EMERGING SCIENCE FOR ENVIRONMENTAL HEALTH DECISIONS

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# The Biology of You

*–by National Research Council staff*

What makes you, you? From a biologic perspective, a common answer is, your genes. The answer seems simple enough—or is it? Certainly, many of our traits are coded in our genome and passed down from parent to child. But as scientists explore questions about why people differ—what makes us healthy, and what makes some of us susceptible to developing a disease—evidence suggests that there is more involved than just "your genes." Variation in human populations is enormous, so either the possible genetic sequences are indefinite or perhaps there is more to "you" than genetics alone.

Understanding human variability is important in medical and public-health communities. Discussions about the appropriate public exposure limits for environmental pollutants or the effectiveness of vaccines and medical treatments can be better informed with improved insight into who is and how many are at risk because of biologic differences. Consequently, some scientists argue that more research on human variability is both practical and urgent.

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# Biologic Factors That Underlie Individual Susceptibility

*–by Kellyn Betts, edited by National Research Council staff*

On April 18–19, 2012, the National Academy of Sciences Standing Committee on Use of Emerging Science for Environmental Health Decisions (ESEH) hosted a public meeting on the state of the science regarding biologic factors that govern how people vary in their responses to environmental exposures. A 2010 National Research Council report, *Science and Decisions: Advancing Risk Assessment,* noted that it is difficult to estimate average population risk without understanding individual risks. In other words, to address population susceptibility to environmental stressors, it is critical to address individual variability. Thanks to emerging molecular techniques, scientists are gaining a new understanding of inherent differences among people. That information can be used to predict how people will differ in their susceptibility to environmental stressors and to inform risk-assessment and public-health practitioners who are tasked with protecting vulnerable populations.

> ***Understanding individual variability is central to understanding susceptibility, identifying vulnerable populations, and understanding mechanisms so that we can identify and develop methods to intervene for the most vulnerable. It may lead to novel treatments and public-health interventions for environmental health problems.***
>
> **—John Balbus**

Why does one person fall ill after exposure to a particular environmental stressor and another remain unharmed? Variability, the true differences in people's attributes, holds the answer. Variability can be

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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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caused by external factors, such as duration of exposure to a pollutant or a person's workplace environment. Endogenous biologic factors, such as genetics and pre-existing illness, are also sources of variability, William Farland, of Colorado State University, explained. Understanding variability is extremely important because "variability is inherent in populations," he emphasized; it's not going to disappear. John Balbus, of the National Institute of Environmental Health Sciences (NIEHS), noted that the ability to characterize variability at the individual level in human and laboratory animals is essential for the protection of human health and understanding variability is therefore the second goal of the newly released NIEHS 5-year strategic plan. Previous ESEH forums have addressed tools and technologies for characterizing exposure; the current meeting would focus on new methods and insights to help to characterize individual biologic variability, Farland said.

How much variability is there in human populations? Meeting participants described a number of endogenous sources of variability. Nathaniel Rothman, a senior investigator at the National Cancer Institute, discussed the scope of human genetic variation and described how genetic variations may contribute to disease. The amount of variability in humans "is striking," Rothman said. Genetic variance between people ranges from such very small differences as single-nucleotide polymorphisms (SNPs; variations in which a single nucleotide in the genome sequence is altered) to such very large differences as chromosomal rearrangements. It is estimated that there are about 10–12 million common SNPs, which have more than a 10% minor allele frequency (the ratio, in a population, of the number of chromosomes that

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## Variability, Susceptibility, and Vulnerability

**Variability**—the true difference in attributes due to heterogeneity or diversity. Variability is usually not reducible by further measurement or study, although it can be better characterized.


**Susceptibility**—the capacity to be affected. Variation in risk reflects susceptibility. An individual can be at greater or less risk relative to the an individual in the population who is at median risk because of such characteristics as age, sex, genetic attributes, socioeconomic status, prior exposure to harmful agents, and stress.

**Vulnerability**—the intrinsic predisposition of an exposure element (person, community, population, or ecological entity) to suffer harm from external stresses and perturbations. Vulnerability is based on variations in disease susceptibility, psychological and social factors, exposures, and adaptive measures to anticipate and reduce future harm, and to recover from an insult.

*To set the stage, Farland and other meeting participants referenced the 2012 National Research Council report* **Science and Decisions: Advancing Risk Assessment,** *which provides practical scientific and technical recommendations for improving risk assessment, including the definitions given above.*

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The Standing Committee on Use of Emerging Science for Environmental Health Decisions (ESEH) has explored many of the facets of human variability. This newsletter focuses on emerging science and approaches to identification and characterization of biologic variability in humans. ESEH meetings have focused on epigenetics, the microbiome, and how environmental exposures influence these aspects of human biology. Recently, the ESEH committee delved into genomic plasticity and the non-DNA elements of the genome that enable humans to adapt to environmental changes. The meetings have made it clear that the biology of what makes us individuals is complex.

2012 marks the fifth year of ESEH meetings that explore the new science of the human genome, epigenome, microbiome, and other biologic factors and how they interact with our environment. So, what makes you, you? The answer is not simple. Please join us in 2013 as we continue to explore the scientific advances that can help us to answer this question and the implications of the new science for environmental health decisions.

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carry a less common gene variant to the number that carry the more common variant); there may be 30–50 million uncommon SNPs, which have minor allele frequencies of 1–10%; and it is possible that there are more than 100 million rare SNPs, with frequencies down to 1%. In other words, variation is inherent in our genome.

Claudia Miller, of the University of Texas at San Antonio, emphasized the need to consider genetics and human exposure to environmental chemicals in the context of evolution. Most chemical pollutants are "novel substances" that were developed after World War II, Miller said. We must ask, What is the variability in human ability to metabolize and excrete these substances that were so recently introduced into our environment?

> ***There are so many SNP variants that some people wonder whether there might be variation in almost every one of the 3.1 billion base pairs in the human genome.***
>
> **—Nathanial Rothman**

## Emerging Technologies

Current methods for detecting genomic variability have focused mainly on DNA, such as the use of off-the-shelf chip technologies, candidate genes, and the newer "agnostic scans" that are possible with genomewide association studies (GWASs). The technologies for detecting DNA variance span molecular genetic methods for sensing smaller differences and cytogenetic methods for detecting larger differences, Rothman said. Today, off-the-shelf chip technologies provided by such companies as Luminol and Affymetrix are capable of interrogating about 10% of the most common SNPs. Rothman stressed that there is a "tremendous amount of genetic variation that so far has not been analyzed in association and genetic epidemiology studies." However, GWASs are enabling scientists to better discover links between genetic polymorphisms and obesity and diseases, including hepatic cancer, chronic leuokocytic leukemia, prostatic cancer, diabetes, and coronary arterial disease. Rothman emphasized that he expects an "explosion in the number of new genetic findings" as technologies for interrogating uncommon SNPs become available.

Rothman cautioned that genetic studies should not be conducted in isolation from other factors that contribute to variability. Integrating all factors that contribute to variability into the same study has the potential to provide mechanistic insight, clarify dose–response relationships, and make it possible to evaluate low-level risks more effectively. For example, Rothman and colleagues recently discovered that overlaying multiple risk factors for bladder cancer allowed them to differentiate risk subgroups. They developed weighted "gene scores" based on SNPs known to be associated with bladder cancer. The gene scores allowed Rothman and colleagues to sort people into quartiles of low, medium, and high genetic risk for bladder cancer. They applied the gene scores to male 50-year-old never, former, and current smokers. Whereas absolute risk for male 50-year-old current smokers is 6.2%, Rothman's method estimated a 9.9% risk for current smokers in the high-genetic-risk subgroup as determined by the gene score. In public- health terms, "eliminating smoking in 100,000 people who have the highest genetic risk could eliminate 8,000 cases of bladder cancer," Rothman said. Rothman hopes that the gene-score approach in his bladder-cancer research will serve as a model for looking at genetic and environmental factors involved in other diseases, but he noted that the methods used in the bladder-cancer research first need to be

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### Sources of Biologic Variability

- Sex
- Genetics and epigenetics
- Health status (new and pre-existing health conditions)
- Life stage and aging
- Microbiome

*Rothman, Farland, and other meeting participants described some of the biologically based factors that contribute to human variability and thus population heterogeneity. Much of the current research is focused on characterizing the sources of variability such as those listed above and their interplay with human behavior and environmental factors that give rise to a person's disease susceptibility.*

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replicated. Rothman added that integration may also identify new environmental health hazards and ultimately help researchers to develop more effective prevention, screening, and treatment strategies.

Scientists are also beginning to use cutting-edge technologies that go beyond DNA—including technologies that involve RNA, proteins, and metabolites—to explore other dimensions of the biologic variability of living organisms. Eric Schadt, of the Mount Sinai School of Medicine, has been focusing on identifying tools for investigating how perturbations affect living systems by looking beyond DNA. Pacific Biosciences has created what Schadt terms a super high-resolution microscope, officially known as Single Molecule Real-Time, or SMRT®, that capitalizes on recent advances in nanotechnology, molecular biology, and optics. The instrument enables researchers to observe the activity of single molecules, such as DNA or RNA polymerases, in real time, Schadt explained. The aim of using such technology is to get a better handle on the complexity of living systems to identify changes within and differences between individuals that may be caused by external perturbations. "Once we can understand the networks, we can move away from a one-dimensional single-gene view and look at how changes in one part of the system give rise to changes in other parts of the system.," Schadt said. The technology also enables researchers to look beyond internal molecular states and microenvironments to look at, for example, how a person's microbiome or the microbiota that the person encounters in the environment interacts with his or her DNA.

> ***We want to start modeling how perturbations, whether genetically or environmentally induced, are being propagated across the system.***
>
> **—Eric Schadt**

Schadt also explained how the technology has direct use in connection with public health. For example, in a single day, SMRT was able to sequence the *E. coli* strain from a 2011 virulent outbreak in Germany and compare it with strains collected from around the globe. The results, published in the *New England Journal of Medicine* last year, showed definitively that the virulent *E. coli* were enteroaggregative, not enterohemorrhagic as other researchers had suspected. Schadt's research group also discovered that the German outbreak strain acquired plasmids—including a shiga toxin gene—that caused greater virulence than other *E. coli* strains. The inserted viral genes caused epigenetic changes throughout the *E. coli* genome and as a result increased virulence in the host, Schadt explained. In short, the SMRT technology enabled Schadt and his colleagues to see where a bacterial virus punched into the bacterium and added its own genome and how the viral genome integrated into the host system. The researchers also found that the German outbreak strain exhibited increased antibiotic resistance because of horizontal gene transfer with enterohemorrhagic strains.

SMRT may be useful for real-time pathogen monitoring. In a pilot study, Schadt and colleagues analyzed sewage samples from a community in California. They were able to detect respiratory viruses and loosely correlate the increasing load of influenza virus in sewage with an influenza outbreak. They were also able to detect pathogens that are commonly associated with foods, such as peppers, tomatoes, and chicken. On the basis of that information, they could roughly estimate the dietary intake of the community, and this could be useful for characterizing nutritional differences between different populations in molecular-epidemiology studies, Schadt said. Such information is "directly actionable," he argued. Real-time pathogen monitoring not only facilitates outbreak detection but could provide information about environmental conditions, such as nutrition, that could serve as the basis of public-health interventions or other decisions.

## Testing for Variability

In vitro screening (cell-based or tissue-based assays) can fill in important gaps in 21st century toxicity testing related to individual variability, said Fred Wright, of the University of North Carolina at Chapel Hill. In vitro screening with human cells can be particularly useful in heritability analyses, identification of mechanisms that might underlie variability via genetic mapping, and characterization of average responses and variations among chemicals for priority-setting. Many of the principles established through in vitro work with pharmacogenomics, particularly cytotoxicity screening

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of anticancer agents, can be applied to the testing of chemical agents, he said. Screening of many human cell lines can unmask sources of heterogeneity that would otherwise be hidden. Chemicals that vary in their effects in the population may need to be ranked for further testing by using additional in vitro or in vivo approaches.

Harvey Clewell, of the Hamner Institutes for Health Science, cautioned that scientists must take care in the choice of cells to be used in vitro studies. He conducted a literature search of arsenic exposure and genomics that revealed that immortalized cell lines yielded results similar to those with primary cells, but tumor-derived cell lines did not.

In vitro systems yield only a partial view of variability and susceptibility to chemical hazards, noted Weihsueh Chiu, of the US Environmental Protection Agency (EPA). However, variability at the molecular, cellular, and tissue levels is integrated in animal and epidemiologic studies that examine whole organisms and populations, respectively. The integration can be probed by using measures of dose and effect biomarkers and clinical outcomes, which provide systemic linkages between exposure and tissue dose (pharmacokinetics); between tissue dose and systemic response, such as a change in hormone concentrations (pharmacodynamics); and finally between systemic response and the likelihood of a disease outcome. By linking to clinical outcomes, Chiu said, animal and population studies can incorporate integrated information on baseline risk and susceptibility, including variability in an organism's robustness in the face of perturbations and its ability to return to homeostasis after a challenge.

Animal and epidemiologic testing has some drawbacks in assessing individual variability, given that, as many meeting attendees commented, both the *dose* and the *host* determine whether an exposure acts as a poison. Animal studies have been handicapped in their ability to assess individual variability by their general use of a single strain of one or two species or an out bred stock, said John. E. French, of the National Toxicology Program. In addition, studies to evaluate the effects of chemical exposures typically are conducted only on young healthy members of inbred animal strains that have little genetic diversity, said Joel Schwartz, of Harvard University.

However, French proposed a laboratory-mouse resource, the Jackson Diversity Outbred (J:DO) stock available through Jackson Laboratories, that could be used to improve the assessment of individual variability and to develop population-based models for environmental exposures, toxicity, and disease. The J:DO mouse was created by Gary Churchill and colleagues from the Collaborative Cross stock, an advanced recombinant intercrossed line developed over the last decade by mouse geneticists led by David Threadgill, of North Carolina State University. The Collaborative Cross stock was created by random outcrossing of eight unique and genetically diverse inbred mouse strains: five laboratory-derived and three wild-derived. When the genetic diversity of the first Collaborative Cross inbred lines were developed and assessed, researchers

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## The J:DO Mouse

Siblings from randomly bred J:DO mice created from the Collaborative Cross random 8-way outcross. *(Images courtesy of Dr. Karen Svenson, The Jackson Laboratory)*

*French described some of the features of the J:DO mice that make them very useful for determining the wide range of variability and response to toxic exposure. The J:DO mice have obvious phenotypic differences, like size and coat color, representative of their genetic diversity. Every mouse also has either equal to or greater than 10% minor allele frequency. This helps illuminate the consequence of rare allele variants that occur very frequently, French said.*

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were able to observe over 45 million segregating SNPs—a number similar to that in humans, French said.

The J:DO stock's founding population was created from random outcross mating of 144 pre–Collaborative Cross male and female mice. In contrast, most outbred stocks used in toxicology have small founding populations—no more than two or three males or females each—and thus "limited genetic diversity," French emphasized. French and colleagues are testing the J:DO mouse's ability to represent individual variability in response to exposure to benzene. Their findings suggest that the mice can function as a tool to help scientists to analyze and define the range of variations in susceptibility or resistance to toxicity and disease. Their work also shows that the mice can aid in identifying candidate genes and regulatory sequences of causal mechanisms and functional validation through hypothesis-based research testing.

Schwartz outlined how epidemiology studies are useful for looking at sources of variability and susceptibility. For example, epidemiology studies have demonstrated that the association between bone lead and heart-rate variability is pronounced in patients who have metabolic syndrome. They have also demonstrated that air pollution is associated with many health outcomes that are common in people who have diabetes. The collection of such studies indicates that diabetes may be an important modifying risk factor in the effects of air pollution or lead exposure.

Schwartz emphasized that humans obviously have much more diversity in age, health status, genetics, and environmental exposures than is captured by classical animal toxicology studies. Consequently, if there is a threshold dose (such as a no-observed-effect level) of a particular toxin in humans, "we expect it to vary" from one person to another, he said. Classical toxicology studies often identify a threshold below which exposure to a given substance does not cause harm. Although individual people may have thresholds, a growing body of evidence suggests that such thresholds may not exist for the human population as a whole, Schwartz said. He argued that the conglomerate of variability factors in human populations (and hence in epidemiology studies) suggests a linear no-threshold response to environmental exposures at low doses. As an example, he pointed out that the latest research on lead and cognitive effects suggests that there is no threshold. Similarly, a followup that he conducted of the Harvard Six Cities study, which linked excess mortality to exposure to increasing concentrations of particulate air pollutants, showed that the effect of exposure to particles is linear down to extremely low concentrations, approaching the background concentrations. Farland commented that "the impact of individual thresholds on population distributions of response is something that we really need to look at."

> ***If we can identify the sources of variability and the distribution of susceptibility, we can provide important information for decision-making.***
>
> **—Joel Schwartz**

Schwartz pointed out that evaluating geographic distributions of risk and of incremental increases in risk would be extremely valuable. Such a strategy would allow researchers to evaluate both socioeconomic and biologic factors that modulate risk and could be an important tool for planning interventions and improving public health. He also argued that regulators need to start thinking about how to use epidemiology studies in the exposure range of interest in setting standards and about how to use information from the studies in identifying sources of variability.

"The challenge is to integrate different levels of biologic organization when you have different data streams that are interrogating different levels," Chiu said. As you move from the molecular level to the level of the whole organism—that is, to greater levels of biologic organization—more and more sources of variability come into play, he explained. Testing with molecular biochemical assays can identify variability in the rates of reaction in situations in which people who have different genetic backgrounds have enzyme affinities that differ slightly. In a cell-based assay you can also detect differences in the intracellular network that is responding to a given chemical concentration, which can be used to generate some sort of bioactivity measure. However, variability in one enzyme is integrated into systems and networks as you move up to the whole-organisms

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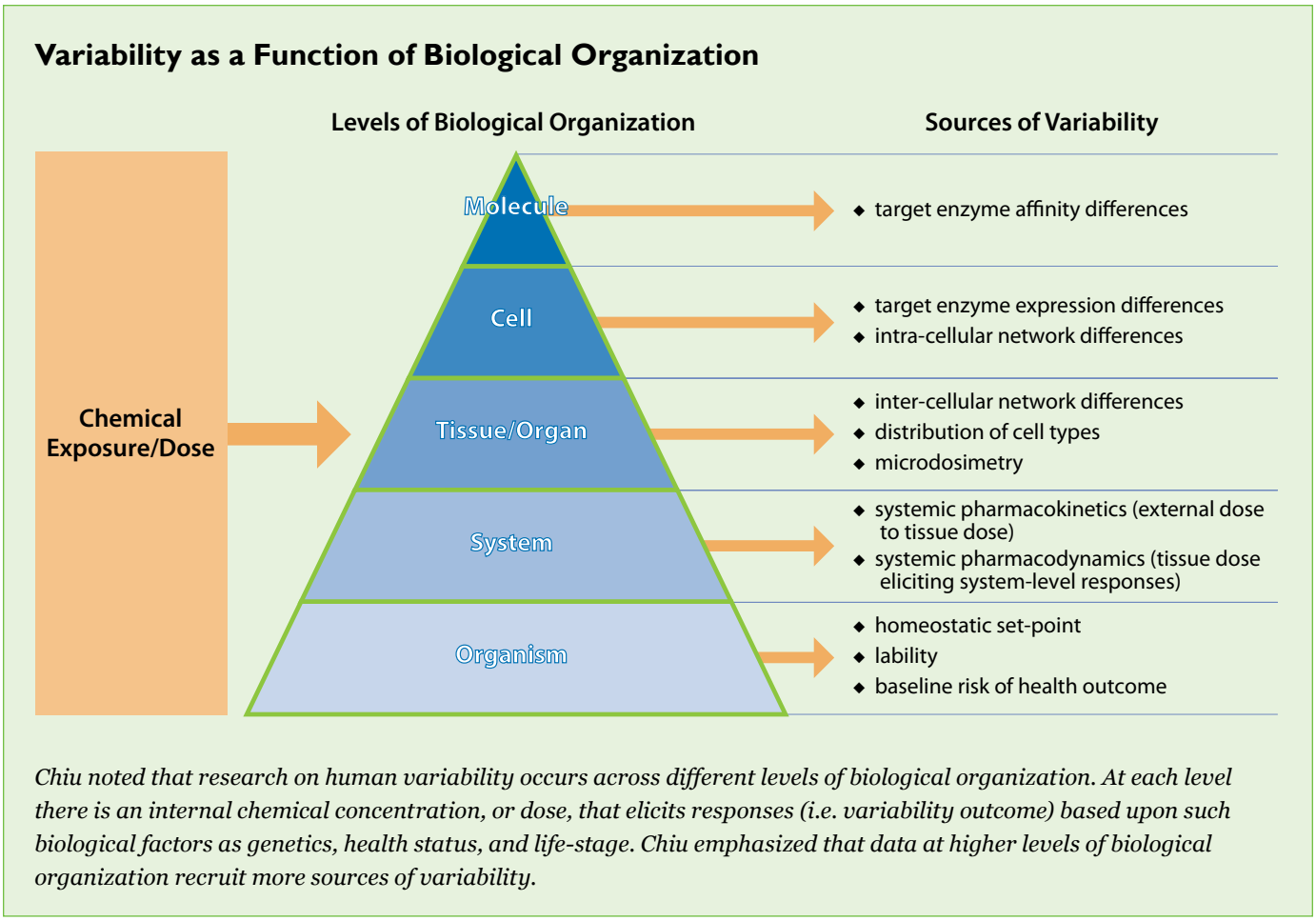


*Chiu noted that research on human variability occurs across different levels of biological organization. At each level there is an internal chemical concentration, or dose, that elicits responses (i.e. variability outcome) based upon such biological factors as genetics, health status, and life-stage. Chiu emphasized that data at higher levels of biological organization recruit more sources of variability.*

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level. "That integration may amplify or dampen individual sources of variability," Chiu cautioned. Tools to model how sources of variability propagate through the system would help scientists to integrate the available data, he said.

## Variability Informing Risk Assessment

"Risk assessment is preventive medicine," said Mike Dourson, of Toxicology Excellence for Risk Assessment. If done appropriately, risk assessment prevents disease and reduces the workload of clinicians, he explained. In a typical assessment, risk assessors pinpoint a critical effect of a pollutant exposure, defined as the first adverse event or its known and immediate precursor that occurs as the dose increases. Risk assessors try to determine the most likely outcome in sensitive groups—not individuals—often on the basis of data on experimental animals or another group of humans, Dourson said. However, the available data that can be used for risk assessments are often insufficient. Consequently, EPA, the Food and Drug Agency (FDA), and other risk assessors use defined uncertainty factors (also called safety factors) when toxicity data or other data are unavailable. Uncertainty is typically addressed by dividing a risk calculation, such as a no-observed-adverse-effect level (NOAEL) of the critical effect, by a default uncertainty factor of 10, which is generally considered to be conservative. "The practice of dividing the NOAEL by 10 implies population variability greater than [a factor of] 10," Dourson said. However, the degree to which uncertainty factors are overprotective or insufficient is usually unknown.

Duncan Thomas, of the University of California Los Angeles, reasoned that uncertainty about population variability has two dominant sources: imperfect knowledge about biologic systems and fundamental randomness in biologic systems. He described mathematical methods for quantifying uncertainty that are based in part on direct biologic measurements. However, he said, the greatest challenge is in dealing with "the unknown unknowns," the factors that contribute to heterogeneity that we do not know about and therefore cannot measure.

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## SCIENTIFICALLY SPEAKING

*Lauren Zeise is the deputy director for scientific affairs in the Office of Environmental Health Hazard Assessment of the California Environmental Protection Agency. She is also a member of the Standing Committee on Emerging Science for and contributed greatly to the planning of the meeting on biologic variability. Her research focuses on human individual variability, dose–response relationships, uncertainty, and risk. She shared her views on biologic variability, environmental health, and research looking forward.*

**Lauren Zeise**

Q. Why study biologic variability?

A. Protecting the public's health from exposure to environmental chemicals cannot be accomplished without explicit consideration of biologic variability. Government agencies, medical professionals, and businesses all make assumptions about biologic variability in their decision-making that affects intentional and collateral human exposures. The assumptions are often based on understanding developed in the 1980s of how much and why people respond differently. Emerging molecular and apical evidence from epidemiology, in vivo and in vitro toxicology, and systems biology is providing newer understanding and reasons to reassess current approaches. It is also showing the way to more targeted interventions both for medical decision-making at the individual level and for environmental decision-making for communities and other groups with regard to age, pre-existing health conditions, economic disadvantage, and other factors.

Q. Did you gain any surprising ideas or insights from the meeting on biologic variability?

A. First, I think that the meeting deepened my appreciation of the broad range of tools that can be brought to bear to understand variability. Single-nucleotide polymorphisms (SNPs) and genome-wide association studies have provided insight into genetic variability and how it may be related to susceptibility, as Nat Rothman discussed. But the meeting also highlighted some profound limitations of current tools that can be quite crude in identifying important genetic and epigenetic modifications that can affect disease states. Eric Schadt's talk reminded us through a case example that SNP analyses led the medical community astray in understanding gene targets for leukemia therapy. The silver lining was that the mistaken inference was corrected by using a powerful new approach that enabled the analysis of larger sequences and gene relationships. The meeting also discussed tools for examining variability due to epigenetic differences, but they clearly are limited in the scope, circumstances, and transience of epigenetic changes that they can examine. Second, at the population and subgroup levels, molecular biology is enabling improved inferences regarding dose–response relationships and sensitive groups. Joel Schwartz and Nat Rothman provided striking examples of reduced and enhanced susceptibility associated with genetic polymorphisms that code for activation and detoxification enzymes, behavior, and other nongenetic factors.

Q. Considering the importance of exposure variability in environmental health, is focusing research on biologic variability putting the cart before the horse?

A. Exposure variability clearly is important. Biomonitoring, -omics methods, and new environmental monitoring tools to improve understanding of individual exposures, environmental-exposure hot spots, and variation clearly hold promise and have been discussed in previous meetings. Biologic variability drives population risk that occurs from exposure, so to protect public health and target interventions wisely we need to pay attention to it. In addition to genetic factors, Joel Schwartz showed substantial variability in response seen epidemiologically due to socioeconomic factors and how one might use this and other information to model risk in susceptible populations. His theoretical simulation demonstrated, using reasonable assumptions, that some sensitive groups may face an inordinately high risk of heart attack do to exposure to particulate matter—greater than a 20% absolute risk—whereas the

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Thomas demonstrated mathematically how an "unknown" genetic risk factor or risk modifier could contribute substantially to population heterogeneity without being accounted for by default uncertainty factors in risk assessment. He argued that the use of default uncertainty factors is inadequate to regulate "residual genetic heterogeneity" but acknowledged that risk assessors might be able to use GWAS-based heritability estimates to inform their efforts.

Human variability data may be able to reduce uncertainty in risk-assessment calculations, Chiu argued. In most cases, risk assessment begins with animal toxicology data. Risk assessors use modeling and other techniques to quantify a benchmark dose for a point of departure based on animal dose–response data, he explained. The next step is to derive a human equivalent dose from the benchmark dose. That can be done through various empirical approaches, such as dividing by an uncertainty factor or allometric scaling (a method of accounting for differences in body size), physiologically based pharmacokinetic

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> ***I have great difficulty with the idea of using arbitrary safety factors of 10 to pretend that we are protecting the most sensitive individuals in the population.***
>
> **—Duncan Thomas**

**SCIENTIFICALLY SPEAKING,** *cont. from page 8*

majority of the population faces a considerably lower risk. That raises equity as an additional concern for public-health intervention and further motivates us to understand the basis of biologic variability in assessing environmental-health strategies.

Q. Will a shift in focus from population variability to individual variability require a change in risk-assessment paradigms?

A. An appreciation of individual variability can affect risk assessment in various ways. Stakeholders and decision-makers who gain an understanding of large susceptibility differences will call for the groups at greater risk to be identified and ask for some appreciation of quantitative differences. A number of environmental regulations require that susceptible groups be addressed in mitigation and standard-setting. Second, the average risk, or "population risk," is driven by the array of individual risks, so understanding of the risks in the susceptible groups provides a better basis for calculating population risk. Joel Schwartz illustrated how a variety of factors can increase risk; some groups face heightened risk, and others face very high risk. The people at increased risk are captured in the right "tail" of the risk distribution. Risk in the "median" person can then be a lot lower than the population risk. People in the right tail are targets for intervention. Finally, for common health conditions, such as asthma and cardiovascular disease, that are affected by environmental toxicants, a better understanding of biologic variability leads to better descriptions of the dose–response relationship at low environmental levels and of the need to depart from the assumption of a population threshold (below which no harm is expected). Those dose–response relationships can be used in economic assessments to estimate the benefits of possible regulatory actions.

Q. What research steps would you like to see next?

A. A number of subjects for research that resonated with me were raised at the meeting, and I will just highlight and elaborate on one. I would like to see research focus on how to manage the integration and interpretation of the large volume of emerging findings from the various relevant fields—medicine, informatics, basic biology and applied epidemiology and toxicology, and demographics. Data relevant to biologic variability in response to environmental stressors are being generated at different levels of biologic organization and at a deep and specialized level in different scientific disciplines, and the volume of data is enormous. The individual is a complex biologic system, and at the population level the complexity is magnified. Research on institutional and other structures that would facilitate progress to answer key questions related to public-health interventions in the face of such complexity is at the top of my list.

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(PBPK) modeling, or pharmacodynamic modeling. The last step is to derive a human equivalent dose for a sensitive population by using approaches similar to those noted above for an animal-to-human derivation. Chiu suggested three ways in which human variability data could be used to improve risk estimates, given the current risk-assessment paradigm: to develop default empirical human variability factors (in the absence of chemical-specific data), to derive chemical-specific or end point–specific variability factors, and to develop biologically based models that incorporate human variability.

Dourson, Clewell, and other meeting participants advocated for the development and use of chemical-specific adjustment factors (CSAFs) in lieu of default uncertainty factors. The World Health Organization's International Programme on Chemical Safety first developed the concept of CSAFs to have an agreed-on quantitative process for replacing the usual uncertainty factor of 10 with a factor that is more chemical-specific, Clewell said. The CSAF for toxicokinetic variability is based on a comparison of a directly measured or modeled surrogate for an internal exposure with a compound. Examples are the comparison of the area under the dose–response curve for an animal with that for a human and the comparison of an average "normal" person with a more sensitive person or population.

Pharmacokinetics vary in a population because of a number of interacting factors, such as height, weight, body fat, and health status, Clewell explained. He emphasized that it is particularly important to consider population variability when studying early life. With PBPK modeling, toxicologists can incorporate the wealth of data on age-dependent changes in organ weights, blood flows, and other well-studied biologic and biochemical processes into a model whose parameter values change with age. After determination of which enzymes metabolize the chemical of interest, it is possible to use a ratio to estimate early-life values on the basis of adult levels and to model the blood concentration of the chemical at different ages for the same exposure dose.

Clewell discussed data that illustrate average blood concentrations of two compounds, tetrachlorodibenzodioxin (TCDD) and nicotine, over the course of a human lifetime. Nicotine, which is water-soluble, mimics what you generally see with water-soluble drugs: exposure early in life tends to be proportionally greater than exposure of adults because of the ontogeny of the enzymes responsible for the clearance of the chemical. The time course for TCDD, which is highly lipophilic, is much more complex because a number of factors become important at different ages, he said.

Clewell also described an approach to modeling of population variability in toxicodynamics that is based on individual-level in vitro data. The National Institutes of Health is actively pursuing the use of induced pluripotent stem cells from a large number of people to investigate variation in susceptibility to disease. The same technology can be applied to investigate human individual variability in toxicodynamics. Clewell is working with induced pluripotent stem cells to see to whether they might offer a way of looking at population variability in susceptibility to chemicals

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## Risk Assessment–*Speak*[1]

**Benchmark dose:** a dose that produces a predetermined change in the response rate of an adverse effect in comparison to background.

**Dose–response assessment:** the component of risk assessment that examines the relationship between exposure to different doses of a substance and their effects.

**Hazard identification:** the determination of whether a stressor has the potential to cause harm to humans or ecologic systems and, if so, under what circumstances.

**Risk assessment:** the process of characterizing the nature and magnitude of health risks to humans or ecologic receptors posed by chemical contaminants and other stressors in the environment.

**Uncertainty factor:** a default factor (usually 10) used to derive reference doses or concentrations (doses or concentrations of exposure that are likely to pose no appreciable risk of deleterious effects during a lifetime) from experimental data. Uncertainty factors are used to account for such characteristics as variations in susceptibility among members of a population and uncertainty in extrapolating animal data to humans.

[1] Definitions are based on the EPA risk-assessment glossary available at http://www.epa.gov/risk/glossary.htm.

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by using cells that are in some sense normal.

Rothman noted that his research on overlaying multiple risk factors for bladder cancer also calls into question whether a safety factor of 10 is adequate. He predicted that studies similar to the one he described will uncover groups whose susceptibility is more than 10 times greater "very soon." From a public-health regulatory perspective, Rothman said, the goal is to think about the whole population to make the workplace safe for everyone, not just for the least susceptible. Many meeting participants agreed that the data presented by Rothman and others made a good case for using chemical-specific adjustment factors more widely. Dourson commented that if toxicologists have amassed chemical-specific data on a given substance, "we expect them to use them" for risk assessment.

> ***Traditional risk-management processes can consume considerable resources with little clarification of uncertainties, especially when there is large individual variability.***
>
> **—Nicholas Ashford**

Chiu also thought that human variability data could inform pathway-based approaches to dose–response assessment, as characterized by the 2007 National Research Council report *Toxicity Testing in the 21st Century: A Vision and a Strategy.* That report championed the concept of identifying and testing *toxicity pathways,* biologic pathways that, when sufficiently perturbed by an exposure, lead to toxicity or disease. Chiu asked whether panels of in vitro (cellular) assays could be used to assess individual variability in a high-throughput manner that would be consistent with the vision of the National Research Council report.

Nicholas Ashford, of the Massachusetts Institute of Technology, cautioned that an overcomprehensive and protracted risk-assessment process may unjustifiably postpone the implementation of desirable risk-reduction measures. He contended that a more synchronized risk-management process is needed. Rather than the sequential process currently used, he suggests a dual parallel approach for clarifying risk information and generating information about safer technologic alternatives. He also argued that if the technologic alternatives are substantially different, rather than marginally different, comparative, rather than full, risk assessments can be used. He suggested that chemical structure–activity relationships could be especially useful in such cases.

At the end of conducting a risk assessment of a compound, regulators try to identify critical uncertainties, said William Slikker, of FDA's National Center for Toxicological Research. With each review cycle, FDA risk assessors look at the literature that has been published since the last time a risk assessment or a review of a particular pollutant was conducted. Assessors are sometimes frustrated by finding that the followup needs that they identified in previous reviews "got lost in the documentation," he said. He pondered how to bridge the gap between the academics who often conduct the research and the risk assessors who conduct reviews—how to inspire both to investigate issues that could resolve key uncertainties and recognize that additional details on populations could be valuable in refining risk estimates. Slikker believes that the research and development arm of EPA does a good job of trying to bridge those communities, but the dots are not always connected.

## Implications for Personal Health Decisions

Advances in tools and approaches to measure human variability have implications beyond regulatory risk assessment. Peter Shaw, of Merck, discussed how improved information about human variability is helping the pharmaceutical world to develop more targeted therapies—in other words, personalized medicine. The optimal situation, he said, is "when you understand the biology at the start of drug development, and you have a target that either is expressed in a fraction of the population or is active in a population." In the optimal situation, both a drug and tests to identify populations that can benefit most from the drug can be developed at the same time. Shaw named several cancer treatments for which the optimal situation occurred—trastuzumab for breast cancer, crizotinib for non–small-cell lung cancer, and vemurafenib for late-stage skin cancer. But typically there is insufficient biologic evidence "to associate a molecular marker with a drug response" at the beginning of drug development, he said. Often,

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genetic or metabolic data about who may be best suited to receive a specific treatment is discovered after a drug has been approved by FDA. At that point, Shaw said, it is difficult to change clinical practice even when it is clear that some patients will benefit more than others from specific treatments. As a result, the pharmaceutical industry "is under pressure to produce medicines with improved benefit: risk profiles." New advances in genomic and health-information technologies are facilitating the pharmaceutical industry's ability to develop more personalized medicines.

Barbara Biesecker, of Johns Hopkins University and the National Human Genome Research Institute, discussed human variability in the context of genetic counseling. She said that genetic counselors help people to make personal health decisions—whether to continue pregnancies, whether to face a biologic risk and have more children, and whether to learn about their risk of the diseases for which there are genetic tests. But genetic risk assessment is based largely on rudimentary tools, such as family history or a specific phenotype, she said. The tools are limited in that they fail to include all risk factors, because many are still unknown. Biesecker emphasized that even when someone has a recognized pathogenic mutation in a known gene associated with disease, there remains variability in whether the person will develop the disease. She expressed excitement about how researchers are combining environmental and genetic factors to predict disease risks more accurately. It is unclear what the future paradigm for developing guidelines to interpret and provide genetic risk assessment will be, Biesecker said. We need to determine whether the information mediators will be health-care providers, health-care systems, regulators, the public, or sets of people who have been identified as at increased risk. Then we can begin to figure out how to communicate what they need to know because each group will require different approaches, she said.

## Moving Science Forward

Meeting participants discussed a variety of avenues to consider as the science on human variability moves forward. "We are clearly at a point, in terms of what kind of targeted research can be conducted, to advance this science," Farland said. Data integration and interdisciplinary problem-solving will both be important, he emphasized. Richard Woychik, of the National Institute of Environmental Health Sciences, called for a true systems-biology approach. Currently, research silos, including the genomics people who are sequencing genomes to find things like SNPs and experts in proteomics and transcriptomics, believe that they are conducting systems biology. However, systems biology encompasses everything that all these experts are doing, and we need better integration among different disciplines, he said.

Deborah Winn, of the National Cancer Institute, remarked that a large human population study with vast amounts of data on exposures, individual susceptibility factors, and multiple health outcomes is needed. In epidemiology, we often worry about generalizability, she said, but there may be times where we would benefit from focusing on groups, such as breast-cancer survivors or women who are at high risk for breast cancer. Nsedu Witherspoon, of the Children's Environmental Health Network, added that characterizing the range and distribution of biologic variability in children will help to protect both children and the general population.

Jim Kaput, of the Nestlé Institute of Health Sciences, asked how population studies can be designed to look at gene–environment interactions. If you look at genetic diversity maps, it is clear that most of our case–control studies probably lack sufficient power to detect differences because of genetic heterogeneity of populations, Kaput argued. He suggested that evaluating metabolic variability may be a better method for separating participants on the basis of responses to an intervention. He also emphasized that nutrition is an important aspect of the environment that bears on individual variability but often is not measured. Food compositions vary depending on where you grow the food, how you process it, and how you cook it; and there are bioactive substances in food that alter the expression of genes that are involved in the metabolism of toxins, drugs, and nutrients, explained Kaput. So you can have chemicals in food—such as fatty acids, sterols, and sterol esters—that bind transcription factors and alter the expression of genes that we know about. But when we measure toxic effects and drug effects, we rarely, if ever, measure nutritional environment, he said.

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# Toxicant-Induced Loss of Tolerance

*–by Kellyn Betts, edited by National Research Council staff*

Individual variability in response to chemical exposures may play a role in explaining why some people report being very intolerant of or susceptible to the presence of chemicals in their environment. Claudia Miller, a professor of environmental and occupational medicine at the University of Texas Health Sciences Center in San Antonio, told meeting attendees that what she calls "toxicant-induced loss of tolerance" affects millions of people around the globe.

Miller's research shows that the intolerance begins with an event, such as exposure to cleaning agents or pesticides. Some people who are exposed to such agents develop what Miller calls "loss of specific tolerance" and begin to respond more intensely to exposures to extremely low concentrations of substances in air, food, and drugs that did not bother them previously and do not generally affect most people. Miller has documented the syndrome in military personnel, industrial workers, people living in communities where they were exposed to chemicals, people exposed to chemicals in their homes, and occupants of so-called sick buildings.

Most people spend about 90% of their days indoors, and indoor air can include some unusual chemicals, Miller said. For example, complex mixtures can form indoors as a result of such phenomena as interactions of different volatile organic chemicals with each other and adherence of ozone to particles.

In the United States, near 15% of the population reports chemical intolerances, and 5% are afflicted by severe sensitivity that dramatically affects their lives. Cases have been documented in an array of demographic groups in more than a dozen countries.

Miller has developed a tool that she calls the Quick Environmental Exposure and Sensitivity Inventory (QEESI), which is used clinically in many countries to identify patients. The tool can be self-administered. A second tool for working with patients who have toxicant-induced loss of tolerance is the Environmental Medical Unit, Miller said. These units do not yet exist despite multiple recommendations by Congress, the National Research Council, and professional organizations, but Miller described how they would be constructed from materials that do not out-gas. Appropriate materials include granite floors and walls and porcelain ceilings, and the units would have an optimal ventilation rate. "Our achievements in genomics, proteomics, and so on over the last 20 years have only heightened the enormous potential of such a facility to help us understand the biologic effects of chemical exposures and the subtleties of individual exposure," she concluded.

> ***Just as adverse reactions to drugs have increased with the increased use of pharmaceuticals, we are seeing the phenomenon of chemical intolerance increase as xenobiotics increase in our environment.***
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> **—Claudia Miller**

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**INDIVIDUAL,** *cont. from page 13*

Farland noted that meeting presentations and discussions had made it clear that scientists need to do a better job of characterizing variability. "Susceptibility needs to be discussed in the context of variability," he said—both the quantitative and qualitative differences in susceptibility. New technologies may pave the way forward, but we must be careful "not to trade knowns for unknowns," he cautioned. Farland also urged participants to move beyond arguments about "whether a safety factor of 10 is great enough." Future environmental-health research needs to address sources of uncertainty. The science and discussion should focus on the "differences that we have not yet recognized because of our lack of understanding of variability but that would cause us to take different approaches to decision-making or communicating with the public," Farland said in closing.

**Presentations and Discussions** from the **Biologic Variability meeting** are available at http://nas-sites.org/emergingscience/meetings/individual-variability/

## National Academies Reports on Risk Assessments


- Risk Assessment in the Federal Government: Managing the Progress (the "red book," 1983)
- Science and Judgment in Risk Assessment (1994)
- Applications of Toxicogenomic Technologies to Predictive Toxicology and Risk Assessment (2007)
- Toxicity Testing in the 21st Century: A Vision and Strategy (2007)
- Phthalates and Cumulative Risk Assessment (2008)
- Science and Decisions: Advancing Risk Assessment (the "silver book," 2009)

The National Research Council has published many reports on risk assessment, beginning with the 1983 "red book," *Risk Assessment in the Federal Government: Managing the Progress,* through the more recent "silver book," *Science and Decisions: Advancing Risk Assessment,* in 2009. Meeting participants referred to these books as laying the framework for the use of molecular information to inform science-based toxicity decisions. To download free PDF copies of these books or to purchase them in hard copy, please visit http://www.nap.edu/.

## ESEH on YouTube

www.youtube.com/EmergingScience/

Beginning in 2013, all videos from emerging science meetings will be accessible through a dedicated YouTube channel. Please check out the presentations and discussions from our most recent meeting on *Integrating Environmental Health Data to Advance Discovery.*

# Gulf of Mexico Program on Environmental Protection and Human Health

*The Coast Guard attempted to burn off oil leaking from the sunken Deepwater Horizon rig, April 28, 2010.*

@iStockphoto.com/EdStock

The U.S. Department of Justice recently announced two legal settlements arising from the 2010 Deepwater Horizon disaster. BP Exploration and Production, Inc., and Transocean Deepwater Inc., the operator of the oil drilling platform, have agreed to pay the federal government $4 billion and $1.4 billion, respectively, in civil and criminal fines. Under the settlements the National Academy of Sciences (NAS) will receive a total of $500 million to establish a 30-year Gulf of Mexico (GoM) program. The GoM program will draw upon the nation's science, engineering, medical, and public health expertise to conduct studies, projects, and other activities that will contribute to the protection of human health and environmental resources in the Gulf of Mexico and on the United States' outer continental shelf.

Chris Elfring, the former Director of the Board on Atmospheric Sciences and Climate and the Polar Research Board within the National Research Council of the National Academies, is the director of the new GoM program. Chris is one of the NAS's most seasoned board directors and will bring to her new role sound judgment and enthusiasm for this new endeavor. Under Chris's directorship, the GoM program will be conducted solely at the direction of the NAS, based on scientific merit and integrity with emphasis on freedom of inquiry and independent, nonpartisan advice and recommendations. The settlement calls for the GoM program to engage in three areas of work: research and development, education and training, and environmental monitoring. Among its activities, the Gulf program will fund projects in the public interest. Neither BP nor Transocean will be involved in any decisions related to the program.

## MEETING INFORMATION

### Meeting Presentations

Would you like more details about individual variability 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 meetings, available newsletters, and other Emerging Science for Environmental Health activities. For more information, visit:

**http://nas-sites.org/emergingscience/**

### Next Meetings

***June 6 – 7, 2013*** ***Biological Platforms***

***September 19 – 20, 2013*** ***Topic to be determined***

Do you have an idea for a meeting topic? We would love to hear it. Please send us an email with your suggestion at eseh@nas.edu.

### Previous Meetings

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

***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 HEALTH DECISIONS NEWSLETTER

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