GENES, ENVIRONMENT AND HEALTH INITIATIVE:
EXPOSURE BIOLOGY PROGRAM

EXECUTIVE SUMMARY

The Genes, Environment and Health Initiative (GEI) was established by the Department of Health and Human Services and the NIH in 2006 to lay a foundation for investigating the interaction between environmental and genetic underpinnings of human disease. The Initiative consists of two major components: identifying genetic susceptibility factors for diseases that have a high public health impact and developing new technologies for accurate measurement of environmental exposures and lifestyle factors. This four-year effort will create a capacity for further research which will lead to better predictions of disease outcomes, more precise therapies for the treatment of many illnesses, and new strategies for disease prevention.

Two GEI programs were developed to achieve these goals – the Genetics Program, led by the National Human Genome Research Institute, and the Exposure Biology Program, led by the National Institute of Environmental Health Sciences. The Genetics Program is focused on the identification of genes implicated in complex human diseases. The parallel Exposure Biology Program (EBP) will develop a set of tools for assessing individual exposure to environmental stresses including airborne chemicals, psychosocial stress, use of addictive substances, diet and physical activity as well as measures of the biological response to those stressors.

Exposure Biology Program: Overview and Scientific Progress

To determine how environmental exposures, including drug use, diet and physical activity, contribute to human disease, the EBP made 32 awards in new technologies that can accurately measure personal exposures across the spectrum from exposure to response. The products developed through these efforts will provide the improved accuracy and precision needed to determine how environmental and lifestyle factors interact with genetic factors to determine the risk of developing disease. In keeping with the developmental, capacity-building nature of the EBP, progress within the program can be seen from two standpoints: scientific progress and outreach. To date, several tangible successes have been achieved on both fronts.

Scientific progress is often quantified through an analysis of publications. Through the first year and a half of support, 7 review articles and 15 primary research articles were published by investigators in the Exposure Biology program. The BRI is the most traditional research program and as a result is the most amenable to publication; however grantees of both the chemical sensors and psychosocial stress programs have also been successful in publishing their efforts.

Perhaps a greater measure of the early success of the EBP is the effort made to translate the developmental activities into future application. Several workshops, symposia and presentations have been given to increase awareness of the program, discuss limitations

of current technologies and opportunities to address them with EBP products and to build interest from the eventual end users of these products, particularly the early adopters who are likely to be the first to applying these tools in their ongoing and planned studies. Through plenary scientific presentations featuring grantees at conferences such as the International Society of Exposure Sciences and the International Society for Environmental Epidemiology, we have worked to increase awareness of the end-user community of our efforts. Likewise, we have used more focused brainstorming sessions with a smaller group of invited participants to explore more meaningful one-on-one connections and future directions for the products of our program.

A final demonstration of the success of the EBP is the initial seeding of integration of efforts across the four component areas. Through the annual 'opportunity fund' investigators have begun to integrate capacity across the program.

Specific Program Highlights:

Sensors for Assessment of Chemical Exposures (SACE): This initiative is focused on the development of portable, self-contained, easy-to-use sensing devices that provide quantitative measures of exposure to potentially toxic airborne chemicals. The products being developed will be 'user friendly' wearable sensors that can generate quantitative, time-and-space resolved measurements of environmental exposures in the breathing zone. The eight awards made within this program include efforts to detect particulate matter, allergens, pesticides and volatile organics. Each of the projects is felt to be on-track to meet their product development milestones; some highlights and notable achievements to date include;

- NJ Tao's group at Arizona State University has completed the design of a prototype sensor array for detection of major volatile aromatic compounds including benzene-specific and total BTEX (Benzene, Toluene, Ethylbenzene and Xylene). This array has been integrated into a first generation sensor device and applied in preliminary field studies measuring environmental exposures at specific locations including campus 'hotspots', airport and gas station.

- Steve Chillrud's Group at Columbia and Pacific Northwest Labs has completed a first generation prototype with a six station filter unit for particulate and integrated GPS sensor. Extensive effort has gone into selection of components for high performance and low energy consumption. In particular, extensive testing of GPS chips for performance in 'urban canyons' was conducted to insure fast 'cold' and 'warm' starts to enable cycling of the chip and maintain optimal battery life. This unit lacks the real-time optical counter for particulate matter as well as the target 36 location sampler.

- SangYoung Son's group at the University of Cincinnati has completed most of the efforts required for miniaturization of the sampler design from the existing 1.7 kilogram unit by almost 20 fold to a final unit which will be approximately the size and weight of a cell phone and which will enable real time detection of

particulate matter from 10 nm to 1 micron. The prototypes will be tested in an existing children's asthma cohort NIEHS supports in Cincinnati during the fourth year of the project.

Improved Measures of Diet and Physical Activity (DPA): This initiative, led by the National Cancer Institute and National Heart, Lung and Blood Institute, is promoting the development of reliable and economically feasible technologies for accurate measurements of diet and physical activity and, in general measures of total energy expenditure, either individually or in an integrated package. These efforts include three projects focused exclusively on developing direct measures of physical activity and analysis of activity patterns, three projects focused on camera-based analysis of dietary intake and improved 24 hour recall assessments, and one project focused on integrating both diet and physical activity assessments into a single device. Highlights of this effort to date include:

- Calculation of food volume to estimate portion size from images of foods taken with mobile phones is progressing well—several factors affect accuracy and most are controllable.

- A series of papers describing the diet projects are drafted and will be submitted for publication in a Journal of American Dietetic Association special issue on assessment methods

- Significant progress made on integration of accelerometers with mobile phones and real-time recognition of different types of physical activity using multiple sensors.

- Working Group meeting planned for July, 2009 to bring together experts to define best practices for calibration and validation of activity monitors. This meeting is co-sponsored by NHLBI, NCI, GEI, and the American College of Sports Medicine (ACSM).

Network for Quantifying Exposures to Psychosocial Stress and Addictive Substances (NEPSAS): This initiative, led by the National Institute on Drug Abuse, is targeting the development of measurement technologies that can improve detection and quantify personal exposure to psychosocial stress and/or addictive substances. Psychosocial stressors include acute events like daily stresses and traumatic events, as well as chronic events such as crowding or isolation, discrimination, or family violence. The awards issued through the NEPSAS program include a combination of ecological momentary assessment and biological measures of stress and addictive substance use. Highlights from this effort to date include:

- Vivek Shetty's group has produced a 2nd generation portable biosensor with field deployable features. This sensor system uses a colorimetric 'dipstick' approach to detect salivary alpha amylase rapidly and with minimal user action.

- Mark Rea's group has developed a phasor analysis for assessing circadian entrainment to the light-dark cycle. This new method quantifies, in a simple, robust way how well people respond to the environmental light-dark pattern. They have published a description of this technique this year in the *Journal of Circadian Rhythms.* Of note, the paper is already number 8, all time, on the list of most accessed papers from that journal.

- Santosh Kumar's team has developed a skin-patch sensor system with embedded micro-processors and wireless transceivers that will enable simultaneous measurement of stress markers and alcohol within the field without requiring active participation from subjects. Their measure of alcohol is an interstitial-fluid (ISF) based sensor that is strapped on the arm; their physiological stress measurements are composed of several sensors strapped on a chest band which measures skin conductance, heart rate, respiratory rate, temperature and physical activity.

Biological Response Indicators of Environmental Stress (BRI): This initiative focuses on the development of robust biomarkers for detecting subtle changes in biological systems following exposure to environmental stressors. These biomarkers reflect changes in key biological pathways, such as inflammation, oxidative stress, DNA damage, endocrine disruption, immune activation and epigenetic regulation, which are known to be influenced by environmental stressors and are linked to the pathogenesis of common diseases. Twelve awards were issued through the BRI program including two centers, which include an additional element of biosensor development in addition to the biomarker discovery activities. Highlights of this program to date include:

- Tim Huang's group at Ohio State University is studying persistent global methylation patterns in breast stem progenitor and breast tumor cells following exposure to xenoestrogens (e.g., DES, 17β-estradiol, and daidzein). Their hypothesis is that methylation patterns serve as a "molecular relic" of prior exposure. Early results from massive parallel sequencing efforts suggest clusters of hyper-methylation in selected areas of genome suggesting coordinate methylation and gene silencing with xenoestrogen exposure.

- Avi Spira at Boston University is developing gene expression signatures as biomarkers of host response to tobacco smoke in bronchial, nasal and buccal epithelium. Early comparisons of patterns from cells from bronchial brushings show that nasal epithelium patterns correlate well with those of bronchial epithelium.

- Yuehe Lin from the U54 Center at PNNL has developed a prototype biosensor for detecting cotinine, nitrated proteins and other protein modifications that result from obesity and cigarette smoke-induced oxidative and inflammatory stress. The biosensor is an ELISA-type device that uses nanoparticle-conjugated antibodies to enhance signal from binding by cotinine or nitrated proteins. The cotinine assay is based on competitive binding using a simple

absorption pad, a QD-labeled cotinine conjugate and a secondary anti-cotinine antibody. The prototype has been tested successfully with cotinine and nitrated fibrinogen (as a general biomarker of inflammation) and will be tested with other modified proteins identified in projects 1 and 2.

- Rich Mathies lab at UC Berkeley has developed a single cell genetic analysis method that can be used to sequence DNA and PCR-amplify targets in single cells. The method relies on the generation of uniform nanoliter emulsion droplets that contain (statistically) a single cell's worth of DNA, reverse primer beads and dye-labeled forward primers (see slide).

Opportunity Fund: From the beginning of the Exposure Biology Program, it was felt that the investigators needed to have a high degree of freedom to conduct their studies: to capitalize on opportunities to meet their product development goals faster, to add new capabilities to their products, to integrate the efforts of others into their products or to explore future directions to advance the goals of the EBP. Therefore, an annual pool of $1 million was established for a supplement program to advance the goals of the EBP and improve the quality and capability of the products we will be developing. These funds have gone to purchase new equipment, to support new research directions, to support cross-grantee and cross-program collaborations and to hold workshops to strengthen the EBP effort. Highlights of the 2008 opportunity fund include:

- A collaboration between investigators in the Chemical Sensors program (Chillrud and Rodes) and Physical Activity program (Initille, Raab, and Haskell) to integrate accelerometers and activity analysis into particulate matter sensors to inform not only about subject compliance but also to add potential analysis on commuter behavior and correlations between activity and exposure underlying an adverse response.

- A research supplement to Spira's group has begun studying effects of tobacco smoke exposure on microRNA expression. A recently published PNAS paper supported by this effort shows a clear negative correlation between increased gene expression and decreased MAGF expression (transcription factor found to be differentially expressed in smokers) with smoking.

- An opportunity fund supported collaboration between Vivek Shetty (NEPSAS) and Ashok Mulchandani (SACE) has established proof of principle that functionalized nanowires can be used as multiplexed biosensing platforms for measuring larger panels of salivary stress indicators.

- Investigators from 9 of the GEI funded projects (DPA, NEPSAS and SACE) will come together to share experiences with and plans for the use of Global Positioning System (GPS) and Geographical Information Systems (GIS) data in their research. The aim is to begin dialogue on the potential for common measures, analytical strategies and outputs from the overall GEI initiative.

GEI Future Directions

The intent of the GEI was to use the initial four year funding period, from 2007 to 2011, to establish a capacity in tools, methods and expertise which could then be applied in population based studies to understand the interaction between genetic and environmental factors. Both programs are well on their way to achieving those goals. We are, however, faced with a reality that progressing with this vision will be cost-prohibitive in the current fiscal environment. A second reality is that while progress on EBP product development is proceeding very rapidly, acceptance of these tools by the end user community will require a level of validation and commercialization that was never intended to be supported in the current GEI activities.

The ideal next step would be a continuation of the GEI effort focused on establishing a population-based proof of principle that integrates measures of exposure and genetic variation can allow testing of additional hypotheses and the generation of improved understanding of the disease process and susceptibility. To make this feasible, it would be best to target this to a particular area, such as asthma, where there is sufficient evidence that both genetic and environmental factors contribute and there is sufficient targeted tool development proceeding in the EBP to strengthen the definition of exposure.