parasympathetic tone in regulating HR. The significance of the current results suggest that PM-induced HR regulatory changes may ultimately depend on the degree of physiological aging (48).

Research Foci 3: Public Health Interventions and Prevention

The *Public Health Interventions and Prevention Research Focus* group, led by Dr. Paul Strickland, fosters the development and validation of biomarkers for exposure, effect, and susceptibility in experimental and human investigations. These tools are applied in studies of disease etiology and evaluation of preventive interventions in at-risk populations. Investigating the etiology of many diseases of current interest has proved challenging for conventional epidemiologic approaches. The temporal sequence of causation and disease can be particularly difficult to investigate. In some diseases, such as cancer, multiple etiological agents act at different points during a multi-stage progression from the earliest stages to diagnosis. Further, intrinsic susceptibility factors may greatly affect individual risk and the time course of disease development. Extensive research on biomarkers linking exposure and susceptibility to risk of disease is now in progress. Validation of biomarkers requires studies in both experimental systems and in exposed human populations. This need forms the basis of a number of collaborations between members of this group and the other research foci in the Center. Thus, researches among the faculty in this program serves to bridge and complement the disciplines of epidemiology, toxicology, and exposure assessment in order to develop tools for identifying individuals at-risk for the development of disease. It is the ultimate objective of the Core investigators to translate their scientific findings into prevention strategies in community settings, especially in urban populations and some examples of this work is found in the following sections that relate to the translation to risk assessment.

Demolition and Community Air Quality Impacts

Reports about current residential demolition practices received from residents and plans for large-scale urban redevelopment in East Baltimore provided impetus for a collaborative study with the *COEC* to assess community concerns and develop approaches to addressing them. Dr. Farfel and Lees working with the *COEC* developed the study regarding residents' experiences with demolition and gut rehabilitation of older housing performed as part of urban redevelopment. Issues examined included; lack of notification and awareness about protective measures, concerns about environmental and safety hazards, psychosocial impact from displacement, disruption in daily life, and inattention to community concerns and recommendations to improve redevelopment practices, including ideas to control neighborhood exposure to environmental hazards potentially exacerbated by residential demolition and gut rehabilitation. The findings from focus groups substantiated and deepened an understanding of earlier anecdotal reports of residents' concerns and emphasized the need for including community perceptions and ideas in addressing environmental and psychosocial issues related to urban redevelopment (49).

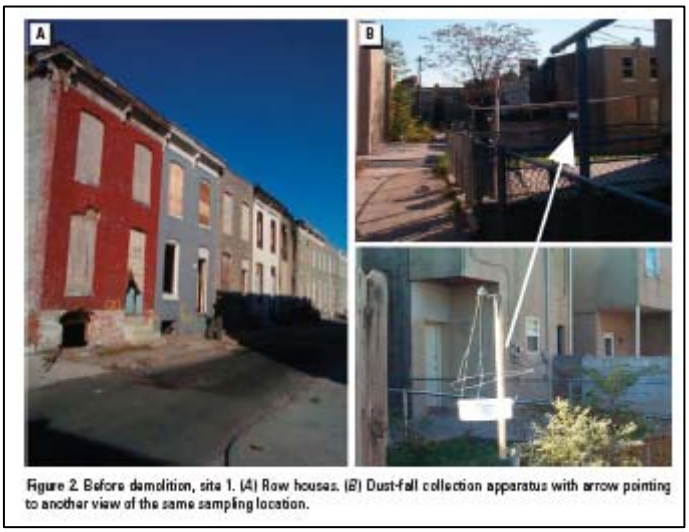

Figure 2. Before demolition, site 1. (A) Row houses. (B) Dust-fall collection apparatus with arrow pointing to another view of the same sampling location.

Figure 10

Demolition of older housing for urban redevelopment purposes benefits communities by removing housing with lead paint and dust hazards and by creating spaces for lead paint-free housing and other community resources. However, given the evident health concerns from contaminated dusts, a study was conducted to assess changes, if any, in ambient dust lead levels associated with demolition of blocks of older lead-containing row houses in Baltimore. The results of dust-fall samples collected from fixed locations within 10 m of three demolition sites were characterized to determine dust lead changes on streets, sidewalks, and residential floors within 100 m of the demolition sites (see accompanying Figures). Geometric mean (GM) lead dust-fall rate increased by greater than 40-fold during demolition to 410 μg Pb/m^2/hr (2,700 μg Pb/m^2 per typical work day) and by more than 6-fold during debris removal to 61 μg Pb/m^2/hr (440 μg Pb/m^2 per typical work day). Lead concentrations in dust fall also increased

during demolition (GM, 2,600 mg/kg) and debris removal (GM, 1,500 mg/kg) compared with baseline (GM, 950 mg/kg). In the absence of dust-fall standards, the results were compared with the U.S. EPA's dust-lead surface loading standard for interior residential floors (40 µg/ft^2, equivalent to 431 µg Pb/m^2); daily lead dust fall during demolition exceeded the U.S. EPA floor standard by 6-fold on average and as much as 81-fold on an individual sample basis. Dust fall is of public health concern because it settles on surfaces and becomes a pathway of ambient lead exposure and a potential pathway of residential exposure via tracking and blowing of exterior dust. The findings highlight the need to minimize demolition lead deposition and to educate urban planners, contractors, health agencies, and the public about lead and other community concerns so that society can maximize the benefits of future demolition activities nationwide (50).

Figure 3. Demolition in progress. Site 1: (A) during demolition; (B) after demolition and before debris removal; and (C) debris removal. Site 2: (D) debris removal; (E) after debris removal; and (F) vacant lot.

Figure 11

This group has continued to examine changes in ambient dust lead levels associated with the demolition of older row houses containing lead paint in Baltimore because of the ongoing development of the Biotechnology Park adjacent to the Johns Hopkins campus. In the follow-up study, the acute increases in Pb loadings and dust loadings after demolition and debris removal showed that streets and alleys had the greatest increases in Pb loadings and the highest levels overall. At one site, geometric mean (GM) Pb loadings immediately after demolition increased 200% for streets to 8080 µg/ ft^2, 138% for alleys to 6020 µg/ ft^2, and 26% for sidewalks to 2170 µg/ft^2. One month after demolition, the GM Pb loadings for streets, alleys, and sidewalks were reduced on average by 41-67% from post-demolition levels and were below baseline levels for alleys and sidewalks. Exterior dust is a public health concern because it is a pathway of ambient Pb exposure and a potential source of residential exposure via tracking and re-aerosolization and re-deposition. Our findings highlight the need to control demolition-related Pb deposition and to educate planners, contractors, and health and housing agencies. This is particularly important given the large numbers of aging US dwellings that will be razed as part of future urban redevelopment efforts (51).

Policy Interventions

Drs. Buckley and Burke linked risk estimates from the US EPAs National Air Toxics Assessment (NATA) to racial and socioeconomic characteristics of year 2000 census tracts in Maryland to evaluate disparities in estimated cancer risk from exposure to air toxics by emission source category. In Maryland, the average estimated cancer risk across census tracts was highest from on-road sources (50% of total risk from non-ackground sources), followed by non-road (25%), area (23%), and major sources (< 1%). Census tracts in the highest quartile defined by the fraction of African-American residents were three times more likely to be high risk (> 90th percentile of risk) than those in the lowest quartile (95% confidence interval, 2.0-5.0). Conversely, risk decreased as the proportion of whites increased ($p < 0.001$). Census tracts in the lowest quartile of socioeconomic position, as measured by various indicators, were 10-100

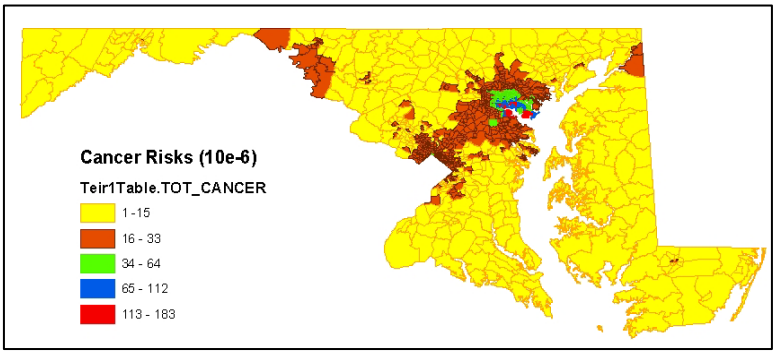

Figure 12

times more likely to be high risk than those in the highest quartile. They observed substantial risk disparities for on-road, area, and non-road sources by socioeconomic measure and on-road and area sources by race. There was considerably less evidence of risk disparities from major source emissions. We found a statistically significant interaction between race and income, suggesting a stronger relationship between race and risk at lower incomes. This research demonstrates the utility of NATA for assessing regional environmental justice, identifies an environmental justice concern in Maryland, and suggests that on-road sources may be appropriate targets for policies intended to reduce the disproportionate environmental health burden among economically disadvantaged and minority populations (52).

Cumulative risk assessment, concerned with the multiple health effects of chemical mixtures, challenges the utility of existing single-chemical regulatory references (53). Drs. Burke and Groopman compare example cumulative risk assessments for 40 HAPs; one based on single-effect toxicological data from EPA, and another based on a multiple-effect toxicological database we developed. For the 40-chemical HAP subset, the multiple effect database contains information on approximately seven effects per chemical and contains a total of 290 toxicological values. Seven health effects are represented in the IRIS data. Seventeen health effects are represented in the multiple-effect data. Respiratory and neurological effects ranked first and second in both cumulative analyses, regardless of the source data. In addition to respiratory and neurological effects, gastro-intestinal/hepatic, renal/kidney, and immunologic effects were identified as effects of concern on the basis of the multiple effect data. Immunologic effects are not found in the 40-chemical IRIS dataset. Cumulative risk assessment has the potential to expand our understanding of the public health impacts of environmental exposures. Advancements in toxicological resources will improve cumulative risk assessment. Cumulative risk assessment will reduce risks to the extent that it can be integrated into prevention strategies to track and protect the public's health.

The work on indoor air quality and health risk of asthma in children was continued in a follow-up investigation led by Dr. Eggleston (54). Dust mites are the primary indoor allergen risk for increasing asthma attacks and morbidity and adherence to allergen avoidance recommendations decreases bronchial reactivity and asthma morbidity. This study compared the knowledge and practice of environmental control advice of families of children with asthma seen by an allergist or a pediatrician. Prior studies suggested that knowledge and practice of environmental control recommendations were inconsistent. In this investigation, the subjects were aged 6 to 17 years, diagnosed with asthma, and had positive skin test to dust mites. There were 114 eligible pediatric patients, and 69 had also seen an allergist before the study. All of the subjects were residents of Baltimore City. An in-home evaluation was completed during which parents were asked about environmental control knowledge and practice. An environmental technician (*Facility Core A: Environmental Exposure and Health Assessment)* then completed a walk-through evaluation to observe which recommendations were implemented in the home. Families who saw an allergist demonstrated significantly greater awareness of environmental control recommendations for dust mite allergens than those who had not. Knowledge and placement of allergen-proof mattress and pillow covers was significantly higher in these families. However, 30% of families who saw an allergist reported no knowledge of any environmental control recommendations for dust mites. Less than half of the allergist families (48%) who were advised to use mattress encasements actually had encasements on their children's beds. The parents of dust mite-sensitive, asthmatic children who saw an allergist were more aware of dust mite allergen control recommendations and made more indoor environmental changes. This particular report illustrates the importance of education as an integral part of the prevention and intervention aspect of the paradigm shown in Figure 3.

Prevention of Liver Cancer

Hepatocellular carcinoma (HCC) is a major cause of cancer morbidity and mortality in many parts of Asia, including China and Thailand, and in sub-Saharan Africa, where there are upwards of 600,000 new cases and deaths each year (1). The impact of HCC is exacerbated by a median age of diagnosis of between 45 and 50 years and it is nearly always fatal. The major known etiological factors associated with development of HCC in these regions are infection with hepatitis B (HBV) and/or hepatitis C (HCV) virus and lifetime exposure to high levels of aflatoxin B_1 (AFB_1) in the diet. HCC is also the most rapidly rising solid tumor in the US and is overrepresented in minority communities, including African-Americans, Hispanic/Latino-Americans and Asian-Americans (see NIH PA-07-258). In the male Asian-American community HCC is 4-times higher than in the

white male population. Collectively, the molecular epidemiology investigations of HCC are among the most detailed sets of data available that link environmental chemical exposures (aflatoxin) and a virus (HBV) to disease outcome.

Several studies have now demonstrated that DNA isolated from serum or plasma of cancer patients contains the same genetic aberrations as DNA isolated from an individual's tumor (55). The process by which tumor DNA is released into circulating blood is unclear but may result from accelerated necrosis, apoptosis or other processes. Recently, Drs. Groopman and Kensler have found that a specific codon 249 *p53* mutation and an HBV $1762^{T}/1764^{A}$ double mutation were not only detectable in plasma samples at the time of HCC diagnosis, but that they were also present in some individuals almost a decade prior to diagnosis (56, 57). To date, the detection of the HBV $1762^{T}/1764^{A}$ double mutation in plasma has been examined in studies in China and Thailand (57, 58). The Thailand studies have been very important, since for the first time infection with HBV, whose pathogenesis is exacerbated by the acquisition of mutations that accelerate carcinogenesis, HCV and dietary exposure to risk. In this study, the occurrence of an HBV $1762^{T}/1764^{A}$ double mutation, the aflatoxin-specific $249^{G\rightarrow T}$ mutation of the p53 gene, and HCV in plasma of 34 HCC cases and 68 age/gender-matched controls, and in 25 liver tumors from northern Thailand were determined. In total, fourteen cases, 5 controls, and 19 tumors had detectable levels of HBV DNA. All 14 cases, 2 controls (2.9%), and 17 tumors (89.5%) were positive for the HBV double mutation. Nine cases (26.5%), 10 controls (14.7%), and 6 tumors (24%) were positive for the p53 mutation. Five cases (14.7%), no controls, and 4 tumors (16%) had both mutations. The median age of HCC diagnosis in these 5 cases was 34 years versus 51 years for other cases. Five cases (14.7%) and 1 control (1.5%) were HCV positive. Thus, specific HBV, HCV and aflatoxin biomarkers reveal the complexity of risks contributing to HCC in northern Thailand and suggest further application of these biomarkers as intermediate endpoints in prevention, intervention trials and etiologic investigations (58).

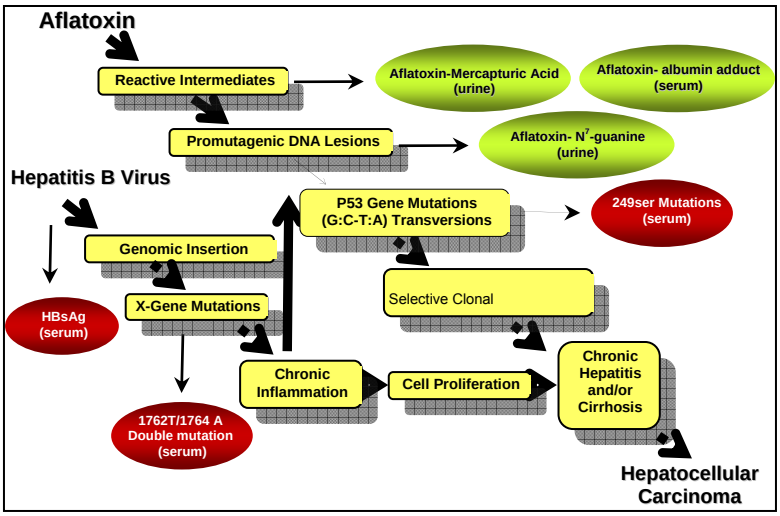

Figure 13

Translation to Chemoprevention Trials

The translational of basic scientific findings to human prevention and clinical trials has also been supported by our NIEHS Center. To a large degree translational research is most easily understood when it involves the direct development of a drug that has evolved from a laboratory investigation for the treatment of a disease. The success measures for these translational efforts are then characterized by clinical effectiveness. In environmental health sciences, these translational efforts are frequently more subtle since the endpoints might involve a prevention trial of a chronic disease endpoint where case reduction might take several decades to enumerate. However, the use of biomarkers as intermediate efficacy endpoints in high risk populations is a fertile area for environmental health sciences translational research. Center members, Dr.

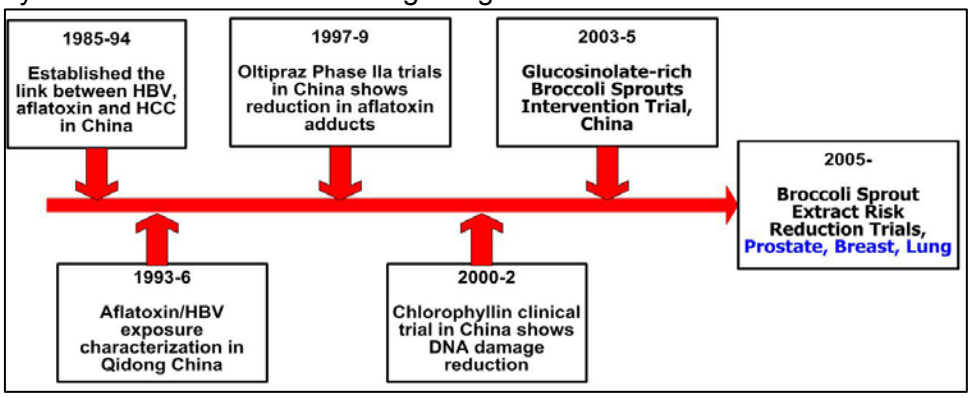

Figure 14

Thomas Kensler and Dr. John Groopman have conducted several decades of basic research that has provided the foundation for the translation of validated biomarkers (using mass spectrometry capacity supported by the NIEHS Center) to clinical trials in high-risk areas of liver cancer.

The basic biology underpinning chemoprotective actions of agents such as Oltipraz and sulforaphane has led to a series of collaborative efforts which have revealed the critical role that the Nrf2-Keap1 system plays in the biology of these compounds as previously described. This basic research has provided insights into the role of the signaling pathway in chemoprevention and these findings not only have importance in cancer research but also been extended to the larger field of inflammatory response to toxic and exposures, as described in a previous section. In these investigations, the dithiolethione, Oltipraz, was the lead compound. A multidisciplinary team worked to obtain a phase II chemoprevention contract from the NIH and the five-year effort culminated with a proof of principle Phase IIa clinical trial in China that showed for the first time that the metabolic pathways leading to aflatoxin induced cancers could be modulated in a protective fashion in people. Specifically, these studies demonstrated for the first time that glutathione-*S*-transferases, critical Phase 2 enzymes, could be induced in people affording a change in the pharmacokinetics and pharmacodynamics of an environmental carcinogen, AFB_1. Further, these findings demonstrated a mechanistic recapitulation of observations in a well-characterized rodent model of liver cancer that was directly translatable to human investigations. The translation of mechanistic studies to human chemoprevention trials requires both the basic scientific information along with public health sensitivity to the economic constraints of an at-risk population

In 2003, this group conducted a placebo-controlled, randomized clinical chemoprevention trial of broccoli sprouts (as a glucosinolate-rich (GR) hot water extract) in residents of Qidong, PRC, who are at high risk to develop HCC associated with consumption of aflatoxin-contaminated foods (59). In this chemoprevention trial we tested whether drinking hot water beverages of 3-day old broccoli sprouts, containing defined concentrations of glucosinolates, could alter the disposition of aflatoxin and phenanthrene. Two hundred healthy adults drank infusions containing either 400 µmole or < 3 µmole GR nightly for 2 weeks. Adherence to the study protocol was outstanding; no problems with safety or tolerance were noted. For example, all blood chemistry analyses were normal. Urinary levels of aflatoxin-N^7-guanine were not different between the two intervention arms (P = 0.68). However, measurement of urinary levels of DTCs (sulforaphane metabolites) indicated striking inter-individual differences in bioavailability. Shown in Figure, an inverse association was observed for excretion of DTCs and aflatoxin-DNA adducts (P = 0.001; R = 0.31) in individuals receiving broccoli sprouts glucosinolates. Moreover, *trans, anti*-phenanthrene tetraol (PheT), a metabolite of the combustion product phenanthrene, was detected in urine of all participants and showed a robust inverse association with DTC levels (P = 0.0001; R = 0.39), although again no overall difference between intervention arms was observed (P = 0.29). Both dose and placebo sprout beverages were analyzed by multiple complementary methods in order to confirm that the dose contained the expected GR levels, and that the placebo contained at least 100-fold lower levels of this glucosinolate. Shown in Fig. 6, GR was the primary glucosinolate in the dose, accounting for 61% of the total glucosinolates, followed by glucoerucin (18%), glucoiberin (14%), glucosinalbin (3.9%), glucoiberverin (1.7%) and 4-hydroxyglucobrassicin (1.7%). Total glucosinolates measured by direct HPLC amounted to 5.3 µmol/ml, of which 3.2 µmol/ml was GR. Thus, the administered 125 ml dose delivered about 400 µmol of GR. Only 0.024 µmol/ml of GR could be detected in the placebo (no other glucosinolates were detectable). Measurement of the DTC metabolites of isothiocyanates in urine was performed by cyclocondensation with 1, 2-benzenedithiol. Shown in Fig. 7, the

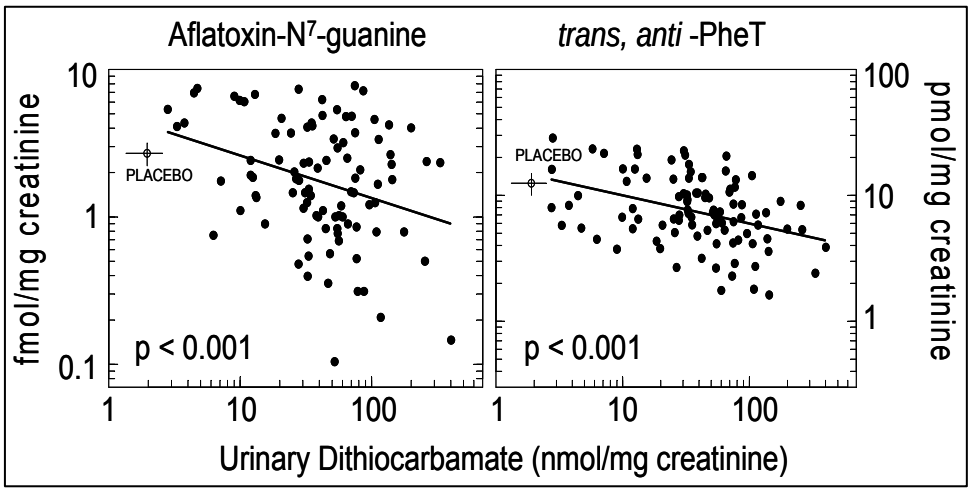

Figure 15

levels of DTC in the placebo and broccoli beverage groups during the run-in period were 0.28 and 0.35 μmol/12 h urine, respectively (P = 0.427). At day 9 of the intervention the urinary levels were 1.55 and 50.37, respectively (P < 0.0001). Thus, the study was successful in providing sulforaphane to the broccoli sprouts arm of the intervention.

However, there was unexpectedly high interindividual variability in the exretion rates, ranging from 4.1 to 180.4 μmol (1-45% of the administered dose). Interestingly, repeated measures of DTC elimination on days 5, 9, 10 and 12 in individuals receiving the broccoli sprout beverage indicated a moderately strong intraclass correlation with a value of 0.29 (95% C.I., 0.17 – 0.45), indicating that DTC "tracks" within individuals over time. This tracking can be readily observed in Fig. 8, which depicts the excretion of urinary DTCs in the ten individuals with the highest excretion rates and the ten individuals with the lowest rates. Overall then, although sulforaphane bioavailability was reasonably consistent between doses within an individual, there was 3-fold greater variability in DTC excretion rates between participants. This variability may reflect inter-individual differences in the composition of intestinal microflora catalyzing the hydrolysis of GR to sulforaphane. These results serve as the basis for proposing Aim 1 in this project, to better optimize the dose and formulation of broccoli sprouts to be used in subsequent clinical trials.

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