N*-acetyltransferases1 and 2 (*NAT1* and *NAT2*), glutathione-*S*-transferases M1 and T1, and *CYP2D6*, and performed a multilocus genotyping assay cardiovascular panel B on more than 1,000 individuals, which detects 32 polymorphisms from 23 candidate genes. In addition, 200 individuals have been assayed for polymorphisms *(VEGF)* associated with angiogenesis.

Combining state-of-the-art genotyping methods with rigorously designed population-based epidemiologic data collection, the case-control research design included 5,000 cases and controls and focused on neural tube defects, selected heart malformations, orofacial clefts, limb defects, gastroschisis, and intestinal atresias. The analytic plan combined maternal interview data with multiplex polymerase chain reaction-based genotyping for nearly 40 candidate genes on more than 7,200 samples.

A study from this research group relates a human genetic polymorphism to risk of spina bifida. Folate binding protein 1 (Folr1) knockout mice with low maternal folate concentrations have been shown to be excellent animal models for human folate-responsive neural tube defects (NTDs). Maternal folate supplementation upregulates the expression of the PCMT1 gene in Folr1 nullizygous neural tube tissue during neural tube closure. PCMT1 encodes the protein repair enzyme PIMT that converts abnormal d-aspartyl and l-isoaspartyl residues to the normal l-aspartyl form. The researchers hypothesized that a known functional polymorphism (Ile120Val) in the human PCMT1 gene is associated with an increased risk of folate-responsive human NTDs. A case-control study was conducted to investigate a possible association between this polymorphism and risk of spina bifida. Compared to the Ile/Ile and Ile/Val genotypes, the homozygous Val/Val genotype showed decreased risk for spina bifida (adjusted odds ratio = 0.6, 95% confidence interval: 0.4-0.9). Results showed that the Ile120Val polymorphism of PCMT1 gene is a genetic modifier for the risk of spina bifida. Val/Val genotype was associated with a reduction in risk for spina bifida.

Zhu, Yang, et al., 2006. A known functional polymorphism (Ile120Val) of the human PCMT1 gene and risk of spina bifida. *Mol Genet Metab* 2006;87(1):66-70.

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

Barry A. Finette, University of Vermont

STAR Grant R830757

The objective of this study was to evaluate the utility of biomarkers of effect and susceptibility for studying cancer risk in a pediatric population following genotoxic exposures. The aim was to determine if children from an exposed population with elevated cancer incidence have an increase in chromosome aberrations as well as the following changes in the hypoxanthine phosphoribosyl transferase (*HPRT*) mutational spectrum: (1) an increase in frameshift mutations reflective of exposure to anthraquinone-based dyes and styrene-acrylonitrile trimers; (2) an increase in point mutations reflective of exposure to benzidine-based dyes, epichlorohydrin, and trichloropropane; and (3) an increase in V(D)J recombinase-mediated deletions reflective of exposure to aromatic hydrocarbons. Researchers were looking to determine if specific DNA polymorphisms of 11 carcinogen-metabolizing enzymes are associated with increased susceptibility to genotoxic exposure.

This study was designed to measure biomarkers of effect and susceptibility in exposed siblings of children in a pediatric cancer cluster (as defined by the Centers for Disease Control and Prevention) linked to exposure to contaminated groundwater from two EPA-designated Superfund sites in Dover Township, New Jersey. The exposed siblings were the focus of the study rather than the children with cancer due to the genotoxic effects of cancer treatment. However, biomarkers of susceptibility were also measured in children with cancer. Biomarkers of effect (chromosomal aberrations and *HPRT* mutations) in the exposed siblings were compared to measurements in unexposed children from neighboring communities. Biomarkers of susceptibility (DNA polymorphisms for carcinogen metabolizing enzymes) in the exposed siblings and unexposed children are being compared to children with cancer to determine if the latter have a higher prevalence of specific metabolic genotypes. In addition, the relationships between biomarkers of effect and susceptibility in exposed siblings and unexposed children were examined to see if the effects of exposure are modified by certain metabolic polymorphisms.

The somatic mutant frequency (Mf) of the HPRT gene has been widely used as a biomarker for the genotoxic effects of exposure, but few studies have found an association with environmental exposures. In a recent publication, researchers measured background Mfs in 49 current and former residents of Dover Township exposed during childhood to industrially contaminated drinking water. The exposed subjects were the siblings of children who developed cancer after residing in Dover Township, where the incidence of childhood cancer has been elevated since 1979. Mfs from this exposed group were compared to Mfs in 43 age-matched, presumably unexposed residents of neighboring communities with no known water contamination and no increased cancer incidence. The mean Mf for the exposed group did not differ significantly from the unexposed group (3.90×10^{-6} vs. 5.06×10^{-6}; $P = 0.135$), but unselected cloning efficiencies were higher in the exposed group (0.55 vs. 0.45; $P = 0.005$). After adjustment for cloning efficiency, lnMf values were very similar in both groups and age-related increases were comparable to those previously observed in healthy children. The results suggest that *HPRT* Mf may not be a sensitive biomarker for the genotoxic effects of environmental exposures in children, particularly when substantial time has elapsed since exposure.

Vacek, Messier et al., 2005. Somatic mutant frequency at the HPRT locus in children associated with a pediatric cancer cluster linked to exposure to two superfund sites. *Environ Mol Mutagen* 2005;45(4):339-45.

GSTM1 Status Determines Cardiovascular Susceptibility to Particulate Matter Effects

Petros Koutrakis, Joel Schwartz, Sung Kyun Park, Marie S. O'Neill, Pantel S. Vokonas, and colleagues, EPA Center for Ambient Particle Health Effects at Harvard University

STAR Grant R827353

Air pollution by particulate matter (PM) has been associated with cardiovascular deaths, although the mechanism of action is unclear. One proposed pathway is through disturbances of the autonomic control of the heart, or changes in heart rate variability (HRV). The parasympathetic and sympathetic stimulation of the heart produces variations in the time intervals between normal heartbeats; analysis of this variability is therefore an estimate of cardiac autonomic regulation. HRV is a noninvasive measure that independently predicts cardiovascular mortality in patients with and without underlying cardiovascular disease.

Overall, studies have generally found significant associations with HRV in elderly subjects, but weaker associations were found in younger subjects, suggesting that age-related decreases in toxic defenses play a role in susceptibility. In a recent review, researchers at the Harvard PM Center (one of the five PM Centers currently supported by EPA) found that the only consistent PM association was with the high-frequency (HF) components of HRV, either HF in the frequency domain, or root mean squared differences between adjacent RR intervals (rMSSD) or proportion of adjacent NN intervals differing by more than 50 ms (PNN50) in the time domain. In contrast, low frequency was not associated with particles in four of five studies. This suggests a significant effect on the parasympathetic nervous system, perhaps because the vagus nerve innervates the lung.

In a recent study, Harvard PM Center researchers tested the hypothesis that such disturbances are mediated by PM exposure increasing oxidative stress by examining the association between PM and the HF component of HRV as modified by the presence or absence of the allele for glutathione-S-transferase M1 (GSTM1), and the use of statins, obesity, high neutrophil counts, higher blood pressure, and older age. The investigators examined the association between particles less than 2.5 µg in aerodiameter ($PM_{2.5}$) and HF in 497 participants in the Normative Aging Study, using linear regression controlling for covariates.

A 10 µg/m3 increase in $PM_{2.5}$ during the 48 h before HF measurement was associated with a 34 percent decrease in HF (95% CI, -9%, -52%) in subjects without the allele, but had no effect in subjects with GSTM1 present. Among GSTM1-null subjects, the use of statins eliminated the effect of $PM_{2.5}$. Obesity and high neutrophil counts also worsened the PM effects with or without GSTM1. The conclusion is that effects of $PM_{2.5}$ on HF appear to be mediated by reactive oxygen species (ROS). This may be a key pathway for the adverse effects of combustion particles.

Schwartz, Park, et al., 2005. Glutathione-S-transferase M1, obesity, statins, and autonomic effects of particles: gene-by-drug-by-environment interaction. *Am Respir Crit Care Med* 2005;172(12):1529-33. Related Editorial: pp. 1482-85.

Genetic Basis of the Increased Susceptibility of Children to Inhaled Pollutants
Terry Gordon, Lung Chi Chen, Albert F. Gunnison, and Eric Tang, New York University
STAR Grant R830755

The objective of this study was to determine the biological mechanism underlying the increased susceptibility of children to inhaled pollutants. The hypothesis was that there is a genetic basis for the differential response of neonatal and adult rodent lungs to inhaled pollutants. The objectives of this research are to: (1) quantify the contribution of genetic vs. environmental factors; (2) identify candidate genes that play a critical role in the molecular pathways leading to the increased susceptibility of the neonatal lung; and (3) compare these genes to those involved in adult lung toxicity. Earlier research demonstrated that ozone produces greater inflammation and injury in neonatal lungs, and this study expanded on those findings to identify genes responsible for the age-related differential response to inhaled ozone.

Ten inbred strains of neonatal mice were exposed to ozone and examined for lung injury and inflammation. To ensure that strain differences in response are genetic in nature, interstrain differences in dose, as measured by ^{18}O in the lung, also were assessed in neonates. To identify candidate genes that play a critical role in the differential response of neonatal and adult mice to ozone, investigators identified quantitative trait loci (QTLs) associated with the response of neonatal mice to ozone by using both a classical genetic cross-breeding method and a state-of-the-art computational genomics method. To identify the most likely candidate genes within these chromosomal loci, QTL results were linked to microarray expression data.

The investigators found that as hypothesized, ozone appears to produce more adverse effects in neonatal mice than in adult male or female mice, and focused further research on the genetic factors that control this increased susceptibility.

III. Novel Exposure Tools and Methods

Novel Exposure Modeling Method To Assess Pesticide Dose in an Agricultural Community
University of California at Berkeley Children's Center
STAR Grants R826709, R831710

The Children's Center at the University of California at Berkeley (UC Berkeley), established in 1998, is a fully coordinated, multidisciplinary Center that integrates exposure, health, and community-based prevention research to address the environmental health risks to low-income children living in agricultural communities in California. The Center's research is focused on pesticide exposures, potential health consequences of those exposures and community-based exposure prevention strategies.

The core of the Center is the CHAMACOS (Center for the Health Assessment of Mothers and Children Of Salinas) project, conducted in collaboration with a coalition of community health care providers and agencies. CHAMACOS (which means "small child" in Mexican Spanish) is a longitudinal birth cohort study of pregnant women and children living in the agricultural community of the Salinas Valley, Monterey County, California. The cohort includes primarily low-income Mexican immigrant farmworkers and their families, thus providing a unique opportunity to examine prospectively the influence of prenatal and early childhood exposures encountered in an agricultural environment, such as to pesticides and bioaerosols, on the health of children.

Approximately 230,000 kg of organophosphate (OP) pesticides are applied annually in California's Salinas Valley (see Figure 1 below), and these activities raise concerns about exposures to area residents. Researchers collected three spot urine samples from pregnant women (between 1999 and 2001) enrolled in the CHAMACOS study and analyzed them for six dialkyl phosphate metabolites. Urine from 446 pregnant women was used to estimate OP pesticide doses with two deterministic steady-state modeling methods: Method 1, which assumed that the metabolites were attributable entirely to a single diethyl or dimethyl OP pesticide; and Method 2, which adapted U.S. EPA draft guidelines for cumulative risk assessment to estimate dose from a mixture of OP pesticides that share a common mechanism of toxicity. Pesticide use reporting data for the Salinas Valley was used to approximate the mixture to which the women were exposed.

Based on average OP pesticide dose estimates that assumed exposure to a single OP pesticide (Method 1), between 0-36.1 percent of study participants' doses failed to attain a margin of exposure (MOE) of 100 relative to the U.S. EPA oral benchmark dose$_{10}$ (BMD$_{10}$) , depending on the assumption made about the parent compound. These BMD$_{10}$ values are doses expected to produce a 10 percent reduction in brain cholinesterase activity compared with background response in rats. Given the participants' average cumulative OP pesticide dose estimates (Method 2) and regardless of the index chemical selected, 14.8 percent of the doses failed to attain an MOE of 100 relative to the BMD$_{10}$ of the selected index. An uncertainty analysis of the pesticide mixture parameter suggests that this point estimate could range from 1 to 34 percent.

This report presents one of the first case studies using EPA's cumulative risk assessment framework for OP pesticides. This study used only the portions of the guidelines dealing with dose aggregation, as the dose estimates were based on biomonitoring data.

Castorina, Bradman, et al., 2003. Cumulative organophosphate pesticide exposure and risk assessment among pregnant women living in an agricultural community: a case study from the CHAMACOS cohort. *Environ Health Perspect.* 2003;111(13):1640-48.

A recent study by researchers from the UC Berkeley Children's Center, the EPA National Exposure Research Laboratory, and others tested field methods that could be useful for large longitudinal studies such as the National Children's Study. These included methods for collecting house dust, indoor and outdoor air, residues from surfaces, clothing, toys and food, as well as urine samples, in a group of 20 farmworker children in the Salinas Valley of California 5-27 months of age. Researchers measured 29 common agricultural and home use pesticides and a subset of organophosphorus (OP), organochlorine (OC) and pyrethroid pesticides were measured in food. They also analyzed urine samples for OP pesticide metabolites. Finally, researchers administered four field-based exposure assessment instruments: a questionnaire, a food diary, home inspection and a self-administered child activity timeline. Pesticides were detected more frequently in house dust, surface wipes, and clothing than other media, with chlorpyrifos, diazinon, chlorthal-dimethyl, and cis- and trans-permethrin detected in 90 to 100 percent of samples. Pesticide loading on socks and clothing was higher for the group of 10 toddlers compared to the 10 younger crawling children. Several OP pesticides, as well as 4,4'-DDE, atrazine, and dieldrin were detected in the food samples. The child activity timeline, a novel, low-literacy instrument based on pictures, was successfully used by participants. Future uses of the data include the development of pesticide exposure models and risk assessment.

Bradman, Whitaker, et al., 2007. Pesticides and their metabolites in the homes and urine of farmworker children living in the Salinas Valley, CA. *J Exp Sci Environ Epidemiol* 2007 Jul;17(4):331-49. Epub 2006 May 31.

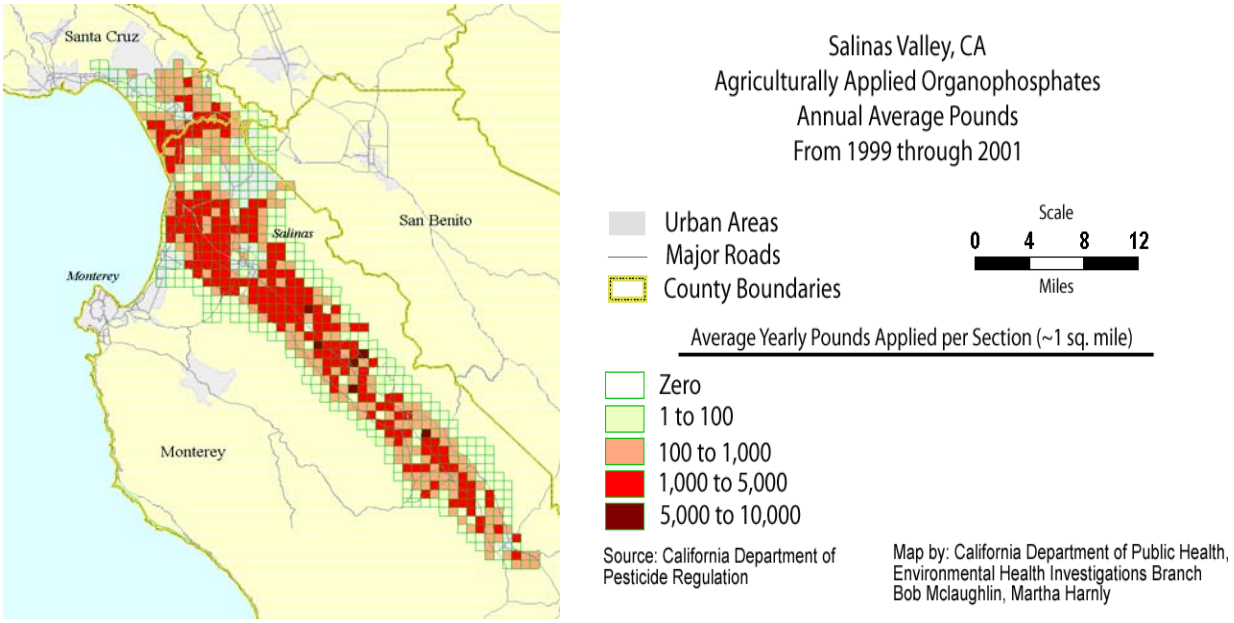


Figure 1. This diagram shows the amount of agriculturally applied organophosphate (OP) pesticides in the Salinas Valley, in average pounds, for the years 1999 through 2001. Used by permission of the California Department of Public Health.

Longitudinal Study of Children's Exposure to Permethrin

Ye Hu, James Raymer, Research Triangle Institute (RTI) International
STAR Grant R829397

The objectives of this research were to: investigate the time course of the redistribution of pyrethroid pesticides in various media following application and factors affecting the redistribution; investigate the functional relationships across time between environmental media, personal measurements, and biological media; estimate aggregate exposure after application and the importance of each exposure pathway; and investigate the difference between the time course of pyrethroid pesticide metabolism between adults and children. Fifteen young children from 13 homes with indoor application of pyrethroids were successfully followed for 12 months. Samples collected included air, surface wipes, toy wipes, hand wipes, body suits, duplicate diet, leftover food, morning urine samples, video (eating and playing events), and questionnaires.

The researchers found that there is substantial surface loading of target compounds in the homes. Also, preliminary analysis of data from hand wipes and body suits indicates potential for dermal uptake from surfaces. The data suggests that pyrethroids are quite stable in the indoor environment, that these compounds are adsorbed to dust particles, and that mechanical redistribution is the primary transport mechanism in the home. Researchers also have developed a method to extract and analyze pyrethroid metabolites from commercially available diapers.

Feasibility of Disposable Diapers as a Tool for Exposure Assessment
Ye Hu, James Beach, James Raymer and Mike Gardner, RTI International

Disposable diapers are widely used in the United States and many other areas in the world; therefore, they are ideal media for urine collection for measurement of young children's exposure to pesticides. However, disposable diapers normally contain polyacrylate polymers that make the extraction and analysis of urine very difficult. The objectives of this research were to evaluate whether disposable diapers that contain polyacrylate granules can be extracted using salt solutions, and whether they can be used for the collection and quantitative measurements of selected urinary pyrethroid pesticide metabolites and creatinine. The storage stability of the metabolites and creatinine in a wet diaper at body temperature and at refrigeration temperature also was evaluated. Salt solutions were tested for efficiency of polymer shrinkage. Pyrethroid metabolites including 3-(2,2-dichlorovinyl)-2,2-dimethyl-(1-cyclopropane) carboxylic acid (DCCA) were analyzed using LC/MS/MS and evaluated for recoveries in the urine released from the diapers. The study found calcium chloride dihydate to be satisfactory in releasing urine and metabolites from the polymers. The percent recoveries for the three tested pyrethroid metabolites were mostly in the range of 65-130. The percent recoveries for creatinine were in the range of 71-133. The detection limit for each of the three metabolites was 0.1 µg/L. The pyrethroid metabolites and creatinine were stable on the diaper for at least 72 h. Researchers concluded from this study that calcium chloride dihydrate can successfully release urine and metabolites from polyacrylate-containing diapers, and the method is promising for studies of pyrethroid metabolites.

Hu, Beach, et al., 2003. Disposable diaper to collect urine samples from young children for pyrethroid pesticide studies. *J Expo Anal Environ Epidemiol* 2004;14(5):378-84.

Saliva Biomonitoring for Organophosphorus Pesticide Exposures in Children
Richard Fenske, University of Washington
STAR Grant R828606

The objective of this study was to evaluate the feasibility of measuring children's exposure to organophosphate (OP) pesticides through saliva biomonitoring. Three pesticides—chlorpyrifos, diazinon and permethrin—were included in the study.

Using animal models, a good correspondence was found for diazinon concentrations in blood and saliva samples, demonstrating that diazinon concentrations in saliva are a reliable indicator of internal dose. Chlorpyrifos was not detected in saliva, probably because of its rapid metabolism. Detection of permethrin in saliva was based on a new analytical method, and results were inconsistent. A study of pesticide applicators and their children in Nicaragua provided an opportunity to evaluate saliva sampling in an exposed population. Analysis of urine samples indicated exposure to chlorpyrifos for both applicators and children, but saliva samples did not. This finding was consistent with the animal studies. Exposure to diazinon occurred among applicators, but not among their children. A good correspondence was observed between diazinon concentrations in the workers' blood and saliva, which is consistent with findings from the animal studies. Diazinon concentrations in saliva also corresponded with excretion of the primary urinary metabolite of diazinon in these applicators.

Saliva sampling is an attractive biologic monitoring method for both children and adults because it is simple, non-invasive, and measures pesticides rather than pesticide metabolites. This study demonstrates that saliva sampling is suitable for some compounds, but not for others, and that such sampling is feasible in adults and children.

Lu, Irish et al. 2003. Biological monitoring of diazinon exposure using saliva in an animal model. *J Toxicol Environ Health* 2003;66(24):2315-25.

Measurement of Non-Persistent Pesticides in Postpartum Meconium as a Biomarker of Prenatal Exposure: A Validation Study
Robin Whyatt, Columbia Children's Center
STAR Grant R828609

Meconium, which represents the intestinal contents of a fetus starting in the second trimester, is being studied as a potential biomarker and dosimeter for fetal exposure to xenobiotics. This research, which was part of the EPA/National Institute of Environmental Health Sciences-funded Columbia Center for Children's Environmental Health (CCCEH), was based on the samples and exposure data collected from 100 mothers/newborn pairs. Indoor air monitoring, questionnaire data, and biological samples (e.g., maternal blood, umbilical cord blood, maternal urine, newborn urine, and meconium) were collected between the third trimester of pregnancy and delivery.

Researchers detected chlorpyrifos, diazinon, and propoxur in nearly 100 percent of the indoor air samples, and chlorpyrifos or its chemical-specific metabolite, TCPy, was consistently detected across all environmental and biologic matrices in the study. In addition, there was a consistent and highly significant correlation seen between TCPy levels in repeat maternal urine samples during pregnancy. TCPy levels in meconium were significantly associated with chlorpyrifos levels in both maternal and umbilical cord blood collected after delivery.

Researchers had hypothesized that measurements of insecticides in meconium would provide a dosimeter of fetal prenatal exposure. The results for chlorpyrifos indicated that this hypothesis was correct. Chlorpyrifos or its chemical-specific metabolite, TCPy, was the only compound that was detected consistently across all environmental and biologic matrices in the current study. Although the researchers saw only a weak correlation between chlorpyrifos levels in indoor air samples and TCPy levels in meconium, a consistent and often highly significant correlation was seen between TCPy in meconium and TCPy levels in repeat maternal urine samples during pregnancy, and with chlorpyrifos levels in maternal and umbilical cord blood collected after delivery. Thus, the measurements of TCPy levels in meconium appear to provide a valuable internal dosimeter for chlorpyrifos exposure during pregnancy.

Results showed a dramatic decrease (by year of enrollment into the study) in both chlorpyrifos and diazinon levels in environmental and biological samples following the U.S. EPA regulatory action to phase out residential use of OP pesticides; the decrease in chlorpyrifos was consistent across all of the environmental and biologic matrices assessed. These research results—along with the other research being performed at the Columbia Children's Center—indicate that EPA regulatory action has been successful at reducing prenatal exposures to certain insecticides among African American and Dominican mothers and newborns in New York City.

Fenske, Bradman et al., 2005. Lessons learned for the assessment of children's pesticide exposure: critical sampling and analytical issues for future studies. *Environ Health Perspect* 2005;113(10):1455–62.

Using GPS and GIS Technology To Enhance Accuracy of Exposure Assessment
University of Washington Children's Center
STAR Grants R826886, R831709

Global positioning system (GPS) technology offers promise for human time-location studies to evaluate potential exposure to environmental contaminants. In this project, researchers focused on the development of a novel GPS instrument suitable for tracking the movements of young children. Eleven children in the Seattle area (2 to 8 years old) wore custom-designed data-logging GPS units integrated into their clothing. Location data were transferred into geographic information systems (GIS) software for map overlay, visualization, and tabular analysis. Data were grouped into five location categories (in vehicle, inside house, inside school, inside business, and outside) to determine time spent and percentage reception in each location. Additional experiments focused on spatial resolution, reception efficiency in typical environments, and sources of signal interference. Significant signal interference occurred only inside concrete/steel-frame buildings and inside a power substation. The GPS instruments provided adequate spatial resolution (typically about 2-3 m outdoors and 4-5 m indoors) to locate subjects within distinct microenvironments and distinguish a variety of human activities. Reception experiments showed that location could be tracked outside, proximal to buildings, and inside some buildings. Specific location information could identify movement in a single room inside a home, on a playground, or along a fence line. The instrument, worn in a vest or in bib overalls, was accepted by children and parents. Durability of the wiring was improved early in the study to correct breakage problems. The use of GPS technology offers a new level of accuracy for direct quantification of time-location activity patterns in exposure assessment studies.

Elgethun, Fenske et al. 2003. Time-location analysis for exposure assessment studies of children using a novel global positioning system instrument. *Environ Health Perspect* 2003;111(1):115-22.

Development of a Physiologically Based Pharmacokinetic/Pharmacodynamic Model To Quantitate Biomarkers of Exposure for Organophosphate Insecticides

Charles Timchalk, Batelle–Pacific Northwest Division

STAR Grant R828608

Age-dependent differences in a child's physiology and metabolic capacity may significantly impact responses to chemical insult, possibly resulting in health effects. Physiologically based modeling integrates age-dependent changes into a comprehensive model that can quantify dose and response across all ages. This STAR-funded research resulted in the development of an age-dependent physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model for the organophosphate (OP) insecticide, chlorpyrifos. The objective of this research is to develop a model that could be used to predict physiological response in humans from exposures to OP pesticides at environmentally relevant levels.

These studies found CYP- and PON1-mediated metabolic profiles in microsomes from liver and intestinal enterocytes, suggesting that intestinal metabolism may impact the dosimetry of OP insecticides, especially at low-dose oral exposures. The findings suggest that a peripheral binding site may play an important role in determining the biological interaction between AChE and the oxon resulting from CYP activation; this may be relevant in understanding the dynamics associated with low-level, environmentally relevant exposures. The PBPK model developed through this research has been used to successfully simulate the disposition of chlorpyrifos in rats and humans and the resulting inhibition of AChE following acute, chronic, oral, and dermal exposure.

At low-level exposures, results indicate that other esterase detoxification pathways may compensate in situations where genetic polymorphisms in PON1 detoxification result in lower chlorpyrifos-oxonase activity. The developed age-dependent rat model behaves consistently with the general understanding of OP toxicity and the model is consistent with experimental findings in neonatal and adult rats. This model suggests that neonatal rats are more sensitive to high-dose acute effects of OP exposure. However, at low exposure levels the neonatal rat is not substantially more sensitive.

Timchalk, Poet et al., 2006. Age-dependent pharmacokinetic and pharmacodynamic response in preweanling rats following oral exposure to the organophosphorus insecticide chlorpyrifos. *Toxicology* 2006;220(1):13-25.

Other Investigations of Interest

There are a number of other recent STAR projects involving susceptible subpopulations and gene-environment interactions that may be of interest. For example, Delfino and colleagues at the University of Californina at Irvine are examining oxidative stress response to particulate matter (PM) exposure in an elderly population. Kipen and colleagues at the University of Medicine and Dentistry of New Jersey/Robert Wood Johnson Medical School have investigated the effects of PM on susceptible subpopulations such as the elderly. The MESA Air Pollution Study is examining how air pollution may affect the development of atherosclerosis and heart disease in a multi-ethnic population. In addition, a group of STAR grantees is examining the exposures and health of tribal populations.

Oxidative Stress Responses to PM Exposure in Elderly Individuals With Coronary Heart Disease

Ralph J. Delfino, Susan Neuhausen, Nosratola D. Vaziri, Norbert Staimer, and Victor Gastanaga, University of California at Irvine
STAR Grant R832413C004 (Southern California Particle Center Project 4)

The overall goal of this study (Project 4 of the Southern California Particle Center) is to advance knowledge of the importance of particle size and composition to the induction of oxidative stress responses in a high-risk population of elderly people with coronary heart disease (CHD). The investigators hypothesize that biomarkers of oxidative stress responses will be associated with indoor and outdoor home PM mass and total particle number concentration, which will support the view that PM leads to systemic inflammatory responses. The investigators also hypothesize that biomarkers will be more strongly associated with predicted indoor exposure to PM of outdoor origin (from source tracer analyses). They are evaluating the effects of exposure to specific metals, elemental and organic carbon, and specific organic components used as source tracers. A further hypothesis is that biomarker associations with ultrafine and fine PM will be better explained by chemical assays that measure reactive oxygen species (ROS) and electrophilic activity.

Intensive exposure assessments include indoor and outdoor home PM mass, number, concentration and particle composition. Accumulation and ultrafine mode PM is being extracted to measure concentrations of transition metals and tracer compounds for use in apportioning PM exposures to specific sources including vehicular emissions, photochemical activity, cooking, and wood smoke. Circulating biomarkers indicating systemic oxidative stress responses are being measured. Changes in these biomarkers are expected to be associated with cardiovascular outcomes and with the inflammatory biomarkers measured in the parent study. The panel study is being coupled with Project 2 through the study of PM-induced oxidative modification of LDL and HDL, leading to altered pro-inflammatory and anti-inflammatory properties, respectively. The study will examine the possible association between biomarkers of oxidative stress and exposure to a variety of PM measures including elemental and organic carbon, and specific metals and organic components used as source tracers. Statistical analysis of biomarker associations with ROS and electrophilic activity provides a direct linkage to Project 3. In addition, genetic susceptibility to oxidative damage will be explored, by genotyping each study subject for polymorphisms in genes likely to be involved in oxidative stress responses.

The investigators expect to clarify findings in the epidemiologic literature of associations between ambient PM and cardiovascular mortality and hospital admissions. This will advance knowledge of the acute effects of ultrafine and fine particles on biomarkers of oxidative stress responses relevant to acute and chronic cardiovascular outcomes. Results will inform policymakers on the sources, particle components, size fractions, and concentrations that affect key intermediate endpoints in the progression of atherosclerosis and in acute changes in cardiovascular function and thrombosis. This study is expected to advance understanding of the adverse effects of particulate air pollutants on the cardiovascular health of high-risk individuals living in ethnically diverse neighborhoods with high exposures to airborne pollutants.

Southern California Particle Center Website: http://www.scpcs.ucla.edu/projects.html

Responses to Fresh Aerosol in Susceptible Subjects
Howard Kipen, Tina Fan, Paul Lioy, and colleagues
University of Medicine and Dentistry of New Jersey/Robert Wood Johnson Medical School
STAR Grant R832144

Increased occurrence of unstable angina and myocardial infarction, particularly in individuals vulnerable from preexisting atherosclerotic cardiovascular disease (ASCVD), follow even hourly increases in particulate air pollution. The mechanisms underlying these acute cardiovascular effects are unknown. Thrombosis, closely related to endothelial cell dysfunction and platelet activation, is now widely recognized to play an important role in acute exacerbations of cardiovascular disease. In experimental studies, endothelial changes are observed within minutes following a 2-hour exposure of humans to inhaled particulate air pollution. Similarly, platelet activation and thrombosis are observed in rodents within 30 minutes of intratracheal instillation of various ultrafine particles. In these models, both the endothelial and platelet responses appear to be independent of lung inflammation, suggesting an immediate and direct effect of ultrafine particles on these cells. The overall objective of this study is to explore mechanisms mediating acute particle-induced prothrombotic effects. Researchers hypothesize that the acute increase in risk of cardiac events following inhalation of ultrafine and fine particles is mediated by a rapid and direct passage of the particles from the lung into the blood, leading immediately to platelet activation and endothelial dysfunction, measured by a decrease in brachial artery reactivity. Moreover, individuals with genetically increased risk for ASCVD and endothelial dysfunction due to a single nucleotide polymorphism (SNP) in endothelial Nitric Oxide Synthase (eNOS) are likely to be more sensitive to these effects of ultrafine and fine particles on the endothelium.

To test this hypothesis, researchers are using pollutant models of freshly generated diesel exhaust (DE) or a secondary organic aerosol (SOA), both of which consist predominately of particles less than 1 micron in diameter but have a chemically different composition. The effects of the two fine and ultra-fine aerosols, using a clean air control, are being compared in 25 young healthy normal individuals and 25 healthy individuals who are selected for the SNP.

The specific objectives of this project are to: (1) determine if exposure of healthy, young, nonsmoking volunteers for 2 hours to freshly generated aerosols will lead to abnormalities in endothelial and platelet function that are independent of pulmonary inflammation; and (2) determine if individuals with genetically increased risk for atherosclerotic cardiovascular disease and endothelial dysfunction exhibit enhanced sensitivity to diesel exhaust or secondary organic aerosol.

MESA Air Study: Prospective Study of Atherosclerosis, Cardiovascular Disease, and Long-Term Exposure to Ambient Particulate Matter and Other Air Pollutants in a Multi-Ethnic Cohort

Joel Kaufman and colleagues, University of Washington and nine other universities across the United States

STAR Grant R831697

The Multi-Ethnic Study of Atherosclerosis (MESA) Air Pollution Study is an unprecedented investigation of the impact of air pollution on hardening of the arteries and the development of heart disease and mortality. This EPA-funded study is built on the framework of the NHLBI (National Heart, Lung, and Blood Institute)-supported MESA, a 10-year prospective study of the characteristics of subclinical cardiovascular disease (disease detected noninvasively before it has produced clinical signs and symptoms) and the risk factors that predict progression to clinically overt cardiovascular disease or progression of the subclinical disease. The EPA-funded study is integrated with another MESA ancillary study called MESA Family, which is a family study of genetic contribution to cardiovascular disease and progression of measures of atherosclerosis. More than 7,000 subjects ages 50 to 89 (more than 6,000 enrolled in the NHLBI-funded MESA Study, 300 recruited specifically for this study, and up to 1,800 being recruited for the MESA Family Study) are being followed over 10 years for the occurrence of cardiovascular disease events. The diverse, population-based sample consists of asymptomatic men and women. Approximately 40 percent of the recruited participants for the MESA Study are white, 30 percent are African American, 20 percent are Hispanic, and 10 percent are Asian, predominantly of Chinese descent.

The EPA-funded project, which began in July 2004, has two general objectives: (1) to prospectively examine the relation between an individual-level assessment of long-term ambient air pollution exposures (including particulate matter <2.5 micrometers in aerodynamic diameter [$PM_{2.5}$] and gaseous co-pollutants) and progression of subclinical cardiovascular disease in a multi-city, multi-ethnic cohort, by repeated assessment of coronary artery calcification, intima media thickness (IMT) of the common carotid artery, and plasma markers of inflammation, oxidative stress, and endothelial activation; and (2) to examine the relation between an individual-level assessment of long-term ambient air pollution exposures and the incidence of cardiovascular disease, including myocardial infarction and cardiovascular death.

Long-term individual-level exposure to ambient air pollutants for each subject are being assessed using community-scale monitoring, outdoor spatial variation, subject proximity to pollution sources, pollutants infiltration efficiency, and personal time-activity information, with detailed monitoring in a subset of subjects. Approximately 3,600 subjects residing in nine locales will twice undergo computed tomography scanning to assess the presence and extent of coronary artery calcification (CAC), and ultrasound of the carotid artery to determine IMT; CAC and IMT are validated subclinical measures of components of atherosclerosis. Plasma markers of inflammation, oxidative damage, and endothelial function in 720 subjects are being obtained. The study will assess if ambient air pollution (e.g., long-term exposure to $PM_{2.5}$) is associated with changes over time in subclinical measures of atherosclerosis and plasma markers of inflammation, oxidative damage, and endothelial activation in a longitudinal data model, adjusting for age, race/ethnicity, socioeconomic status, and specific cardiovascular risk factors (such as diabetes, hypertension, smoking, and diet). Similarly, the study will assess if the

incidence of cardiovascular events is associated with long-term exposure to ambient air pollution, using a proportional hazards model.

This study is providing new and critically important information to understand the relation between ambient air pollution and cardiovascular morbidity and mortality.

MESA Study Web Sites:
http://www.mesa-nhlbi.org
http://depts.washington.edu/mesaair/

There are already many publications from MESA Study investigators. Several abstracts from those papers are included here.

An early publication from the MESA Study documents that acculturation and socioeconomic factors are associated with differences in the prevalence and amount of coronary calcification within racial and ethnic groups, including whites, Asians, African Americans, and Hispanics. Not being born in the United States was associated with a lower prevalence of calcification in blacks (relative prevalence [RP], 0.75; 95% confidence limit [CL], 0.61-0.94) and Hispanics (RP, 0.89; 95% CL, 0.81-0.98) after adjustment for age, sex, income, and education. Years in the United States was positively associated with prevalence of calcification in non-U.S.-born Chinese (adjusted RP per 10 years in United States, 1.06; 95% CL, 1.01-1.11) and non-U.S.-born blacks (RP, 1.59; 95% CL, 1.22-2.06). Low education was associated with a higher prevalence of calcification in whites (adjusted RP for no high school versus complete college, 1.17; 95% CL, 1.05-1.32) but with lower prevalence of calcification in Hispanics (RP, 0.91; 95% CL, 0.77-1.09). U.S. birth and time in the United States also were positively associated with the extent of calcification in persons with detectable calcium. These differences did not appear to be accounted for by smoking, body mass index, LDL and HDL cholesterol, hypertension, and diabetes. The authors suggest that the presence of this heterogeneity needs to be acknowledged in the quantification and investigation of race/ethnic differences.

Diez Roux, Detrano, et al. 2005. Acculturation and socioeconomic position as predictors of coronary calcification in a multiethnic sample. *Circulation* 2005;112(11):1557-65.

Another publication shows ethnic differences in the frequency of a gene which contributes to blood pressure regulation. The natriuretic peptide system includes atrial and brain natriuretic peptides, which are cleaved into smaller biologically active molecules by corin, a transmembrane serine protease expressed in cardiomyocytes. Sequencing of the human corin gene identified two nonsynonymous, nonconservative single nucleotide polymorphisms (Q568P and T555I) in near-complete linkage disequilibrium, thus describing a single minor I555 (P568) corin gene allele. This allele was present in the heterozygote state in 12 percent of African Americans but was extremely rare in whites (< 0.5% were homozygous for the minor allele). This allele is associated with higher blood pressure and an increased risk for prevalent hypertension.

Dries, Victor, et al. 2005. Corin gene minor allele defined by 2 missense mutations is common in blacks and associated with high blood pressure and hypertension. *Circulation* 2005;112(16):2403-10.

Exposure to airborne particulate matter (PM) has been linked to cardiovascular events. Whether this finding reflects an effect of particulate matter exposure on the triggering of events or development of atherosclerosis remains unknown. Using data from the MESA Study collected at baseline (2000-2002), researchers investigated associations of 20-year exposures to PM with measures of subclinical disease (coronary calcium, common carotid intimal-medial thickness, and ankle-brachial index) in 5,172 US adults without clinical cardiovascular disease. Intimal-medial thickness was weakly, positively associated with exposures to PM_{10} and $PM_{2.5}$ after controlling for age, sex, race/ethnicity, socioeconomic factors, diet, smoking, physical activity, blood lipids, diabetes, hypertension, and body mass index (1-4% increase per 21-microgram/m(3) increase in PM_{10} or a 12.5-microg/m(3) increase in $PM_{2.5}$). No consistent associations with other measures of atherosclerosis were observed. There was no evidence of effect modification by sociodemographic factors, lipid status, smoking, diabetes, body mass index, or site. Results are compatible with some effect of PM exposures on development of carotid atherosclerosis.

Diez Roux, Auchincloss et al., 2008. Long-term exposure to ambient particulate matter and prevalence of subclinical atherosclerosis in the multi-ethnic Study of atherosclerosis. *Am J. Epidemiol.* 2008;167(6):667-675.

Children's Vulnerability to Environmental Immunotoxicant (PCB) Exposure
Philippe Grandjean, Harvard School of Public Health
STAR Grant R830758

Developmental exposure to polychlorinated biphenyls (PCBs) has been implicated as a possible cause of deficient immune function in children. This study was designed to assess whether prenatal and postnatal exposure to PCBs has an effect on antibody response to childhood vaccinations. The objective of this project was to determine the immunotoxic risk in children exposed prenatally and postnatally to PCBs. Experimental animal studies with Aroclor 1254 used by EPA for calculating a Reference Dose (RfD) for PCBs suggest that immunotoxicity may be critical, but current exposures, especially those from breast-feeding, greatly exceed the RfD.

One possible reason that children vary in their response to vaccinations is that the developing immune system may be damaged in some babies by exposure both before birth and after birth through breast milk to immunotoxicants such as PCBs. These stable, man-made chemicals, which have been extensively used as insulators in electrical equipment and as fire retardants, accumulate and persist in the environment where they affect animal and human health. PCB-exposed babies often have a small thymus (the gland where immune system cells mature), make decreased amounts of antibodies, and have more childhood infections.

A birth cohort of 547 Faroese children was established in 1998-2000. Because of consumption of PCB-contaminated seafood such as whale blubber, some mothers have PCB concentrations that exceed U.S. averages up to 100-fold. In a small-scale pilot study, researchers showed PCB-related decreases in antibody response against primary protein antigens from the 12-month vaccination. This study was designed to focus on antibody responses of the children to scheduled vaccinations at age 5. Immune deficits were related to measures of prenatal and postnatal PCB exposure and to *in vitro* activation of the arylhydrocarbon receptor measured in maternal pregnancy serum by a novel approach. This new technique includes a newly discovered promoter sequence to assess different aspects of AhR activation.

Results of the data analyzed so far indicate that increased perinatal exposure to PCBs may adversely impact immune responses to childhood vaccinations. The clinical implications of insufficient antibody production emphasize the need for prevention of immunotoxicant exposures.

Heilmann C, Grandjean P et al., 2006. Reduced antibody responses to vaccinations in children exposed to polychlorinated biphenyls. PLoS Med. 2006 Aug;3(8):e311.

Project Web site: http://www.chef-project.dk

Molecular Epidemiology of Hypospadias
Jeanne Manson, Children's Hospital of Pennsylvania
STAR Grant R828599

Hypospadias, a birth defect of the urethra in males, is one of the most common congenital anomalies in the United States, occurring in approximately 1 in 125 live male births. It is characterized by an abnormally placed urinary opening and altered development of the penis. This project is designed to characterize the genetic and environmental risk factors for hypospadias in the general population. 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]) may be highly associated with the risk for hypospadias. Parental exposure to environmental agents with anti-androgenic activity during pregnancy may further increase the risk for a susceptible genotype, resulting in a gene-environment interaction. The association between gene variants and hypospadias has been evaluated to date only in consanguineous families or small case series. Results from these studies indicate that SRD5A2 gene activity is critical to the formation of the external male genital tract. Allelic variants in the AR and HSD1 7133 genes are expected to be rare but important in modifying effects due to allelic variants in the SRD5A2 gene and from environmental exposures. This is the first study in a large, outbred population to investigate the association between allelic variants in these genes and environmental exposures on the risk for hypospadias.

Concern has focused on whether exposure to endocrine disrupting chemicals (EDCs) with antiandrogenic activity could be a cause of an increase in hypospadias since the 1960s. A clear role for exposure to antiandrogenic environmental chemicals in hypospadias has yet to be established, although results from laboratory animal models indicate that a number of environmental chemicals could be implicated. Studies that simultaneously examine the roles of allelic variants in genes controlling androgen action and metabolism and environmental exposures are needed to elucidate the risk factors for these anomalies and the causes of the increased rate of hypospadias.

A common single nucleotide polymorphism (SNP) in exon 1 of the SRD5A2 gene (V89L) has been identified, and a significant association of the L allele with severity of hypospadias has been found. Efforts continue to assess the relationship between this SNP and the risks for hypospadias. Results are confounded by racial admixture, with African-Americans having a much higher frequency of the L allele than Caucasians.

These results add to the growing body of evidence that common genetic and environmental risk factors have a role in male reproductive tract disorders. Several environmental antiandrogens have been identified in rodent models that interfere with male sexual differentiation at environmentally relevant doses.

Manson and Carr, 2003. Molecular epidemiology of hypospadias: review of genetic and environmental risk factors. *Birth Defects Res Part A: Clin Mol Teratol* 2003;67(10): 825-36.

Tribal STAR Grants

Subsistence populations live disproportionately near Superfund and other sites containing environmental toxicants, and the lifestyles and cultural practices of Tribal populations can modify their risk. An **eco-cultural zone map and guidance manual** have been developed for exposure and quantitative risk assessment based on developing ecoregion-specific subsistence exposure scenarios that integrate ecological data with information on subsistence lifestyle and diet. The guidance manual describes how to modify the scenarios for site-specific and/or individual tribal use to evaluate and reduce tribal risks. One study is examining **the extent of polycyclic aromatic hydrocarbons (PAHs) in the diet of the Inupiat** in northern Alaska while another study has performed a **chemical analysis of whale, walrus, seal, and reindeer tissues in Northern and Western Alaska**, in partnership with Inupiat hunters and Saint Lawrence Island Yu'piks. A Pacific Northwest tribe, the Swinomish, is analyzing **bioaccumulative toxics in shellfish and sediments** in combination with dietary surveys to accurately assess baseline exposure and risk. A **mercury (Hg) risk intervention study** in the Great Lakes region is using geographic information systems mapping to inform Tribal decisions for subsistence fishing.

Lifestyles and Cultural Practices of Tribal Populations and Risks From Toxic Substances in the Environment
Barbara L. Harper, Anna K. Harding, Stuart G. Harris, Therese S. Waterhous
STAR Grant R831046

Risks to Northern Alaskan Inupiat: Assessing Potential Effects of Oil Contamination on Subsistence Lifestyles, Health, and Nutrition
Dana Wetzel, Taquilik Hepa, Todd O'Hara, John Reynolds, Carla Willetto
STAR Grant R831045

Environmental Contaminants in Foodstuffs of Siberian Yu'piks From St. Lawrence Island, Alaska
Pamela K. Miller, John Arnason, David O. Carpenter, Anthony DeCaprio, Lorraine Eckstein
STAR Grant R831043

Bioaccumulative Toxics in Native American Shellfish
Felix A. Basabe
STAR Grant R829467

Reducing Risks of the Anishinaabe From Methylmercury
Jeffery Foran, Kory Groetsch, Neil Kmiecik, Kirk Riley
STAR Grant R831047

These and other Tribal-related projects represent EPA's first efforts to systematically incorporate cultural practices of sequestration, consumption, and use of foodstuffs in risk assessment and risk management.

STAR Program Contacts

For information about these or other STAR grants, please check the NCER website at http://www.epa.gov/ncer/ or contact the Project Officers listed below.

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To find the Project Officer for a specific project, please consult the NCER Web pages at the URL below.
http://cfpub.epa.gov/ncer_abstracts/index.cfm/fuseaction/search.welcome

Note: When searching for a particular grant number, you should include the letter R preceding the number (for example, R829388).