

## Research Foci Areas

### **Research Focus 1: Environmental Exposure and Health Assessment**

Under the leadership of Dr. Jonathan Samet, Professor and Chair of the Department of Epidemiology, *Research Focus 1: Environmental Exposure and Health Assessment* brings together a core of faculty to address the issues of characterizing the occurrence and causes of morbidity and mortality in Baltimore City, the surrounding region and in other high-risk populations. The diseases investigated by research members of this core include primarily non-cancer respiratory endpoints with an emphasis on indoor and outdoor air pollution exposures and health effects. Since the tragic attacks of September 11, 2001, the expertise of this research group and its associated facility core laboratories continue to address health outcomes in workers at the World Trade Center and Pentagon disaster sites (2-5). Most recently, members of this group have been investigating the exposures related to Hurricane Katrina in both New Orleans and along the Gulf Coast.

#### *National Morbidity, Mortality and Air Pollution Study (NMMAPS)*

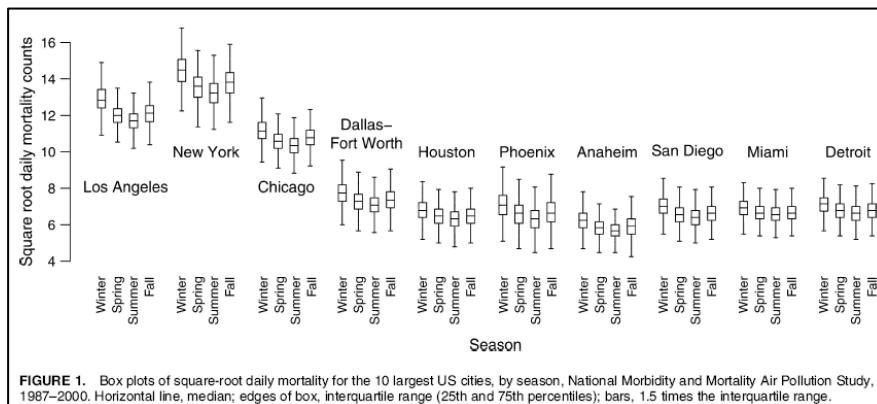
Since 1994, Drs. Samet and Zeger, and more recently Dr. Dominici, have been funded by the Health Effects Institute and more recently by the US EPA supported Johns Hopkins Particulate Matter (PM) Research Center (R832417) to conduct studies of particulate air pollution and mortality. The Johns Hopkins PM Research Center brings together a multidisciplinary research team: biostatisticians, epidemiologists, exposure assessors, lung biologists and respiratory toxicologists, pulmonary clinicians, and atmospheric scientists to address the most critical gap in current understanding of health and PM; the physical and chemical characteristics that determine risk to human health. The PM Center's conceptual foundation lies in mapping health risks of PM across the US, based on analysis of national databases on air pollution, mortality, and hospitalization, and then using the maps to guide detailed monitoring and collection of PM samples for physical, chemical, and biological characterization in assays relevant to pulmonary and cardiovascular outcomes. In addition to Drs. Samet, Zeger and Dominici other members of this research foci participating in the PM Research Center include Drs. Breyse, Geyh, Ondov and Garcia. A cornerstone of team effort is the development of a new, national model for characterizing the health effects of air pollution. In the National Morbidity, Mortality and Air Pollution Study (NMMAPS), national databases were assembled on daily deaths and hospitalizations, air pollution levels, temperature, and city characteristics. The NMMAPS approach, now being extended into a web-based format, offers a useful model, not only for air pollution, but also for other environmental pollutants with widespread but varying exposure. This new Center makes extensive use of our current *Facility Core A: Environmental Exposure and Health Assessment* and in this upcoming renewal period this group will be utilizing the *Integrated Health Sciences Facility Core*.

In the initial report led by Drs. Samet, Dominici and Zeger, the objective was to investigate whether short-term (daily and weekly) exposure to ambient ozone was associated with overall mortality in the United States. Using analytical methods and databases developed for NMMAPS, they estimated a national average relative rate of mortality associated with short-term exposure to ambient ozone for 95 large US urban communities from 1987-2000. They used distributed-lag models for estimating community-specific relative rates of mortality adjusted for time-varying confounders (PM, weather, seasonality, and long-term trends) and hierarchical models for combining relative rates across communities to estimate a national average relative rate, taking into account spatial heterogeneity. This study found that a 10-ppb increase in the previous week's ozone was associated with a 0.52% increase in daily mortality and a 0.64% increase in cardiovascular and respiratory mortality. Effect estimates for aggregate ozone during the previous week were larger than for models considering only a single day's exposure. These findings were robust to adjustment for PM, weather, seasonality, and long-term trends. These results indicate a statistically significant association between short-term changes in ozone and mortality on average for 95 large US urban communities, which include about 40% of the total US population. Thus, this widespread pollutant adversely affects public health (6).

Dr. Samet and Dominici extended this research by using a meta-analysis of time-series studies of ozone and mortality with comparison to the NMMAPS data set (7). They performed a meta-analysis of 144 effect estimates from 39 time-series studies, and estimated pooled effects by lags, age groups, cause-specific mortality, and concentration metrics. These results were compared with pooled estimates from NMMAPS and both meta-analysis and NMMAPS results provided strong evidence of a short-term association between ozone

and mortality, with larger effects for cardiovascular and respiratory mortality, the elderly and current-day ozone exposure. In both analyses, results were insensitive to adjustment for PM and model specifications. In the meta-analysis, a 10-ppb increase in daily ozone at single-day or 2-day average of lags 0, 1, or 2 days was associated with an 0.87% increase in total mortality, whereas the lag 0 NMMAPS estimate is 0.25%. Several findings indicate possible publication bias in the scientific literature: meta-analysis results were consistently larger than those from NMMAPS; meta-analysis pooled estimates at lags 0 or 1 were larger when only a single lag was reported than when estimates for multiple lags were reported; and heterogeneity of city-specific estimates in the meta-analysis were larger than with NMMAPS. Thus, this study continues to provide evidence of short-term associations between ozone and mortality as well as evidence of publication bias.

This same team have also examined the relation between seasonal changes in air pollution and mortality in cities (8). Time series models relating short-term changes in air pollution levels to daily mortality counts typically assume that the effects of air pollution on the log relative rate of mortality do not vary with time. However, these short-term effects might plausibly vary by season. Changes in the sources of air pollution and meteorology can result in changes in characteristics of the air pollution mixture across seasons. This group developed Bayesian semi-parametric hierarchical models for estimating time-varying effects of pollution on mortality in the database for NMMAPS. At the national level, a 10  $\mu\text{g}/\text{m}^3$  increase in PM less than 10  $\mu\text{m}$  in aerodynamic diameter at a 1-day lag was associated with 0.15%, 0.14%, 0.36%, and 0.14% increases in mortality for winter, spring, summer, and fall, respectively. An analysis by geographic region found a strong seasonal pattern in the Northeast (with a peak in summer) and little seasonal variation in the southern regions of the country. These results shown in the accompanying Figure 4 provide useful information for understanding particle toxicity and guiding future analyses of particle constituent data.



**Figure 4: Seasonal Variation in Mortality in Cities**

mortality. Several statistical models were used to estimate the exposure-response curve for tropospheric ozone and risk of mortality and to evaluate whether a "safe" threshold level exists. Methods included a linear approach and subset, threshold, and spline models. All results indicated that any threshold would exist at very low concentrations, far below current U.S. and international regulations and nearing background levels. For example, under a scenario in which the U.S. Environmental Protection Agency's 8-hr regulation is met every day in each community, there was still a 0.30% increase in mortality per 10-ppb increase in the average of the same and previous days' ozone levels. These findings indicate that even low levels of tropospheric ozone are associated with increased risk of premature mortality. It is also important to note that Dr. Bell is a recipient of a ONES award from NIEHS (R01 ES015028) was a post-doctoral fellow at Johns Hopkins.

Collectively the NMMAPS investigations have had and continue to have a major impact on environmental health policy and regulation. This is a measure of translational research supported by this NIEHS Center to the community and country that can have a profound impact on overall health status.

#### *Exposure Assessment of Air Quality and Health*

Research in the exposure assessment component of this group continues to expand covering a spectrum of activities related to the measurement and/or estimation of exposures to a wide variety of chemical, biological and physical agents. Ongoing collaborative efforts in environmental epidemiology includes development and validation of exposure assessment methods, development of appropriate exposure assessment tools for use in epidemiologic studies, and development of mechanistic descriptions of exposure processes. These studies

make extensive use of our current *Facility Core A: Environmental Exposure and Health Assessment* and in this expand into the *Integrated Health Sciences Facility Core* in the renewal period. Thus, a considerable portion of the ongoing exposure assessment research is conducted in conjunction with epidemiologic and clinical studies to identify and quantify exposure-outcome relationships. These studies have been conducted in a wide range of venues, including specific urban and community-based research. The following highlights collaborative investigations related to environmental tobacco smoke, indoor air pollutant and allergen exposures. The indoor air pollution and allergen studies are directly linked to the NIEHS supported Center for Childhood Asthma in the Urban Environment (P01 ES009606) directed by Drs. Breyse and Eggleston.

Drs. Breyse, Samet and a new faculty recruit, Dr. Navas-Acien (see *Career Development for Environmental Health Investigators* section), continue to collaborate on an extensive study of exposure to environmental tobacco smoke (ETS) in South America. This project is being carried out in collaboration with the Pan American Health Organization (PAHO). In 2001, PAHO launched the Smoke-Free Americas Initiative to build capacity to achieve smoke-free environments in Latin America and the Caribbean. In the initial phase, airborne nicotine samples were collected in public spaces and workplaces in seven countries in South America. These samples were analyzed by the current *Facility Core A: Environmental Exposure and Health Assessment*. Based upon the initial success of the project, the investigation has now been expanded to include eight additional countries. Findings from the first seven countries were reported in *JAMA* (10). To assess ETS concentrations in public places in the capital cities of Argentina, Brazil, Chile, Costa Rica, Paraguay, Peru, and Uruguay a total of 633 sampling devices were placed for 7 to 14 days in 1 hospital, 2 secondary schools, 1 city government building, 1 airport (2 in Argentina), and restaurants and bars in each country. Airborne nicotine was detected in most (94%) of the locations surveyed. By country, Argentina and Uruguay had the highest median concentrations in most environments (e.g. in hospitals: 1.33  $\mu\text{g}/\text{m}^3$  and 0.8  $\mu\text{g}/\text{m}^3$ , respectively). Overall, bars and restaurants had the highest median concentrations (3.65  $\mu\text{g}/\text{m}^3$  and 1.24  $\mu\text{g}/\text{m}^3$ , respectively). Nicotine concentrations were also found in a number of key, sentinel buildings, including 95% (155/163) of hospital samples (in the physicians' and nurses' stations the median was 0.27  $\mu\text{g}/\text{m}^3$ ), schools, government buildings, and/or airports in most countries. The finding of airborne nicotine in critical locations in Latin America provides a basis for enforcing smoke-free initiatives and for strengthening the protection of the public from unwanted exposure to secondhand smoke. This work is also part of the efforts of the Institute for Global Tobacco Control directed by Dr. Samet (see Figure 12 in the *Strategic Vision and Impact on Environmental Health* section).

Dr. Samet has also received funding from the Flight Attendants Medical Research Institute (FAMRI) that is being used to develop a global protocol for assessing exposures of women and children to ETS. The current *Facility Core A: Environmental Exposure and Health Assessment* is also supporting this FAMRI effort and a study of ETS in China. These data on exposure have direct impact on policy directed interventions in reducing ETS concentrations. This research team has also recently developed in house capability for assessing nicotine and cotinine biomarkers in hair. Hair nicotine measures will be incorporated into all of the future studies conducting ETS exposure assessments.

The multidisciplinary Center for Childhood Asthma in the Urban Environment, funded since 1998 by NIEHS and EPA (P01 ES009606), shares many common faculty with this NIEHS Center in Urban Environmental Health. Drs. Breyse, Buckley, Ford, Diette, Eggleston and Dominici are all joint collaborators. The Center for Childhood Asthma in the Urban Environment conducts translational research, epidemiologic and intervention trials of asthma in Baltimore City. Drs. Breyse, Buckley, Diette and Eggleston collaborated on a major study of indoor air pollutant concentrations and allergen levels collected from the homes of 100 Baltimore city asthmatic children participating in an asthma intervention trial (11). PM, NO<sub>2</sub>, and ozone samples were collected over three days in the child's sleeping room. Time-resolved PM was also assessed using a portable direct-reading nephelometer. Dust allergen samples were collected from the child's bedroom, the family room, and the kitchen. The mean PM<sub>10</sub> concentration, 56.5 $\pm$ 40.7  $\mu\text{g}/\text{m}^3$ , was 25% higher than the PM<sub>2.5</sub> concentration (N=90), 45.1 $\pm$ 37.5  $\mu\text{g}/\text{m}^3$ . PM concentrations measured using a nephelometer were consistent and highly correlated with gravimetric estimates. Smoking households' average PM<sub>2.5</sub> and PM<sub>10</sub> concentrations were 33-54  $\mu\text{g}/\text{m}^3$  greater than those of non-smoking houses, with each cigarette smoked adding 1.0  $\mu\text{g}/\text{m}^3$  to indoor PM<sub>2.5</sub> and PM<sub>10</sub> concentrations. Large percentages of NO<sub>2</sub> and ozone samples, 25% and 75%,

respectively, were below the limit of detection. The mean NO<sub>2</sub> indoor concentration was 31.6±40.2 ppb, while the mean indoor ozone concentration in the ozone season was 3.3±7.7 ppb. The levels of allergens were similar to those found in other inner city environments. These results indicate that asthmatic children in Baltimore are exposed to elevated allergens and indoor air pollutants and the understanding of this combined insult may help to explain the differential asthma burden between inner-city and non-inner-city children.

Drs. Buckley, Breyse and Eggleston collaborated on an airborne mouse allergen (Mus m1) and asthma study (12). Airborne mouse allergen has not previously been measured in inner-city homes, and its relationship to settled dust mouse allergen levels was unknown. One hundred inner-city school-age children with asthma in Baltimore underwent skin testing for a panel of aeroallergens, and their homes were inspected by a trained technician from this NIEHS Center. Air and settled dust were sampled in the child's bedroom. Mus m1, particulate matter smaller than 10 microns (PM<sub>10</sub>), and particulate matter smaller than 2.5 microns were quantified in air samples, and Mus m1 was quantified in settled dust samples. Mus m1 was detected in settled dust samples from 100% of bedrooms. Airborne mouse allergen was detected in 48 of 57 (84%) bedrooms, and the median airborne mouse allergen concentration was 0.03 ng/m<sup>3</sup>. The median PM<sub>10</sub> concentration was 48 µg/m<sup>3</sup>. Airborne and settled dust mouse allergen levels were moderately correlated ( $r = .52$ ;  $P < .0001$ ), and airborne Mus m1 and PM<sub>10</sub> levels were weakly correlated ( $r = .29$ ;  $P = .03$ ). Having cracks or holes in doors or walls, evidence of food remains in the kitchen, and mouse infestation were all independently associated with having detectable airborne mouse allergen. Airborne mouse allergen concentrations in many inner-city homes may be similar to those found in animal facilities, where levels are sufficiently high to elicit symptoms in sensitized individuals. Exposed food remains, cracks and holes in doors or walls, and evidence of mouse infestation appear to be risk factors for having detectable airborne Mus m1. This work has immediate translational opportunities to the community and our COEC is working with this team to affect this effort.

One of the major collaborations to emerge from these efforts in the inner-city have been the studies of Dr. Jean Ford, who is the principal investigator of a large study of asthma severity referred to as the Baltimore Asthma Severity Study (BASS). The aims of this study are to describe the allelic variants of the IL-4, IL-13 and β<sub>2</sub>-adrenoreceptor genes among African American asthmatic individuals (cases) and controls; and to characterize T cell response phenotypes among African Americans who have asthma. Five hundred and fifty-seven (557) subjects were screened as a part of this study and all of the screening was done in *Facility Core A: Environmental Exposure and Health Assessment*. Three hundred and ninety (390) of the 557 subjects were eligible, and have agreed to participate. Of these 390, two hundred and seventy-eight (278) completed the BASS informed consent process: 101 cases (36%) and 177 controls (64%). Two hundred and sixty-three (263) of the 278 participants also have completed testing visits; this represents 92 cases (35%) and 171 controls (65%). Data collected from the study participants include a standardized questionnaire on factors related to asthma severity, pulmonary function testing (including methacholine challenge testing in a subset of the participants), allergy skin testing and a blood draw for genotyping and ascertainment of immunophenotypes (Th1/Th2 cytokine profiles), including the proliferative responses of peripheral blood mononuclear cells in response to aeroallergens such as cockroach and house dust mite.

#### *Biomarkers in Exposure Assessment*

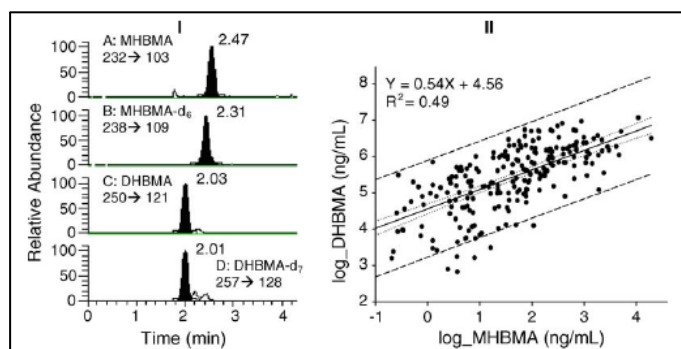
Drs. Buckley and Strickland have been extensively involved in the application of urinary biomarkers of exposure to polycyclic aromatic hydrocarbons (PAHs) in environmental exposure assessment. In a study in Taiwan (13), this group evaluated urinary 1-hydroxypyrene-glucuronide (1-OHP-gluc) as a potential biomarker of exposure to various motor vehicle traffic exhausts. This is a significant problem for all urban environments. The subjects were 47 female highway toll-station workers and 27 female office workers in training for toll-station employment in Taipei, Taiwan. The mean concentration of urinary 1-OHP-gluc was 0.117 µmol/mol creatinine in the exposed group and 0.073 µmol/mol creatinine in the reference group (difference in mean concentrations: 0.044 µmol/mol creatinine [95% confidence interval [CI]: 0.015, 0.072]). In the lanes where tolls were collected from passenger cars, there was a statistically significant relationship between cumulative traffic and 1-OHP-gluc concentration (i.e., average increase of 0.015 µmol/mol creatinine [95% CI: 0.003, 0.027] per 1,000 vehicles). The average increase for truck/bus lanes was similar to that identified for the car lanes (i.e., average increase of 0.011 µmol/mol creatinine [95% CI: -0.024, 0.045] per 1,000 vehicles). The group determined that exposure to various traffic exhausts increased the urinary concentration of 1-OHP-gluc in a

dose-response pattern, which suggests that this chemical may be a useful biomarker for exposure to vehicle exhausts. This study builds upon the findings of the Baltimore Traffic Study (14) that has been supported by *Facility Core A: Environmental Exposure and Health Assessment* in this Center.

An assessment of the relations between exposure to traffic exhausts and indicators of oxidative DNA damage was also conducted using the same group as described above. In this study, urinary 8-hydroxydeoxyguanosine (8-OHdG) was examined as a biomarker of oxidative DNA damage and plasma nitric oxide (NO) was measured as an indicator of oxidative stress related to traffic exhaust exposure. The mean concentration of urinary 8-OHdG was substantially higher among the exposed non-smokers (13.6  $\mu\text{g/g}$  creatinine) compared with the reference group of non-smokers (7.3  $\mu\text{g/g}$  creatinine). The mean concentration of NO among the exposed (48.0  $\mu\text{mol/l}$ ) was also higher compared with the reference group of non-smokers (37.6  $\mu\text{mol/l}$ ; difference 10.4, 95% CI -0.4 to 21.2). In linear regression modeling adjusting for confounding, a change in log (8-OHdG) was statistically significant and these results indicate that exposure to traffic exhausts increases oxidative DNA damage. Thus, urinary 8-OHdG is a promising biomarker of traffic exhaust induced oxidative stress that will need to be validated in future investigations (15).

On-road mobile sources contribute substantially to ambient air concentrations in urban environments of the carcinogens 1,3-butadiene, benzene, and polycyclic aromatic hydrocarbons (PAHs). Dr. Buckley's group measured benzene and 1,3-butadiene at the Baltimore Harbor Tunnel tollbooth over 3-hr intervals on seven weekdays ( $n = 56$ ). Particle-bound PAH was measured on a subset of three days during the week. The 3-hr outdoor 1,3-butadiene levels varied according to time of day and traffic volume. The minimum exposure occurred at night (12 a.m.-3 a.m.) with a mean of 2  $\mu\text{g}/\text{m}^3$  (SD = 1.3,  $n = 7$ ), while the maximum occurred during the morning rush hour (6 a.m.-9 a.m.) with a mean of 11.9  $\mu\text{g}/\text{m}^3$  (SD = 4.6,  $n = 7$ ). The corresponding traffic counts were 1413 (SD = 144) and 16,893 (SD = 692), respectively. During the same time intervals, mean benzene concentration varied from 3  $\mu\text{g}/\text{m}^3$  (SD = 3.1,  $n = 7$ ) to 22.3  $\mu\text{g}/\text{m}^3$  (SD = 7.6,  $n = 7$ ). Median PAH concentrations ranged from 9 to 199  $\text{ng}/\text{m}^3$ . Using multivariate regression, a statistically significant association ( $p < 0.001$ ) between traffic and curbside concentration of these compounds was observed. Much of the pollutant variability (1,3-butadiene 62%, benzene 77%, and PAH 85%) was explained by traffic volume, class of vehicle, and meteorology. These results suggest that vehicles with more than 2-axes emit 60, 32, and 9 times more PAH, 1,3-butadiene, and benzene, respectively, than do 2-axle vehicles. This study provides a model for estimating curbside pollution levels associated with traffic that are relevant to exposures in the urban environment (16).

Although, 1,3-butadiene is a known human carcinogen emitted from mobile sources, little is known about traffic-related human exposure to this toxicant. A pilot collaborative study by Drs. Buckley, Halden and



**Figure 5: Butadiene mercapturic acid biomarkers by mass spectrometry**

1,2-dihydroxybutyl mercapturic acid (DHBMA). The comparison of these two biomarkers is shown in Figure 5. The mass spectrometry was conducted using *Facility Core A: Environmental Exposure and Health Assessment* and the Thermo-Finnigan triple-quad MS/MS instrument. Exposure differed between groups ( $p < 0.05$ ) with median values of 2.38, 1.62 and 0.88  $\mu\text{g}/\text{m}^3$  for toll collectors, the urban-weekday group and the suburban-weekend group, respectively. A refined ID-LC-MS/MS method enabled detection of MHBMA,

Groopman, was designed to characterize traffic-related environmental exposure to 1,3-butadiene and evaluate its urinary mercapturic acids as biomarkers of exposure in these settings. Personal air samples and multiple urine samples were collected on two separate occasions from three groups of individuals that differed by spatial proximity as well as intensity of traffic: (i) toll collectors, (ii) urban-weekday and (iii) suburban-weekend group. Air samples were analyzed using thermal desorption followed by gas chromatography/mass spectrometry and urine samples were analyzed using isotope dilution liquid chromatography tandem mass spectrometry (ID-LC-MS/MS) for two mercapturic acids of 1,3-butadiene: monohydroxy-3-butenyl mercapturic acid (MHBMA) and

previously detected only in occupational settings, with high frequency (see Figure 5). MHBMA and DHBMA were detected in 95 and 100% of urine samples at levels (mean $\pm$ S.D.) of 9.7 $\pm$ 9.5, 6.0 $\pm$ 4.3 and 6.8 $\pm$ 2.6 ng/mL for MHBMA and 378 $\pm$ 196, 258 $\pm$ 133 and 306 $\pm$ 242 ng/mL for DHBMA for the three different groups, respectively. Mean biomarker levels were higher among the toll collectors compared to the other two groups; however, the differences were not statistically significant ( $p>0.05$ ). This study was the first to evaluate 1,3-butadiene biomarkers for subtle differences in environmental exposures (17).

Dr. Halden's group (see *Career Development for Environmental Health Investigators and Pilot Projects Program* section) has developed analytical techniques to assess the biocide triclosan and its biotransformation products in environmental samples and breast milk using liquid chromatography/mass spectrometry and gas chromatography/mass spectrometry (*Facility Core A: Environmental Exposure and Health Assessment*). The topical antiseptic agent triclocarban (TCC) is a common additive in many antimicrobial household consumables, including soaps and other personal care products. Long-term usage of the mass-produced compound and a lack of understanding of its fate during sewage treatment motivated the present mass balance analysis conducted at a typical U.S. activated sludge wastewater treatment plant featuring a design capacity of 680 million liters per day. Using automated samplers and grab sampling, the mass of TCC contained in influent, effluent, and digested sludge was monitored by mass spectrometry. The average mass of TCC (mean  $\pm$  standard deviation) entering and exiting the plant in influent (6.1  $\pm$  2.0  $\mu$ g/L) and effluent (0.17  $\pm$  0.03  $\mu$ g/L) was 3737  $\pm$  694 and 127  $\pm$  6 g/d, respectively, indicating an aqueous-phase removal efficiency of 97  $\pm$  1%. Tertiary treatment by chlorination and sand filtration provided no detectable benefit to the overall removal. Due to strong sorption of TCC to wastewater particulate matter (78  $\pm$  11% sorbed), the majority of the TCC mass was sequestered into sludge in the primary and secondary clarifiers of the plant. Anaerobic digestion for 19 days did not promote TCC transformation, resulting in an accumulation of the antiseptic compound in dewatered, digested municipal sludge to levels of 51  $\pm$  15 mg/kg dry weight (2815  $\pm$  917 g/d). In addition to the biocide mass passing through the plant contained in the effluent (3  $\pm$  1%), 76  $\pm$  30% of the TCC input entering the plant underwent no net transformation and instead partitioned into and accumulated in municipal sludge. Based on the rate of beneficial reuse of sludge produced by this facility (95%), which exceeds the national average (63%), study results suggest that approximately three-quarters of the mass of TCC disposed of by consumers in the sewershed of the plant ultimately is released into the environment by application of municipal sludge (biosolids) on land used in part for agriculture (18). This is a common practice in Maryland and suggests the need to assess the possible health consequences due to resulting ground water contamination.

#### *Metals Exposure and Health*

Lead and cadmium exposure may promote atherosclerosis, although the cardiovascular effects of chronic low-dose exposure are largely unknown. Dr. Navas-Acien led a study to evaluate the association between blood levels of lead and cadmium and peripheral arterial disease. Data were analyzed from 2125 participants who were over 40 years of age in the 1999 to 2000 National Health and Nutrition Examination Survey (NHANES). Peripheral arterial disease was defined as an ankle brachial index less than 0.9 in at least one leg. Lead and cadmium levels were measured by atomic absorption spectrometry (done by CDC). After adjustment for demographic and cardiovascular risk factors, the odds ratios (ORs) of peripheral arterial disease comparing quartiles two to four of lead with the lowest quartile were 1.63 (95% CI, 0.51 to 5.15), 1.92 (95% CI, 0.62 to 9.47), and 2.88 (95% CI, 0.87 to 9.47), respectively ( $P$  for trend=0.02). The corresponding ORs for cadmium were 1.07 (95% CI, 0.44 to 2.60), 1.30 (95% CI, 0.69 to 2.44), and 2.82 (95% CI, 1.36 to 5.85), respectively ( $P$  for trend=0.01). Thus, blood lead and cadmium, at levels well below current safety standards, were associated with an increased prevalence of peripheral arterial disease in the general US population (19). These are also very common metals in the urban environment found in Baltimore City because of industrial uses over the past century.

A follow-up to the previous investigation was conducted to evaluate the association between urinary levels of cadmium, lead, barium, cobalt, cesium, molybdenum, antimony, thallium, and tungsten with peripheral arterial disease (PAD) in a cross-sectional analysis of 790 participants over 40 years of age in NHANES 1999-2000. Metals were measured in casual (spot) urine specimens by inductively coupled plasma-mass spectrometry (ICP-MS done at CDC, but these data encouraged the investment in our own instrumentation as

described in the *Strategic Vision and Impact on Environmental Health* section). After multivariable adjustment, subjects with PAD had 36% higher levels of cadmium in urine and 49% higher levels of tungsten compared with non-cases. The adjusted odds ratio for PAD comparing the 75th to the 25th percentile of the cadmium distribution was 3.05 [95% confidence interval (CI), 0.97 to 9.58]; that for tungsten was 2.25 (95% CI, 0.97 to 5.24). PAD risk increased sharply at low levels of antimony and remained elevated beyond 0.1 µg/L. PAD was not associated with other metals. In conclusion, urinary cadmium, tungsten, and possibly antimony were associated with PAD in a representative sample of the U.S. population. For cadmium, these results strengthen previous findings using blood cadmium as a biomarker, and they support its role in atherosclerosis. For tungsten and antimony, these results need to be interpreted cautiously in the context of an exploratory analysis but deserve further study. Other metals in urine were not associated with PAD at the levels found in the general population (20). In the future, these findings will be explored in the Baltimore City population where the industrial legacy of these heavy metals is causing significant concerns for the urban redevelopment plans.

The health effects of heavy metal exposures also extends to renal disease and an occupational study was directed by Drs. Weaver and Schwartz to compare associations of lead biomarkers with renal function in current and former lead workers. In total, 803 lead workers and 135 controls in South Korea were assessed. Clinical renal function was measured by blood urea nitrogen (BUN), serum creatinine, and calculated creatinine clearance. Urinary N-acetyl-beta-D-glucosaminidase (NAG) and retinol-binding protein were also measured. Mean tibia lead, blood lead, and chelatable lead levels in lead workers were 37.2 µg/g bone mineral, 32.0 µg/dl, and 767.8 µg/g creatinine, respectively. Higher lead measures were associated with worse renal function in 16 of 42 mathematical models explored. When influential outliers were removed, higher lead measures remained associated with worse renal function in nine models. An additional five associations were in the opposite direction. Effect modification by age was also observed. In 3/16 models, associations between higher lead measures and worse clinical renal function in participants in the oldest age tertile was significantly different from associations in those in the youngest age tertile. Overall mean urinary cadmium (CdU) was 1.1 µg/g creatinine (n = 191) and higher CdU levels were associated with higher NAG. These data suggest that lead has an adverse effect on renal function in the moderate dose range, particularly in older workers. Associations between higher lead measures and lower BUN and serum creatinine and higher creatinine clearances may represent lead induced hyperfiltration. Environmental cadmium may also have an adverse renal impact, at least on NAG (21).

The potential gene-environment interaction in health effects from lead exposure was assessed in 798 lead workers in Korea. Lead exposure and polymorphisms in the genes encoding delta-aminolevulinic acid dehydratase (ALAD), endothelial nitric oxide synthase (eNOS), and the vitamin D receptor (VDR) were associated with or modifiers of lead exposure and dose measures with renal outcomes. Lead exposure was assessed with job duration, blood lead, dimercaptosuccinic acid (DMSA)-chelatable lead, and tibia lead (using the XRF capabilities in the Center). Renal function was assessed with blood urea nitrogen (BUN), serum creatinine, measured creatinine clearance, calculated creatinine clearance and urinary N-acetyl-beta-D-glucosaminidase (NAG), and retinol-binding protein. Mean (+/- SD) tibia lead, blood lead, and DMSA-chelatable lead levels were 37.2 +/- 40.4 µg /g bone mineral, 32.0 +/- 15.0 µg/dL, and 767.8 +/- 862.1 µg/g creatinine, respectively. After adjustment, participants with the ALAD(2) allele had lower mean serum creatinine and higher calculated creatinine clearance. This group observed an effect modification by ALAD on associations between blood lead and/or DMSA-chelatable lead and three renal outcomes. Among those with the ALAD(1-2) genotype, higher lead measures were associated with lower BUN and serum creatinine and higher calculated creatinine clearance. Participants with the eNOS variant allele were found to have higher measured creatinine clearance and BUN. In participants with the Asp allele, longer duration working with lead was associated with higher serum creatinine and lower calculated creatinine clearance and NAG; all were significantly different from relations in those with the Glu/Glu genotype except NAG (p = 0.08). No significant difference was seen in renal outcomes by VDR genotype, nor was consistent effect modification observed. The ALAD findings could be explained by lead-induced hyperfiltration (22).

The Korean Lead workers study is important for the assessment of elderly worker exposure and health risk in Baltimore since in the past these lead related jobs were common in the Baltimore industrial base. No previous longitudinal studies have compared and contrasted associations of blood lead and tibia lead with

declines in cognitive function over the course of time in a large sample of subjects with current and past occupational exposure to inorganic lead. From 1997 through 2001, the group led by Dr. Brian Schwartz conducted a longitudinal study of 803 current and former lead workers in South Korea to evaluate effects on the central and peripheral nervous systems. Three study visits occurred during a mean follow-up duration of 2.20 years. Neurobehavioral test scores, peripheral nervous system function, and blood lead were measured at each of the 3 study visits, whereas tibia lead was measured by XRF at the first and second visits. The study was limited the final analysis to the 576 lead workers who completed testing at all 3 visits. Regression analyses using generalized estimating equations showed consistent associations of blood lead with test scores at baseline and of tibia lead with declines in test scores over the next year; mainly in executive abilities, manual dexterity, and peripheral vibration threshold. The results support the inference that occupational lead exposure can cause declines in cognitive function over the course of time. Lead likely has an acute effect on neurobehavioral test scores as a function of recent dose and a longer-term (possibly progressive) effect on cognitive decline as a function of cumulative dose (23).

The Baltimore Memory Study, directed by Dr. Brian Schwartz, is a cohort study of the multilevel determinants of cognitive decline in 50-70-year-old randomly selected residents of specific city neighborhoods. This study is funded in part by the NIEHS (R01AG010785). The goal of this study was to describe differences in neurobehavioral test scores by race/ethnicity, before and after control for a four-dimensional measure of socioeconomic status (SES) and health-related behaviors and health conditions, in a cross-sectional analysis of first visit data. Random samples of households in the study area were selected until enrollment goals were reached. Among the 2,351 persons on whom eligibility was determined, 60.8% were scheduled for an enrollment visit; of these, 1,140 (81.3%) were enrolled and tested. These study participants were 34.3% male and 65.7% female and were from 65 Baltimore, Maryland, neighborhoods. After adjustment for age, sex, and testing technician, there were large and statistically significant differences in neurobehavioral test scores by race/ethnicity, with African-American scores lower than those for whites, for both men and women. After adjustment for individual SES (educational status, household income, household assets, and occupational status), the average difference declined by 25.8%. After additional adjustment for SES, health-related behaviors and health conditions, and blood lead, the average difference declined another 10%, but large differences persisted; African Americans had test scores that averaged 0.43 standard deviation lower than those for whites across all neurobehavioral tests. These differences were present in all cognitive domains, including tests that would not be characterized as susceptible to differential item functioning by race/ethnicity, suggesting that the results are not due to race/ethnicity-associated measurement error (24). This work illustrates a heretofore unrecognized set of long-term effects from lead exposure that could have a significant impact on health status among the aging population.

Due to its cardiovascular benefits, fish consumption is widely encouraged among older Americans. However, this fast-growing population is at increased risk of cognitive impairment and may be particularly sensitive to methylmercury, a neurotoxicant found in fish. A cross-sectional analysis to determine the effect of mercury levels on neurobehavior in 474 randomly selected participants in the Baltimore Memory Study, a longitudinal study of cognitive decline involving 1140 Baltimore residents aged 50 to 70 years, was performed. Dr. Schwartz's group measured total mercury in whole blood samples and used multiple linear regression to examine its associations with neurobehavioral test scores. First-visit data were obtained in 2001-2002 and the data from twenty scores from 12 neurobehavioral tests were analyzed. The median blood mercury level was 2.1  $\mu\text{g/L}$  (range, 0-16  $\mu\text{g/L}$ ). After adjustment for covariates, increasing blood mercury was associated with worse performance on Rey complex figure delayed recall, a test of visual memory (beta, -0.224; 95% confidence interval, -0.402 to -0.047). However, increasing blood mercury levels were associated with better performance on finger tapping, a test of manual dexterity (beta for dominant hand, 0.351; 95% confidence interval, 0.017-0.686). Overall, the data do not provide strong evidence that blood mercury levels are associated with worse neurobehavioral performance in this population of older urban adults and these findings are continuing to be follow-up as part of the overall efforts examining heavy metal exposures and neurologic effects among the elderly (25).