

Exposure Science Advancing

by National Research Council Staff

The science of evaluating environmental exposures is on fire. From sensors embedded in smart phones to gene-expression profiles to exposomics, new technologies that advance our understanding about our exposures to environmental stressors are developing rapidly. Exposure science is even a key theme in the new National Institute of Environmental Health Sciences (NIEHS) 2012–2017 strategic plan. NIEHS will focus research on improving technology for exposure measurement and developing predictive models and informatics tools that can advance the understanding of biologic pathways involved in disease.

Two National Research Council reports, *Toxicity Testing in the 21st Century: A Vision and a Strategy* (2007) and *Science and Decisions: Advancing Risk Assessment* (2009), underline the importance of improving exposure characterization for identifying and understanding early biomarkers of effect, individual variability, susceptibility and vulnerability, and

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Measuring Individual Exposomes

by Elisabeth Stallman Brown, edited by National Research Council Staff

On December 8 and 9, 2011, the National Academy of Sciences Standing Committee on Use of Emerging Science for Environmental Health Decisions (ESEH) held a public meeting on emerging technologies for characterizing the individual exposome—an individual's lifetime environmental (nongenetic) exposures. The exposome concept was proposed in 2005 by Christopher Wild, director of the International Agency for Research on Cancer, as a complement to the human genome in investigations of the causes of human diseases. The ESEH had hosted a forum in February 2010 (<http://nas-sites.org/emergingscience/meetings/exposome/>) to present Wild's exposome concept to a broad audience of scientists, policy-makers, public-health practitioners, and other environmental health stakeholders. At the December 2011 meeting, participants examined recent advances that enable external and internal measurements of a person's exposures from all sources, including lifestyle factors, the microbiome, and environmental pollution. Participants discussed the complementary

roles of such measurements and highlighted two innovative proof-of-concept studies that synthesized multiple sources of exposure data to characterize portions of an individual's exposome. By assessing the utility of the emerging technologies and their potential integration, participants sought to inform researchers and policy-makers about the importance of the exposome concept and the new science and technology for understanding and preventing diseases.

Stephen Rappaport, of the University of California, Berkeley, opened the meeting with a key question: Why do we care about environmental exposures? The simple reason, he said, is that even though two-thirds of the deaths in the world are caused by noncommunicable diseases, especially cancer and cardiovascular disease,

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This newsletter and additional information about the committee and its activities can be found at <http://nas-sites.org/emergingscience/>. The newsletter is prepared by National Research Council staff to keep you informed of activities of the Standing Committee on Emerging Science for Environmental Health Decisions. The views expressed in the newsletter are those of the meeting presenters and participants. The newsletter does not represent either formal consensus conclusions of the attendees or positions necessarily endorsed by the National Research Council.

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we can attribute only about 10% of this mortality to genetic variation. Because current research into disease etiology mainly uses genomewide-association studies (GWASs) to conduct detailed genetic analyses while relying on questionnaires to evaluate diet, lifestyle, and other exposures, Rappaport concluded that it is important to develop -omic approaches that can characterize the nongenetic factors, particularly small molecules and metabolites

We need a much better handle on exposure and exposure variability at the individual level, in the critical states of development, and in people who are more vulnerable.

—Linda Birnbaum

(through metabolomics) and large molecules (such as proteins, through proteomics). Diseases are thought to arise from interactions

between genes and environmental factors, so Linda Birnbaum, director of NIEHS, emphasized that more detailed characterization of nongenetic (environmental) factors could pay big dividends in reducing disease risks. “We can’t trade in the genes we’ve inherited, but we might be able to reduce our risks of some diseases by altering our environment or lifestyle,” Birnbaum held.

The potential to reduce the prevalence of some major diseases, said Paul Elliot, of Imperial College, is driving research to understand the totality of exposures over the course of our lifetimes. But, Elliot cautioned, the ability to capture high-quality environmental-exposure data is challenging. In addition, the pace and sophistication of research on genetic factors associated with disease have far exceeded those of research on environmental factors for more than a decade, said Chirag Patel, of Stanford University. The evolution of GWASs, in particular, has enabled researchers to search the entire genome for genes associated

with particular diseases and to do so without specific hypotheses regarding which genes might be involved. In contrast, research on environmental contributions to disease has remained relatively crude and imprecise, often using self-reporting mechanisms or focusing on only one or a few pollutants, Rappaport noted.

Many participants agreed that for the origins of human disease to be better understood, the time is ripe for environmental health science and exposure science to be better integrated with human genetic research. Rappaport emphasized that characterization of an individual’s exposome is an essential first step in promoting discovery of environmental causes of disease.

Emerging Tools and Technologies

Researchers attempting to characterize the exposome face the daunting task of optimizing and integrating two largely distinct approaches for the measurement

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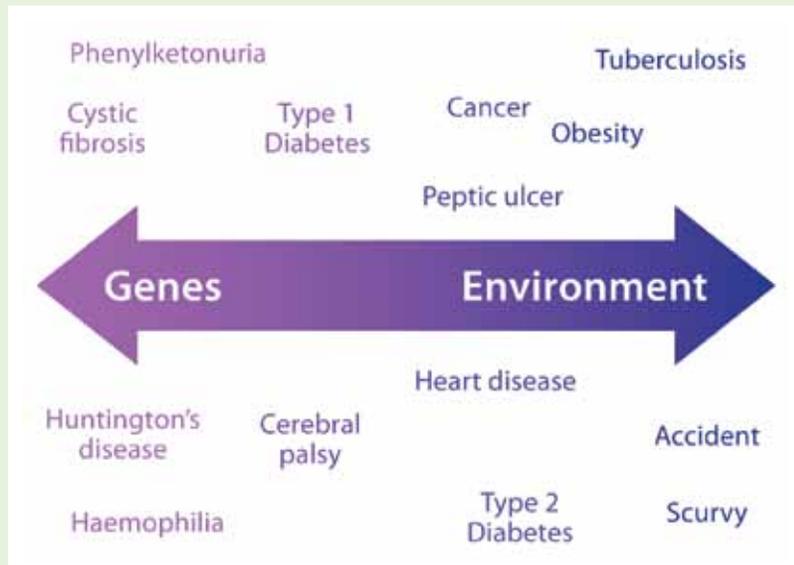
cumulative exposures and risks. A 2012 report, *Exposure Science in the 21st Century: A Vision and a Strategy*, defines the scope of exposure science—the “eco-exposome”—and outlines a roadmap for advancing exposure science through technologic innovations and strategic collaboration (see article on page 13).

The pace of discovery enabled by new tools often far exceeds the pace at which decision-makers—from global leaders to ordinary people—can evaluate the utility and importance of the resulting

findings for personal or public-health decisions. Public meetings of the National Academies Standing Committee on Emerging Science for Environmental Health Decisions have explored the implications of rapid technologic advancement in exposure science. Meetings on the exposome (February 2010), early indicators of disease (October 2010), the microbiome (April 2011), mixtures and cumulative risk (July 2011), and the individual exposome (December 2011) delved into both cutting-edge science and implications for its practical uses. The

meetings attracted large audiences, sometimes with standing room only, and drew considerable post meeting Web traffic (<http://nas-sites.org/emergingscience/> for archived Webcasts and PowerPoint presentations). As one participant in the individual-exposome meeting observed, the “remarkable energy” of the discussions reflect not only that exposure science is a hot topic but the hope that it will address persistent challenges in environmental health and help decision-makers to protect human health and the environment.

Genetic vs Environmental Determinants of Disease



The vast majority of chronic diseases are a result of the interplay between genetics and the environment. Elliot and other meeting participants emphasized that the causes of complex chronic diseases—cancer, heart disease, obesity, type 2 diabetes, and others—responsible for the bulk of disease-related morbidity worldwide all have environmental components. The environmental exposures and lifestyle factors involved in the cause of many chronic diseases are largely unknown. Meeting participants stressed the importance of elucidating the environmental causes so that preventive measures can be put into place to reduce the incidence and prevalence of chronic diseases.

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of exposures, said Rappaport. On one hand, researchers measuring exposures in external media (such as air, soil, or water) are increasingly able to do so in a nearly continuous manner. However, with today's technology external measurements are taken in a *knowledge-driven* approach, limited to environmental factors that have known or suspected links to disease. On the other hand, Rappaport continued, researchers measuring exposures by sampling biologic specimens (such as whole blood, serum, plasma, or urine) could theoretically measure all chemicals in a given class (for

example, all small molecules) that are present in a *data-driven* (or "agnostic") approach that lacks specific hypotheses. The latter type of study, which generally uses -omic techniques to identify biomarkers, typically relies on one or a few samples to assess agents that can fluctuate widely. Ideally, data-driven exposomics research would identify both external and internal environmental exposures that require targeted study. Meeting participants examined the techniques used in both approaches to try to understand their complementary roles in elucidating environmental determinants of disease.

Personal sensors enable a comprehensive and realistic assessment of many of the external exposures that we face, said Michael Jerrett, of the University of California, Berkeley. The devices—with remote-sensing-based spatial referencing technologies and modeling—enable the continuous, real-time, real-world assessment of exposures that vary according to an individual's location, activity, and lifestyle. For example, one's level and type of activity in particular locations can affect the inhalation of air pollutants (for example, by a bicycle commuter vs a car commuter) and the extent of dermal or oral contact with harmful substances (for example, by a crawling infant vs a walking toddler), Jerrett explained.

In recent years, wearable personal sensors that measure environmental exposures and other stressors have become much smaller, less expensive, more accurate, and more user-friendly, said Nongjian Tao, of Arizona State University. The development of portable, relatively unobtrusive sensing devices and their integration with smart phones, which many people already carry, Jerrett explained, opens the door for *ubiquitous* sensing systems: the complete embedding of sensing technology into everyday life. By leveraging the existing features of smart phones—including Internet access and networking capabilities, data transmission and storage, GPS, an accelerometer, and user-friendly graphic interfaces—researchers are advancing personal sensing technology at minimum cost, said Tao and Jerrett. For example, Tao's team is working on a personal sensor system for the quantitative,

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Developing a Common Language

The development and consistent use of such terms as environmental exposure, exposome, and exposure assessment was an important discussion theme during the meeting. The terms are often used differently by researchers depending on discipline (for example, toxicology vs epidemiology vs clinical research). Rappaport, Elliot, and other meeting participants focused in part on the development of a common language and key research characteristics important for environmental health science.

Environmental exposure: contact with agents that have external sources (such as air, water, diet, infection, radiation, and stress) or internal sources (such as hormones, inflammation, and pre-existing health conditions).

Environmental exposures change, are continuously distributed, and have a wide dynamic range, all of which make them difficult to measure.

Exposome: the accumulation of a person's environmental exposures from conception onward. Measuring a person's exposome is important for understanding disease etiology. Exposome research calls for data-driven and untargeted analysis of biospecimens.

Exposure assessment: measurement of environmental agents outside an organism. Exposure assessment is important for understanding environmental exposures that might cause or be linked to health outcomes. Exposure assessment calls for targeted knowledge-driven investigations of the external environment.

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real-time measurement of multiple volatile organic compounds and other chemicals. The detection module of the system works in conjunction with a smart phone. The sensor is worn on the arm so that it can provide data on exposure within a person's breathing zone. Real-time exposure data are displayed and stored in the cell phone, which transmits them to a computer server accessible to the researchers.

Portable chemical sensors, Tao explained, must be able to detect the target compounds selectively in the presence of multiple other compounds and in environments of highly variable humidity and temperature. To provide high sensitivity and selectivity at low cost, Tao's device uses tuning-fork-based sensing elements that can translate a chemical signal into

a mechanical signal. The device selectively detects hydrocarbons, acids, and humidity in harsh environments. Tao's team is validating its performance in indoor and outdoor field tests. In one such test, the team used the device to measure and map hydrocarbons in the Gulf of Mexico shortly after the BP oil spill. Although average hydrocarbon concentrations were similar to those detected by a nearby fixed US Environmental Protection Agency monitoring station, they found dramatic spatial and temporal variability; spikes in localized hydrocarbon concentration could have implications for people who live or work in the area.

Stephen Intille, of Northeastern University, and his colleagues are developing the Wocket System—a personal sensor system that measures physical activity and

sends the data to a person's cell phone. The accurate measurement of physical activity is important for exposomic research because physical activity can modify a person's exposure and response to pollutants. The Wocket system is being designed to continuously measure physical activity—including its intensity, duration, and location—and could potentially do so for months or years. A pattern-recognition algorithm is used to translate spikes of activity from ankle and wrist sensors into specific movements. The cell phone uploads summary data to a central server hourly, allowing researchers to provide feedback to the wearers; at night, while the phone is charging, it uploads the raw data. The ultimate goal of Intille's project is to enable the measurement of physical activity in cohort studies. Intille and his team are planning a validation study with 50 subjects who will wear the Wocket system daily for 4 months.

Jerrett described a pilot project that demonstrates an application of personal exposure and activity sensing systems in Barcelona. Researchers outfitted volunteers with two physical-activity monitoring devices and smart phones that contained a novel software program, CalFit. CalFit is a mobile phone application that uses the phone's built-in accelerometer and GPS to record activity and the time and location in which it occurs. Researchers obtained objective measures of physical activity and location and integrated the data with air-pollution maps to estimate individual inhaled doses of nitrogen oxides. They found that travel accounted for a

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disproportionate percentage of participants' inhaled dose primarily because they were more active while walking and biking.

The devices developed by Tao, Jerrett, and Intille allow researchers to gather critical data on exposures or outcomes at the individual level in much more seamless and reliable fashion than ever before. Jerrett terms this critical

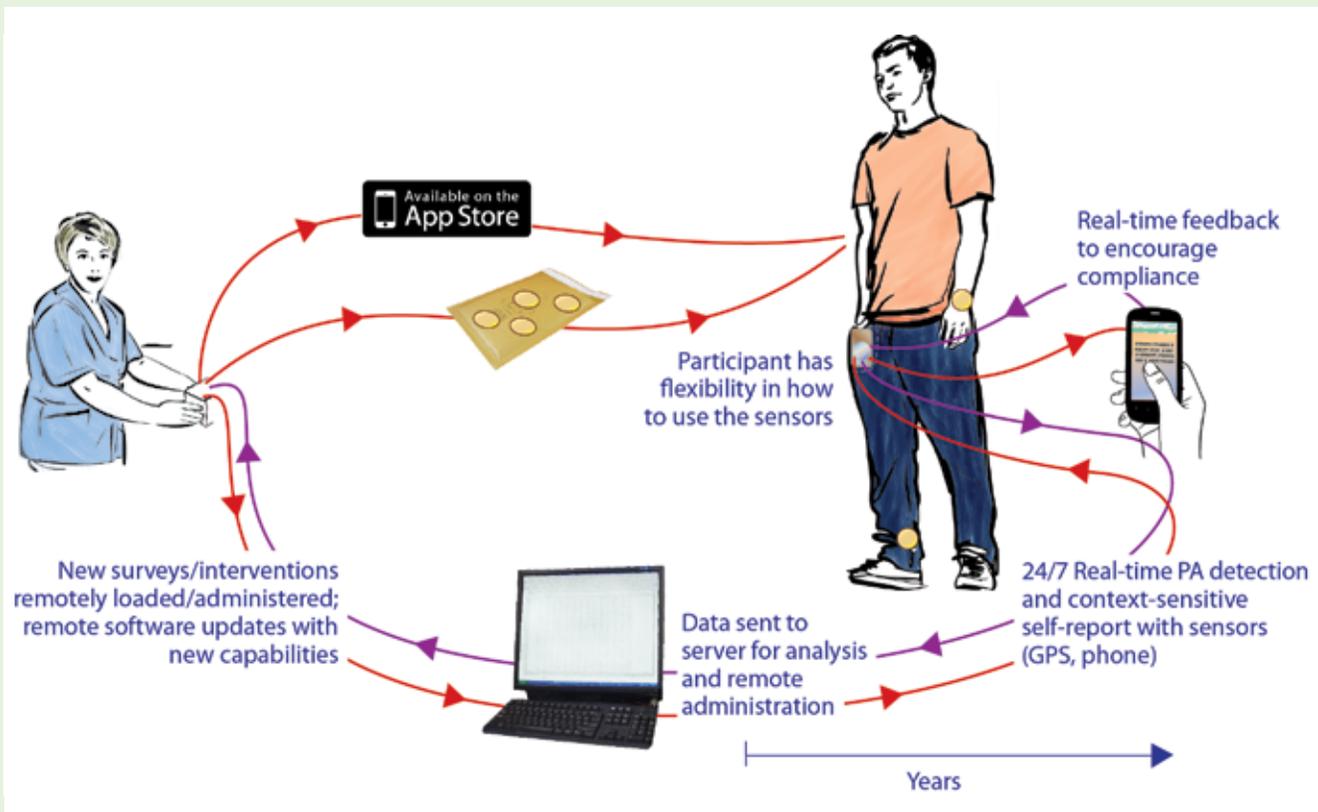
information “the five Ws of exposure”: Who is exposed? To what is a person exposed? When did exposure occur? Where did exposure occur? Why did exposure occur? However, to complete the pathway from source to effect, a sixth W becomes important: Which exposures cause disease?

Technologies for characterizing a person's internal chemical environment, and ultimately an

individual's exposome, range from monitoring a person's metabolic profile in response to external environmental stimuli to evaluating environmental chemicals in blood, urine, or other biospecimens. Rajeshwari Sundaram, of the National Institutes of Health, described a new, simple tool useful for understanding the association between environmental exposures

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The Wocket — Personal Measurement of Physical Activity



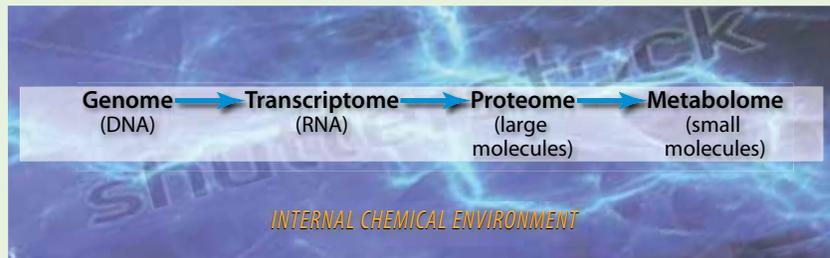
Intille's Wocket system is designed to minimize the burden on participants and maximize data quality and completeness. It is designed to provide the wearer with information about his or her physical activity to help, for example, in meeting personal health goals. The Wocket system consists of two thin, waterproof sensors designed to be worn on the upper and lower body (one on the ankle and one on the wrist), two thin bands with pockets to hold the sensors in each location, an extra set of sensors that can be charged while the first pair is in use, and a charger. The sensors can be worn comfortably at night, reducing the potential for missing data that can result, for example, when a person forgets to put a device on in the morning. They are also thin and unobtrusive enough to be worn comfortably under one's clothing for months. The sensors send data to a smart phone that is preloaded with an application that detects data quality and missing data in real time. The phone application also provides feedback to the wearer both to provide physical-activity information that may be of interest to the wearer and to encourage study compliance.

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and reproductive outcomes. Many reproduction-related events, including ovulation and fertilization, are not readily observable and have high interindividual and intraindividual variability. Sundaram and her colleagues are using commercially available fertility monitors, with a participant's home serving as the laboratory, to measure fluctuations in reproductive hormones—estrone-3-glucuronide and luteinizing hormone (LH)—in women. The cost-effective, user-friendly, validated monitors allow hormone profiles to be directly uploaded to an Internet-based data-analysis system, provide real-time feedback to participants regarding the optimal timing for conception, and identify missing data if a scheduled test is not done. The monitors are an essential piece of the Longitudinal Investigation of Fertility and the Environment (LIFE) Study, which is evaluating whether persistent environmental chemicals affect human reproduction and development. The LIFE Study followed 501 couples for up to 12 months as they tried to achieve pregnancy. Women regularly used fertility monitors that were timed to coincide with their menstrual cycles. Researchers measured a number of chemicals—including pesticides, phytoestrogens, and phthalates—in biologic fluids and assessed some lifestyle factors via interviews. Data for the LIFE Study are being evaluated. Devices like the fertility monitor described by Sundaram provide researchers with high-quality individual-level information on otherwise “inaccessible” health outcomes.

Avi Spira, of Boston University, described his work that is using gene-expression profiles in the

The Molecular Basis of Life and Disease



Rappaport described the molecular basis of life as a “symphony.” The genome (an organism’s complete set of genetic material, DNA) is the director that drives everything that goes on in the process of life. The transcriptome (the set of all RNA molecules) translates the genome’s directions to an orchestra of molecules made up by the proteome (all proteins produced by the genome) and the metabolome (all small-molecule metabolites produced by cellular processes and such noncellular processes as those of endogenous microorganisms). The internal chemical environment, said Rappaport, can interact with all these -omes to give rise to both healthy processes and disease processes. Rappaport emphasized that a key aspect of exposome research is determining which -omes will be the most useful for finding causative exposures.

human airway—that is, the bronchial airway transcriptome—to develop biomarkers of the body’s response to tobacco-smoke exposure. Existing diagnostic tests for lung cancer—a computed tomographic scan coupled with bronchoscopy—detect early-stage lung cancer with only about 50% sensitivity. By running RNA extracted from apparently normal bronchial epithelial cells (obtained via bronchoscopy) on microarrays, Spira and colleagues found 80 genes whose expression in smokers indicated whether they ultimately developed lung cancer. The sensitivity of this biomarker panel for the diagnosis of early-stage lung cancer exceeds 80% and climbs to 95% when it is combined with bronchoscopy. The biomarker panel, which is now undergoing validation in a large independent cohort, is expected to become a diagnostic adjunct to bronchoscopy. In research

with direct therapeutic relevance, Spira’s team found that they could use patterns of gene expression in particular pathways to predict which high-risk smokers (those who had moderate airway dysplasia) are most likely to benefit from treatment with a new dysplasia-reducing drug, myo-inositol. More generally, Spira’s work is advancing the ability to predict a person’s susceptibility to respiratory disease in response to inhaled environmental contaminants.

Rappaport discussed the applicability of -omic technologies to characterizing a person’s exposome. An organism’s external and internal environments give rise to a constantly changing pool of small molecules, which Rappaport termed the *serum exposome*. Those molecules can influence disease processes by interacting with various -omes. For example, they

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can interfere with the genome and produce mutations, with the transcriptome and produce epigenetic modifications, and with the proteome and modify proteins.

-Omic techniques, said Elaine Holmes, of Imperial College, are allowing us to learn how exposures lead to disease, to identify biomarkers useful in disease prevention and diagnosis, and to develop novel therapies tailored to an individual's -omic profiles.

Holmes described a number of metabolomics studies in which she and her colleagues characterized changes in the metabolic profiles of humans and other animals in response to chemical exposures or lifestyle factors or events, such as diet and preterm birth. A key

to those studies, she explained, is the ability to conduct dynamic monitoring, that is, monitoring multiple compounds through time. Holmes and her colleagues use nuclear magnetic resonance spectroscopy or mass spectrometry with principal-components analysis to tease out "multivariate fingerprints" from collected biologic fluid or tissue. Using those techniques, Holmes and her colleagues found, for example, paracetamol, an over-the-counter drug used to reduce pain and fever, in the urine of breast-fed infants whose mothers were taking the drug to treat symptoms of malaria. However, that study captured a very short-term exposure and does not indicate what effects paracetamol exposure may have in the future.

Holmes's research team was also able to observe clear biochemical changes in renal tissue of wild animals exposed to cadmium at concentrations lower than the exposure limits recommended by the World Health Organization.

In a remarkable pilot study, Holmes and her colleagues were also able to differentiate young adults who had a normal or a preterm birth; this emphasizes the importance of evaluating metabolic changes in early life. When a baby is born, Holmes explained, many factors, including preterm birth, influence the development of the gut microbiome (the totality of microorganisms and their genes in the gut). The gut bacteria, which vary widely among people, can affect metabolic profiles associated with surprisingly diverse aspects of health and disease. It is well known that preterm birth is a risk factor for cardiovascular disease, metabolic syndrome, renal disease, and other adverse health outcomes. Holmes and her colleagues evaluated the metabolic profiles of 30 young adults and discovered marked increases in some metabolites produced (or co-produced) by microorganisms in people who had preterm birth. Some of the metabolites responsible for discriminatory signatures are associated with inflammatory or preinflammatory conditions. Those findings suggest that the metabolome may hold an accurate record of lifetime exposures that can be linked to disease outcomes—in other words, the exposome.

Analysis of environmental effects on metabolic profiles, and particularly the contribution of gut flora to the metabolome, is

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The Microbiome

Holmes discussed the importance of including microbial metabolic signatures into disease research. Humans have coevolved with their microbiomes. The gut bacteria, which vary widely among people, are particularly important because they can affect metabolic profiles associated with surprisingly diverse aspects of health and disease, Holmes explained. Some metabolites are produced entirely through the metabolic processes of the microbiota itself; others, such as hippurate, are co-metabolized (synthesized in a process that both the microbiota and the host contribute to). Each person carries about a kilogram of bacteria in his or her intestines. Holmes pointed out that in published metabolic profiling studies of nearly every field of disease, from neuropsychiatric to metabolic to reproductive diseases, about 20–30% of metabolic signatures result from gut microbial activity. The gut microbiome is responsible for either detoxifying potentially harmful chemicals or synthesizing toxins and can also influence other tissues, including the liver, kidneys, heart, and brain. Holmes emphasized that integrating metabolic activity of microbiota into exposome research will "give a better systems-level view" of environmental exposures related to human disease than studying only metabolites derived from human genetics.

The ESEH committee hosted a previous meeting focused entirely on the interplay between environmental stressors and the microbiome. For more information about the implications of the microbiome for environmental health research, visit <http://nas-sites.org/emergingscience/meetings/the-microbiome/>.

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one way to characterize part of the exposome, Holmes argued. In addition, she said, the elucidation of distinct metabolic profiles according to microbiome, diet, and other exposures can contribute to the discovery of biomarkers of exposure and may prove useful in personalized medicine.

N. Leigh Anderson, of the Plasma Proteome Institute, discussed how proteomics has been effective in fields that are directly relevant to the characterization of individual exposomes. For example, Anderson and his team examined the effects of six peroxisome proliferators on the concentrations of more than 100

proteins in the mouse liver. The effects of those diverse compounds on protein expression helped to illuminate the mechanisms by which peroxisome proliferators produce harmful effects.

Proof-of-Concept Studies

Currently, Patel explained, environmental epidemiology tends

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SCIENTIFICALLY SPEAKING

David Balshaw is a program director in the NIEHS Center for Risk and Integrated Science. He is a leader in translational science, overseeing NIEHS-funded research programs on new and innovative tools, technologies, and approaches for understanding complex systems; for discovering and validating exposure biomarkers; and for reducing the risk of exposure and disease. He shared his views on emerging science, environmental health, and the exposome.



David Blashaw

Q. Why is emerging and translational science important to you?

A. The way we typically conduct research in environmental health is carefully controlled: we simplify the questions that we ask so that they are easily and reliably addressed with current technologies, and we formulate concrete hypotheses, test them, and use the results to improve our understanding of how environmental factors influence health. One problem is that we know that the controlled systems will give us only part of the truth. The use of these approaches can be highly informative, but reliance on them can mask factors that can contribute to the linkage between environmental exposures and disease. The hypothesis-driven approach is limited by both mindset and the ability to conduct *discovery-driven* science. Developing new technologies and emerging fields of science can begin to allow us to embrace the complexity needed to advance our understanding—not to replace hypothesis-driven research but to identify novel hypotheses and to strengthen our ability to test hypotheses. Improved understanding can be translated into interventions that will improve individual and public health.

Q. How have the Emerging Science meetings contributed to your work?

A. The Emerging Science committee has covered a tremendous amount of new science, including new ways of doing things that we have done for a long time, such as computational modeling, and fundamentally new fields of science, such as the exposome and the microbiome. The committee has been willing to embrace challenges that we have long known of but have never been able to approach well, such as individual variability and the response to chemical mixtures. The committee has also looks at practical applications of new science. For example, they've explored potential applications of environmental health to improve public health in such fields as green chemistry.

Q. Describe a new insight or idea that stayed with you after the individual-exposome meeting. Were there any surprises?

A. I think that two key messages came out the individual-exposome meeting. First, even with our somewhat limited technical ability, it is possible to identify unexpected and powerful effects of environmental factors; for example, Chirag Patel found an association between heptachlor epoxide and diabetes, and Stanley Hazen discovered an association between trimethylamine *N*-oxide and cardiovascular disease. Second, our ability to identify the *external* exposures to environmental factors lags substantially behind our ability to characterize exposures in blood

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to examine only a few candidate environmental factors at a time for their association with disease, often ignores the statistical problems associated with testing multiple hypotheses, and fails to consider environmental exposures as a system. An environment-wide-association study (EWAS), the

environmental complement to the GWAS, is an untargeted, transparent, data-driven way to examine multiple environmental factors for possible associations with disease, Patel argued.

In the first published EWAS, Patel and his team used data collected from four cohorts of the National Health and Nutrition

Examination Survey (NHANES) to evaluate genetic variations in exposure to environmental factors (such as vitamins, metals, hydrocarbons, phthalates, infectious agents, and pesticides) and in biologic factors (such as fasting blood glucose concentrations, cholesterol concentrations, height,

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and urine. As we saw in Michael Jerrett's presentation and in the panel discussions, that is a major gap in our ability to measure the exposome and a critical need if we want to identify the sources of environmental pollutants and use the exposome as a tool to prevent disease by reducing the burden of exposure.

- Q.** Do you think the environmental health community will benefit from a shift in focus from population-wide exposure estimates to individual-level exposure measurements?
- A.** Certainly, as you look at the border between the individual-variability meeting and the individual-exposome meeting, this becomes clear. The exposome is an incredibly powerful tool for understanding the individual environment, and that will make it a powerful tool for understanding individual risk. If you look at the power of real-time, spatially resolved exposure measurements like the ones that N. J. Tao and Jerrett presented, you can see that "population" metrics are nice for calculating an "average" but can potentially entail massive amounts of error for the individual; given the importance of understanding the individual response, the effect will be a revolution in environmental health sciences. It is a revolution that comes with a price, though. In their current state, the technologies for making these measurements are very expensive, and the data that result from such studies will require massive computational power to analyze and pose challenges for data-sharing.
- Q.** If you could choose the next steps for exposome research, what would they be?
- A.** I feel that the message about the exposome needs to be twofold, and the two may at first sound mutually exclusive. First, we know that we can do this. We have the proof of principle that you can find unexpected associations that you would not have hypothesized on the basis of the current state of the science from reductionist approaches. There is more work to do. The findings need to be confirmed, and we need reductionist research to explain the nature of the associations is—Are they real? Are they causative or correlative? What do they teach us about fundamental biology or the disease process? Second, given the limitations of the proof-of-principle studies, it is clear that we need to invest substantially in the development of new technologies. There is a truism in the computer science community called Moore's law that, in paraphrased form, states that the computational capacity of a chip will double every 18 months. The law has held since the early 1960s; more important, it has been found to hold for any number of technology-related fields, including genomics. Just over a decade ago, a "whole-genome" study consisted of looking at about 300 genes and cost in the neighborhood of \$10,000 per sample; these numbers are almost identical with those in the study presented by Patel. Today, thanks to a focused investment in technologies, any of us can get our complete genome sequence from a commercial source for about \$4,000. If we can mirror that technologic investment, the true power of the exposome will be seen.

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and weight). The researchers were able to identify environmental exposures associated with type 2 diabetes. They conducted regression analysis between each environmental factor and the fasting blood glucose threshold used to diagnose type 2 diabetes (over 125 mg/deciliter), adjusting for demographic factors and body-mass index. As is standard for a GWAS, Patel's team validated significant findings in more than one cohort. Because testing multiple associations can increase the odds of a false positive (a result that erroneously appears to be statistically significant), Patel used the false-discovery rate, a statistical tool roughly similar to but less strict than the Bonferroni

Comprehensive connection of environmental factors to disease is practicable if we use high-throughput analytic methods that are now common in genome-based investigations.

—Chirag Patel

correction used in a GWAS, noted Patel and Ivan Rusyn, of the University of North Carolina at Chapel Hill. Among the novel findings of this EWAS, Patel found that type 2 diabetes was positively associated with heptachlor epoxide, a carcinogenic pesticide, and γ -tocopherol, the main form of vitamin E found in the US diet. Among the known associations confirmed by the EWAS, Patel's group found that type 2 diabetes was positively associated with polychlorinated biphenyls and negatively associated with two types of β -carotene.

Rappaport argued that- omics techniques, perhaps with a focus on small molecules, should be applied to EWAS efforts to aid exposomic research. Patel and other meeting attendees noted that an EWAS also naturally leads to followup longitudinal and toxicologic studies to validate associations, infer causality, and elucidate mechanisms of action. Such followup is important, Patel and Birnbaum said, because an environmental factor associated with a disease in an EWAS might be an *outcome* of the disease, of a disease treatment, or of another environmental factor rather than a *cause* of the disease. In fact, Patel's team found numerous associations among their EWAS-assessed environmental factors. As Ivan

Rusyn, of the University of North Carolina, and others pointed out, such intercorrelated coexposures should be accounted for in future research.

Stanley Hazen, of the

Cleveland Clinic, demonstrated how integrating metabolomics with clinical research methods has illuminated the role of the gut microbiome in the development of cardiovascular disease (CVD). Hazen and his collaborators evaluated whether gut flora interacts with dietary factors in the development of CVD. In the first phase of the project, they used metabolomic techniques to identify small-molecule profiles predictive of risks of major cardiovascular events. Using high-performance liquid chromatography–mass spectrometry, the researchers found 18

metabolites in biologic specimens that distinguished between healthy control subjects and people who had recently experienced a heart attack, stroke, or death. In an independent prospective cohort, they found that three of the metabolites—choline, betaine, and trimethylamine *N*-oxide (TMAO)—were associated with CVD in a dose-dependent manner. Those three *biogenic amines* are all linked to the metabolism of choline and of phosphatidylcholine, a phospholipid—also known as lecithin (a component of egg yolks and other foods)—that is the major dietary source of choline, Hazen explained. The gut microbiome and the human host cometabolize ingested choline or phosphatidylcholine and release trimethylamine and flavin monooxidases (FMOs); FMO3 in particular oxidizes trimethylamine, converting it to TMAO. Betaine is produced when choline is oxidized. In a series of studies in humans and mice, Hazen and his team confirmed that the gut flora is required for the production of trimethylamine and TMAO.

In the second phase of their project, Hazen's team validated their findings in a series of cohorts, confirming the clinical utility of choline, betaine, and TMAO as predictors of CVD. For example, in a prospective cohort of 1,865 cardiology patients, they found that plasma concentrations of choline, betaine, and TMAO predicted CVD risk in a strikingly strong, dose-dependent manner after adjustment for such factors as age, sex, smoking, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol.

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In the third phase, Hazen's team used mechanistic studies to demonstrate the causality of the association between the three metabolites and CVD.

The universe of the microbiome is relatively uncharted when it comes to disease. It participates in many disease processes, but we are just at the tip of the iceberg.

—Stanley Hazen

In rodent feeding studies, they found accelerated atherosclerosis (hardening of arteries, a common symptom of CVD) in mice with an intact gut flora that were fed TMAO or a precursor (choline or phosphatidylcholine). Accelerated atherosclerosis did not occur in mice that were fed choline but could no longer make TMAO because their intestinal microflora was suppressed by oral broad-spectrum antibiotics. Thus, Hazen concluded, both the dietary stressor (high choline) and the gut flora are necessary to produce the increase in atherosclerotic plaques.

Hazen's work demonstrates the use of metabolomics to assess exposures associated with human disease. By further illuminating the microbiota-dependent mechanisms underlying such associations, his research informs the prevention, diagnosis, and treatment of CVD. Regarding prevention, for example, Hazen suggested that it may be helpful to reduce consumption of foods rich in betaine, choline, lecithin, phosphatidylcholine, and TMAO. Birnbaum

cautioned, however, that choline in particular is essential for brain development in children, so some amount of it must remain in the diet. The work by Hazen, Patel, and their colleagues can inform efforts to refine and integrate exposome-relevant technologies and methods.

The Future of the Exposome: Challenges and Opportunities

Meeting participants discussed a number of factors important for establishing the validity of exposomic research. Hunter Young, of Johns Hopkins University, cautioned that the demonstration of causality is complicated by intercorrelations among exposures. Longitudinal prospective cohort studies can provide hints as to causality, said Rappaport and Elliot. As Young argued, however, such studies are not sufficient to demonstrate causality; typically, Spira added, *in vivo* experimentation is also required.

The dynamic nature of the exposome also poses a challenge. Unlike the fixed genome, Elliot and Patel explained, the exposome changes with time and therefore cannot be assessed with a single measurement. The use of repeated sampling of cohorts can help to address that problem, and Nathaniel Rothman, of the National Cancer Institute, argued that modest-size cohorts with repeated samples are far more valuable than much larger, single-sample cohorts. Young added that continuous monitoring with

personal sensors could have great utility in characterizing the time-varying exposome. Exposures also vary quite widely among individuals, said Elliot, and statistical analyses can require large cohorts to ensure sufficient power.

Selection bias may pose a problem for some exposomic approaches. As Young explained, those who volunteer to participate in research may differ in some important respects from those who do not. Well-designed cohort studies should therefore complement volunteer-based data collection. Finally, Rothman and others argued, human studies must recruit from a variety of ethnic groups and regions, in part to allow the identification of universal risk factors.

Among the research gaps in exposome science, Patricia Mabry, of the National Institutes of Health, William Slikker, of the Food and Drug Administration (FDA), and others suggested that stress and diet deserve more attention. External personal

I'm excited about the technologies discussed here . . . They will help the nation to understand better what exposures are affecting population illness and long-term health and enable us to prevent disease better.

—Chris Portier

sensors to measure them would be quite useful. Stephen Edwards, of the Environmental Protection Agency, reminded participants that the real power of exposomic research will be the ability to

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consider how both genetic and environmental factors affect health.

Suzanne Fitzpatrick, of FDA, was among several participants who suggested resources that could greatly facilitate exposomic research, including data repositories, biologic specimen repositories, expanded metabolomic libraries, searchable databases of specimen repositories, and laboratories that are capable of processing particular types of samples. The US Department of Defense (DOD) has a repository with more than 50 million serum samples taken from every service member before and after each deployment, noted Craig Postlewaite, of DOD. Patel encouraged the environmental health community to consider developing a comprehensive, standardized method for storing exposomics data, such as the Gene

to detect a wide variety of toxic industrial chemicals. Such sensors offer the only way to assess individual longitudinal exposures for use in treatment, diagnosis, compensation decisions, and epidemiologic studies. Personal sensors and monitors could also help individuals and communities to learn how to protect themselves from exposures before people become sick, said Jerrett. For example, they could allow those concerned about high pollution exposures to measure and map the exposures in their neighborhoods and to communicate the results via social networking. Jerrett also suggested working with ecologists to try to create exposomes of the natural world; such ecosystem monitoring could provide information relevant to human health.

Ubiquitous sensing raises important privacy concerns, said Richard Denison, of the Environmental Defense Fund,

for additional, research-related uses of some of the same data. Researchers could also, in seeking possible solutions, look to other countries that have already implemented systems for the secure collection, integration, and sharing of individual-level data, Jerrett said. Edwards suggested that environmental health researchers could learn from the approach of personal genetics services (such as genome-sequencing services), which emphasize the potential for people to take control of, and learn from, their own genetic information for the benefit of their health.

Participants considered in some depth the relative merits of the two distinct approaches to exposure measurement—external and internal. In particular, they considered whether both approaches can be considered exposomic, how to integrate them, and how to increase funding for exposomic research. Rappaport argued that because external measurement of exposures is currently possible only for known exposures in a targeted, knowledge-driven manner, such an approach can contribute only indirectly to exposome characterization. External measurement is vital, but truly exposomic research, Rappaport continued, must by definition assess *all* exposures, known and unknown, with a data-driven approach. Only -omic techniques offer the potential to do that, he said. Rusyn and Jerrett argued for parallel efforts that use both major approaches to come closer to measuring the totality of exposure. In particular, they noted that many exposures, even some with important health effects, are not

Integrating with the broader science communities is essential if we are going to push the exposome idea forward. . . . We need to bring world-class scientists into this arena. If they are not going to come to us, then we are going to have to go to them.

—Stephen Rappaport

Expression Omnibus, a public genomics data repository developed by the National Center for Biotechnology Information.

Postlewaite and other participants described additional applications for personal sensing devices. In particular, Postlewaite said, DOD requires personal sensors that are lightweight, rugged, continuously operating, capable of integrating data over time, sensitive, specific, and able

and Christopher Portier of the Centers for Disease Control and Prevention. Jerrett and Intille agreed about the importance of privacy issues but argued that these problems are not insurmountable. For example, Jerrett noted, many private companies already routinely collect information on their customers after obtaining consent; perhaps researchers could work with such companies to obtain consent

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The Eco-Exposome



The National Research Council recently released a consensus report that presents the merits, needs, and future directions of exposure science. *Exposure Science in the 21st Century: A Vision and a Strategy* defines the scope of exposure science and outlines a roadmap to advance the science so that it “fully comple-

ments toxicology and risk assessment and can be used to protect human health and the environment better.” The report puts forth an expanded vision of exposure science, captured by the new term, eco-exposome. The eco-exposome extends exposure science from

the point of contact between a stressor and a receptor inward into the organism and outward to the general environment, including the ecosphere. The new vision drives home the concept that humans and other organisms are embedded together in a larger ecosystem—hence that “human and ecosystem health are inextricably linked.” The report also describes rapid advancement of tools and technologies in four fields—geographic information systems, ubiquitous sensing, biomonitoring, and models and information management—that has the potential to produce more accurate and comprehensive exposure-science data than ever before and that will achieve the broader eco-exposome vision.

More information and a free PDF of this report are available at http://www.nap.edu/catalog.php?record_id=13507.

MEASURING, *cont. from page 13*

yet detectable in biologic samples so are not currently amenable to -omic techniques. Such exposures almost certainly have a potentially quantifiable internal chemical correlate, as Martyn Smith, of the University of California, Berkeley, pointed out, even if that correlate has not yet been identified. Jerrett and others added, however, that external sensing offers the potential for continuous real-time measurement of exposure; this is an important feature for characterizing the temporally variable exposome, and it is not yet possible with -omics. It is clear that efforts to characterize individual exposomes will require both interdisciplinary research teams and integrated technologies.

The potential to integrate -omic approaches with ubiquitous external sensing is particularly exciting. One could, for example, combine measurement of the serum

exposome with neighborhood-level external measurements. Military bases worldwide might provide a good opportunity for this kind of integration, said Postlewaite, especially in light of the availability of serum samples. Mabry noted that communities concerned about increased disease incidence might provide feasible sites for such research. Communities in general might be more interested in being involved in such research if researchers shared data more openly with community members, perhaps through social media, Germaine Buck Louis, of the National Institute of Child Health and Human Development,

suggested. At least one study is already integrating external measurements with -omics, said Spira. Two large cohort studies developed by Spira and his team are using upper-airway biomarkers for tobacco-smoke exposure and personal monitors to provide objective measures of secondhand smoke exposure of children. Such integrative studies, said Elliot and others, will show how we can improve human health through specific environmental and lifestyle alterations and the development of novel pharmaceutical preventions and treatments.

Presentations and Discussions

from the **Individual Exposomes meeting** are available at <http://nas-sites.org/emergingscience/meetings/individual-exposomes/>

The Data Challenge

What types of data are important in environmental health research? How can disparate data sets be integrated? What are best practices for managing multifaceted data sets? These are a few of the recurring data questions that surface at Emerging Science meetings. New and emerging technologies, like the sensors and 'omics approaches described at the individual exposome meeting, generate valuable data. However, as one researcher commented, it takes "months and months to clean the data and merge it," with other data sources like air pollution levels and GPS locations, before scientists can produce knowledge from it. To learn more about these issues please join us for the next ESEH meeting and explore new methods to approach data challenges in environmental health science:

Integrating Environmental Health Data to Advance Discovery

January 10–11, 2013

Keck Center, Room 100 (500 Fifth Street, NW, Washington DC)

Research in biomedical sciences has undergone a dramatic transformation in the past two decades. Science is increasingly data-intensive, computational, interdisciplinary, and collaborative. This trend toward "Big Data" is pervasive throughout science and imposing new challenges for biomedical research along three dimensions, sometimes referred to as the three V's: volume, velocity and variety. Only through the coordination of all three dimensions will the full potential of Big Data be realized. Whereas significant progress has been made in the development of digital technologies, community-wide principles and resource management for some large and rapidly expanding data types

such as genomic sequences, integration of existing heterogeneous data sets (the variety component of the three V's) has lagged. This lag presents particular challenges for environmental health sciences, which is uniquely and inherently cross-disciplinary. This meeting aims to foster discussion about the need for enhanced data integration in environmental health sciences, evaluate the lessons that can be learned from integrative initiatives in other scientific domains, and strategize about how the community can take major steps toward improving data coordination and access to advance understanding about environmental effects on human health.

Session 1: Data Integration – Strategies and Lessons Learned

This session will highlight past and ongoing efforts at data integration and identify lessons that can be learned from them. The goal of this session is to explore how these lessons can inform the environmental health science community about data coordination and access as the scope and diversity of environmental health data continues to grow.

Session 2: Environmental Health Science Data Streams

Session 2 will identify areas of science and data sets that can help advanced environmental health sciences and the associated data challenges.

Session 3: Data Management and Project Coordination

The final session will address strategies for coordinating and managing efforts to integrate heterogeneous data sets. How can environmental health data be better integrated? Will data integration efforts require centralized management? What can be developed and accomplished at the individual laboratory basis?

Creating a Biomarker Pipeline

by Elisabeth Stallman Brown, edited by National Research Council staff

Using the example of proteomics, Leigh Anderson, of Plasma Proteome, discussed the importance of a pipeline framework for biomarker research. Given the important and increasing scientific output in candidate protein-biomarker discovery, one would expect an explosion in the availability of new biomarkers for clinical use, such as cancer diagnosis and treatment. However, Anderson noted, FDA approves only about 1.5 new protein biomarkers per year for clinical use. The mismatch between protein-biomarker discovery and the diagnostic-biomarker approval rate suggests that there is “a defect in the pipeline for effective translation with biomarkers,” said Anderson. By taking a pipeline view, Anderson and his colleagues discovered that there is a major technology gulf between biomarker discovery and diagnostic platforms. The primary impediment to the development of clinically useful tests from candidate protein biomarkers, Anderson continued, is the lack of sensitive, specific assays that are *multiplexable* (able to be used to quantify multiple candidate biomarkers

The Biomarker Pipeline



Anderson encouraged meeting participants to think about -omics research as a pipeline that moves from basic biology and discovery to biomarker validation and translation to diagnostic methods for biomarker use in the clinic or for other purposes. He noted that pipeline thinking is common in the pharmaceutical world for drug development but is not as commonly used in health research. The pipeline framework will encourage researchers to think about the type of technology needed to move efficiently from one area in the pipeline to the next.

simultaneously) and capable of analyzing enough samples (typically around 1,500) to assess true clinical utility. To date, Anderson said, only two of more than 20,000 studies on protein biomarkers may have analyzed enough samples to weed out spurious findings.

If the explosion in biomarker discovery “is a reflection of the dangerous and seductive nature of really effective technology,” the bottleneck is the lack of effective technology to validate and translate discoveries for diagnostic purposes, Anderson said. In protein-biomarker studies, Anderson explained, most proteins are measured with immunoassays. Although very sensitive, immunoassays are expensive and are not easily multiplexable. An alternative technology, hybrid mass spectrometry–based assays such as peptide mass spectrometry,

have a number of advantages: they are multiplexable, insensitive to the complexities of protein structure, and able to quantify small molecules like peptides with high accuracy. Assay sensitivity can be boosted by stable-isotope standard capture with antipeptide antibodies (SISCAPA), a technique that enriches target peptides and depletes other peptides in a sample. For example, using SISCAPA, Anderson and his team improved the sensitivity of thyroglobulin, a protein biomarker used to detect the recurrence of thyroid cancer. SISCAPA also allows one to run peptide mass spectrometry in multiplex with large numbers of samples and potentially to aid biomarker validation.

Pipeline thinking, from basic biology through clinical use, is necessary to advance proteomics and ultimately exposomics.

MEETING INFORMATION

Meeting Presentations

Would you like more details about the individual exposome or other Emerging Science for Environmental Health meeting topics? Descriptions, agendas, presentations, and newsletters for our meeting topics are available through our Web site. We invite you to subscribe to our Listserv for the latest information about upcoming workshops, available newsletters, and other Emerging Science for Environmental Health activities. For more information, visit:

<http://nas-sites.org/emergingscience/>

Upcoming Meetings

Integrating Environmental Health Data to Advance Discovery—January 10–11, 2013

Previous Meetings

Exploring Human Genomic Plasticity and Environmental Stressors: Emerging Evidence on Telomeres, Copy Number Variation, and Transposons—October 4–5, 2012

Biological Factors that Underlie Individual Susceptibility to Environmental Stressors—April 18–19, 2012

Emerging Technologies for Measuring Individual Exposomes—December 8–9, 2011

Applying 21st Century Toxicology to Green Chemical and Material Design—September 20–21, 2011

Mixtures and Cumulative Risk Assessment: New Approaches Using the Latest Science and Thinking about Pathways—July 27–28, 2011

Interplay of the Microbiome, Environmental Stressors, and Human Health—April 27–28, 2011

The Use of In Utero and Post-natal Indicators to Predict Health Outcomes Later in Life—October 14–15, 2010

Stem Cell Models for Environmental Health—June 3–4, 2010

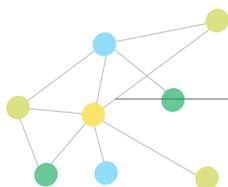
The Exposome: A Powerful Approach for Evaluating Environmental Exposures and Their Influences on Human Disease—February 25–26, 2010

Computational Toxicology: From Data to Analyses to Applications—September 21–22, 2009

Use of Emerging Science and Technologies to Explore Epigenetic Mechanisms Underlying the Developmental Basis for Disease—July 30–31, 2009

About the Committee

At the request of the National Institute of Environmental Health Sciences, the National Academies forms the Standing Committee on Use of Emerging Science for Environmental Health Decisions to facilitate communication among government agencies, industry, environmental groups, and the academic community about scientific advances that may be used in the identification, quantification, and control of environmental impacts on human health.



EMERGING SCIENCE FOR ENVIRONMENTAL
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