

Reflections on 2011

The field of environmental health science is increasingly multi-disciplinary and rapidly expanding. In the first 3 years of the Emerging Science for Environmental Health Decisions project, we have learned about new fields of science, such as epigenetics and stem-cell biology, that have important uses in and implications for environmental health research. We have delved into the intricacies and new discoveries initiated by computational toxicology. We have explored challenges in and new approaches to characterizing environmental exposures and predicting health outcomes of such exposures. And throughout all the meetings, we have observed how multiple scientific advances have the potential to improve our understanding of and change our public-policy approaches to environmental health hazards.

2011 is an exciting year. The year will have seen four meetings on an array of emerging topics and issues. We kicked off 2011 with the new world of the microbiome. The microbiome meeting held April 27–28, 2011 is the sixth in our series and is the theme of this newsletter. It drew attendees from

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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 presenters and participants at the meetings. The newsletter does not represent either formal consensus conclusions of the attendees or positions necessarily endorsed by the National Research Council.

Implications of the Microbiome for Environmental Health

On April 27 and 28, 2011, the National Research Council's Standing Committee on Use of Emerging Science for Environmental Health Decisions brought together experts in microbiology, toxicology, medicine, and ethics to discuss the basic science of and important new research on the interaction between the microbiome and the environment. Scientists increasingly recognize that this vast and complex collection of microorganisms (microbes), collectively known as the microbiome, plays a critical role in our health. The microbiome may strongly influence how our bodies' process and respond to environmental exposures, including food, drugs, and chemical pollutants. And environmental conditions can influence the microbiome's composition and function. Evidence that links disturbances in the microbiome's composition and function to environmentally relevant diseases and disorders—including asthma, obesity, and cancer—is mounting.

In her opening remarks, Linda Birnbaum, director of the National Institute of Environmental Health Sciences (NIEHS), said that research on the microbiome,

enabled by the Human Genome Project and the advances of genomics and related technologies “represents a new frontier...for the field of environmental health sciences.” Birnbaum also told attendees that NIEHS recognizes the enormous implications of the

Clearly, the microbiome is a frontier which we must explore, and exploring it will take a great deal of collaboration among a wide array of disciplines from genomics to microbiology to toxicology.

—Linda Birnbaum

interactions between chemical exposures and the microbiome and has begun exploring these issues.

Birnbaum and Vincent Young, of the University of Michigan, explained how the meeting complemented a November 2010 meeting, “Consequences of Xenobiotic–Gut Microbiome–

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Host Interactions.” Birnbaum said that the November meeting, organized by the NIEHS Superfund Research Program, drew attention to the lack of understanding of how xenobiotic exposures may affect the composition and function of the microbiome and how individual variability in the composition and function of the microbiome may alter the effects of xenobiotic exposures. The current meeting expanded on those concepts and highlighted preliminary research that demonstrated the interplay between the microbiome and the environment. Meeting organizer Helmut Zarbl, of the Robert Wood Johnson Medical School, said that when the planning group began organizing the forum a year earlier, members wondered whether the topic was “too far out on the edge.” But the large number of developments in the field in the intervening year has made it “clear that we were right on the mark and very timely,” he noted.

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across the microbiology, toxicology, and environmental health policy communities. Some of the meeting highlights have been featured in scientific publications and online blogs. Hundreds of people have viewed the presentations and audio recordings available on our website. Our summer and fall meetings delved into how to incorporate pathway-based toxicology into mixtures and cumulative risk assessment and how to integrate 21st century toxicology methods into green chemical and material design processes, respectively. On December 8–9, we held the

A Revolution in Thinking

Lita Proctor, program coordinator of the National Institutes of Health (NIH) Human Microbiome Project (HMP), introduced participants to the key concepts associated with the microbiome. She explained why microbes are important for all life. Proctor and other participants noted that when most people think about microbes, they think of disease. Young said that for well over 100 years the medical community had taught that “the only good bug is a dead bug.” It is true that some bacteria cause major human infection and disease, including cholera, foodborne illnesses, and malaria, but “the majority of microbes that

Are we encountering some unintentional consequences for the collateral damage that we do to several billion of our closest friends every time we give antibiotics?

—Vincent Young

second meeting on the explosive, Emerging Technologies for Measuring Individual Exposomes. More information about all the meetings, including presentations and audio recordings, is available on our website <http://nas-sites.org/emergingscience>

Now, we are in the midst of planning for 2012. Please mark April 18–19 on your calendar for our meeting on individual variability. If you have ideas for future meeting topics, we are happy to hear about them. The “Contact Us” page on our website includes a form for submitting meeting suggestions.

we interact with do not cause disease,” Proctor stressed. In addition to being important for producing foods—such as bread, cheese, and yogurt—and for generating at least half the oxygen in the atmosphere, microbes are key to producing and regenerating soil, and for their ability to degrade and recycle pollutants and toxins in the environment. Equally important, microorganisms play a major role in maintaining human health.

“One of the reasons why microbes are so successful on this planet is that they don’t limit their gene exchange to sex,” Proctor explained. “They share genes promiscuously across all kinds of habitats and under all kinds of conditions.” This gene-swapping in the absence of sex is called horizontal gene exchange. Research demonstrates that “there is lots of horizontal gene transfer occurring in the microbiome,” Proctor said. In addition, Ellen Silbergeld, of Johns Hopkins University (JHU), pointed out evidence that suggests that microbial communities within the microbiome preferentially exchange genetic material with one another. Silbergeld said that these preferential patterns of gene exchange “are an extremely efficient way to have immediate access to genetic information.” Silbergeld likened the exchange patterns to social networking or “tweets” in the microbial world. Proctor and Silbergeld both emphasized that the combination of horizontal gene exchange and widespread antibiotic use over the last 60 years has facilitated the spread of antibiotic resistance among bacteria. It also allows human pathogen reservoirs to exist in environments outside humans, for example, in farm animals. For these reasons and others, “we have to start thinking

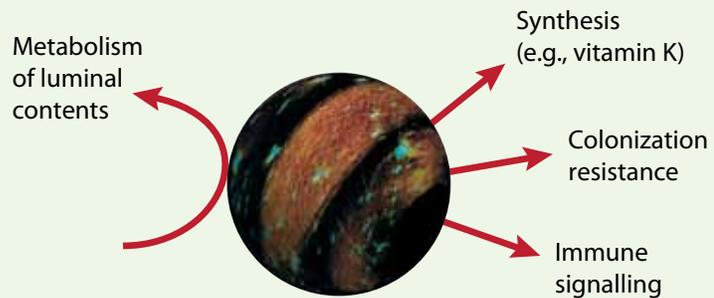
of the human and the environment as a continuum,” said Proctor.

What exactly is the human microbiome? And why is it important?

Proctor defined the human microbiome as “all microbial life and their genes” that live in and on the human body. All body sites—including the gut, the skin, the mouth, the nose, the ear, and the urogenital tract—have their own unique microbial communities. Recent estimates suggest there are around 1,000 species in our microbiomes, and they have roughly 20,000,000 genes. In comparison, the human genome includes 23,000 genes. That shows that “the microbial genetic signal is really important,” Proctor said. However, unlike our genome, which is inherited, each generation acquires its microbiome from the environment. For example, research demonstrates that the microbiome of babies delivered vaginally initially comes from the mother’s vaginal microbiome. But the microbiome of babies born by cesarean section arises from the skin of anyone who has handled the child, Proctor explained. The microbiome also changes with age. She described evidence that the

Key Roles of the Human Microbiome

Functions of the Microbiota



Immunity. Microbes help to develop and maintain our immune system and protect us from opportunistic pathogens.

Digestion and Energy Production. Without our gut microbes, we couldn’t digest a lot of the foods that we consume. The final end product of gut digestion and fermentation is short-chain fatty acids, which our cells need for energy.

Synthesis of Essential Chemicals and Nutrients. Microbes produce many beneficial compounds, including anti-inflammatory compounds, antibacterial products, and vitamins. Synthesized molecules play a role in immunity, digestion, energy production, and nutrition.

*Lita Proctor, Vincent Young, Michael Fischbach, and other a **YHb** participants spelled out fundamental roles the microbiome plays in promoting and maintaining human health.*

microbiomes of infants become increasingly adult-like as infants age, but the microbiomes of the elderly are different from those of infants and adults.

Proctor and other participants highlighted fundamental services

performed by the microbiome in promoting and maintaining human health, including immune defense, digestion, and nutrient production. Those are key functions that “we can’t live without,” Proctor told the audience. Microbes’ unparalleled ability to synthesize a wide array of biomolecules makes them “the best chemists on the planet,” said Michael Fischbach, of the University of California, San Francisco. Some of the biomolecules play roles that are important for their human hosts, and others are important for the microbes themselves.

Fischbach’s group actively studies the “natural products” formed by the microbes in the human microbiome. He pointed

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Human Microbiome Project (HMP)

Lita Proctor briefly described the scope of NIH’s Human Microbiome Project. The HMP aims to characterize microbial communities on different body sites in healthy adults and diseased tissues and to analyze the role of these microbes in human health and disease. HMP is primarily sequencing microbial genes, including genes of bacteria, viruses, bacteriophages (viruses that target bacteria), archeons (single-celled prokaryotic organisms that are genetically distinct from bacteria), and eukaryotic microbes such as fungi. All the data collected through the HMP is publically available for data mining.

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out that microbes both synthesize new molecules and modify existing molecules with which they come into contact, many of which are strong antibiotics or exhibit other medicinal properties and are potential sources of new drugs. For example, many of bacteria-produced molecules that Fischbach has studied are similar to the structures of known psychoactive drugs. Other microbes that Fischbach has studied can decarboxylate tryptophan, an essential amino acid in the human diet, to make tryptamine. Tryptamine can bind to receptors and trigger the production of serotonin, a neurotransmitter that contributes to feelings of well-being. The fact that the microbes invest much of their energy and genetics in producing natural products suggests that these molecules are important ecologically, Fischbach emphasized.

Although our microbiomes clearly are active in our bodies, widespread scientific recognition that trillions of microbes inhabit people dates back only about 15 years, said Margaret McFall-Ngai, a comparative animal biologist

Humans have made two mistakes. One is to forget that they are animals, and the second is to forget that they are embedded in the environment and that they evolved as components of the environment.

—Margaret McFall-Ngai

at the University of Wisconsin–Madison. This is “phenomenal in that we have known about pathogenesis for thousands of years and have known about the other organ systems for . . . hundreds of years,” she said. McFall-Ngai opined that the recent recognition that microbes are what she called “the 11th organ” was a “complete

revolution . . . in thinking about animals and humans.”

McFall-Ngai told the audience that the basic principles of animal-bacterial interactions can be derived by studying the patterns of genetic evolution among all animal species, both invertebrates and vertebrates. Using genetic phylostratigraphy (a statistical method of correlating the evolutionary origins of genes with specific macroevolutionary transitions), scientists have shown that most genes are prokaryotic (derived from single-celled organisms). That is, humans and bacteria share some ancient genetics. She explained that our joint ancestors date back to before the Cambrian explosion, which led to the creation of complex, multi-celled organisms about 520–540 million years ago. McFall-Ngai also pointed out that recent research by Tomislav Domazet-Lošo and Diethard Tautz, of the Max Planck Institute, shows that almost all the genes associated with known human diseases that are linked to genetic mutations are ancient. Those findings support the notion that considering the coevolution of microbes and humans can provide

insights into the role of our microbiomes in health and disease.

McFall-Ngai pointed out that how humans and their verte-

brate predecessors coevolved with their microbiomes, including viruses, may have affected how the adaptive immune system evolved. She hypothesized that the need for vertebrates to maintain a well-functioning community of microbes may have played a key role in promoting the development of the adaptive immune system.

The human immune system may be more a “management system” rather than a system for recognizing and destroying invaders.

Importance for Environmental Health

Young emphasized that the microbiome is a complex community that is essential for maintaining a balanced internal ecosystem. Research increasingly suggests that environmental stressors that disturb the balance between good communities of microbes and their human hosts may underlie many disease states, such as infectious gastroenteritis, obesity, and gastrointestinal cancers. After decades of focusing on eradicating “bad bugs” associated with human illnesses and infections, the medical community is coming to terms with the idea that there are “not only good bugs, but good communities of organisms that are helping with our health,” Young said.

Young proposed three paradigms to frame our thinking about host–microbe interactions and how they are influenced by environmental stressors and affect our responses to such stressors. In the first paradigm, the microbiome can directly influence the host immune response. Young described recent research in mice that demonstrated that intestinal microbes regulate the well-known TH-17 autoimmune response. This finding suggests that environmental stressors that alter the intestinal microbiota’s composition may affect the host’s immune response.

The second paradigm is also related to host immunity. Some microbes can influence the host immune response to prevent colonization of other, potentially pathogenic microbes, Young said. For example, research by Eric

Pamer and colleagues at Memorial Sloan-Kettering Cancer Center found that gram-negative organisms in the microbiome can send a signal to the host to produce an antimicrobial peptide that works against gram-positive microbes, such as Enterococcus bacteria. That is, the data suggest that “one organism can tell the host to produce something that can interfere with another organism’s establishment in that environment,” Young said.

The third paradigm is that the microbiome can influence aspects of the host’s physiology and thus influence how xenobiotics are metabolized. A team of researchers led by Jeremy Nicholson, of Imperial College, London, showed that when germ-free mice acquire microbiota, their expression of cytochrome P450 (CYP; a family of enzymes involved in metabolism of xenobiotics) and nuclear receptors (proteins involved in regulating the expression of some genes) increased. These mice also have an increased ability to metabolize bile salts (an indication of CYP metabolic function).

In addition to highlighting to the relationship between a healthy microbiome and host immunity, the three paradigms that Young discussed suggest that environmental stressors that alter the composition of the human microbiome may have unintended and detrimental consequences for our immune capabilities. Research increasingly suggests that some autoimmune diseases may reflect our altered interactions with the microbiota, and he speculated about whether improved sanitation and the widespread use of antibiotics over the last few decades have changed our interactions with our microbiota. Perhaps our immune

Human Microbiome and Disease

ORAL CAVITY
gingivitis, periodontitis, halitosis, caries

SKIN
psoriasis, cellulitis

RESPIRATORY TRACT
COPD, cystic fibrosis, otitis media, sinusitis, asthma, allergic rhinitis

GASTROINTESTINAL TRACT
IB, AAD, IBS, infectious gastroenteritis, obesity, GI cancer

GENITOURINARY TRACT
bacterial/fungal vaginosis, urethritis

*Young, Huffnagle, and other a *YHJ* participants noted many possible disease–microbiome relationships. Research suggests that perturbation of the microbiome by environmental stressors, including antibiotics, may lead to the development of many well-known and widespread diseases.*

system “gets bored” and has to come up with things like Crohn’s disease and multiple sclerosis because it has to attack something. “If we remove what it normally polices, is it now policing us?” he asked.

Tom Van de Wiele, of Ghent University in Belgium also provided an example of how ignorance of the human microbiome can have severe unintended consequences. About 20 years ago, Japanese physicians found that combining two drugs could be lethal because of how they affected the microbiome. The body metabolizes Tegafur, a drug used for chemotherapy for colorectal cancer, into 5-fluorouracil, which is toxic to cancer cells. Under normal conditions, a liver enzyme, detoxifies excess 5-fluorouracil, and the compound is cleared from the body. However when tegafur was combined with sorivudine, an anti-viral agent that is often provided to

cancer patients, eighteen patients died. Investigation showed that in some patients, gut microbes converted sorivudine into a compound that inhibited the liver enzyme responsible for detoxifying 5-fluorouracil. This example also demonstrates how differences in the microbiome contribute to inter-individual variability Van de Wiele said.

The Microbiome and Disease

Gary Huffnagle, of the University of Michigan, discussed new findings on the microbiome that are relevant to environmental health and challenge thinking about the etiology of disease. Huffnagle described research on a particular histocompatibility allele, HLA-B27, and the risk of spondyloarthropathies (multi-organ inflammatory diseases of the joints) in humans. Rats that are genetically engineered to

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MICROBIOME, continued from page 5 express the human HLA-B27 gene spontaneously develop inflammatory disease similar to the human autoimmune diseases associated with the gene. When the rats were engineered to be germ-free (that is, to have no microbiome), they no longer developed the inflammatory disease in the intestine or peripheral joints. In other words “if you change the microbiome, the rats can be protected,” Huffnagle emphasized.

Traditionally, the medical and research communities have thought of many diseases as being the results of genetic predispositions. More recent thinking is that many diseases result from interactions between human genes and environmental exposures, often referred to as the G x E relationship. Huffnagle and other meeting participants contended

I am going to argue that many diseases we think have genetic associations are really gene–microbiome associations.

—Gary Huffnagle

that the microbiome may be a critical component missing from the G x E discussion.

Huffnagle noted that diet constitutes an important environmental exposure with respect to the microbiome and gastrointestinal diseases. He described a study published in the *British Journal of Nutrition* in 2009 in which researchers showed that rats’ diet could play a major role in whether they developed the colitis associated with inflammatory disease. The rats in the study were fed diets either low in polyphenols (Golden Delicious apples) or high in

polyphenols (Marie Menard apples). The disease was ameliorated in the rats fed the high-polyphenol diet, he said. That finding supports the “idea that one of the ways that we can modulate the microbiome is by what we eat.” Diets that are high in colorful raw fruits and vegetables, which have high phenolic content, may play a favorable role in reshaping the microbiota.

Johanna Lampe, of the Fred Hutchinson Cancer Research Center, described how cancer risk may be influenced by a combination of microbial factors, host-responses, and host-microbe interactions. She emphasized two important themes for thinking about cancer and the microbiome: microbes as infectious agents and microbes as modifiers of exposure. “About 20% of cancers worldwide can be attributed to microbial infections,” Lampe said. Microbial

SCIENTIFICALLY SPEAKING

The Emerging Science a Year brings together scientists from a variety of backgrounds and disciplines to discuss how new tools, advances, and approaches may help to solve some of the pressing issues in environmental health. In this interview, **Linda Birnbaum, the director of the National Institute of Environmental Health Sciences (NIEHS) and the National Toxicology Program**, offered her perspective on the microbiome and its role in environmental-health research and on the committee’s *Life* a *more broadly*.

Q: How do the Emerging Science meetings contribute to environmental-health research and policy?

A: The meeting topics over the last year and a half have been extremely exciting. Some of the topics, such as Mixtures and Cumulative Risk, raise awareness of current research. Other topics, such as Microbiome and the Exposome, change how we think about environmental health. An important component of all the meetings is the interaction between the committee and the federal liaison group. This interaction helps to ensure that new ideas move from the bench to the bedside, from the laboratory to policy-making.

Q: Describe an “Aha!” moment in a recent meetings that stimulated new thinking or gave you a new perspective about environmental influences on health.

A: A few years ago, we knew little about the microbiome. Now we know that it is extremely important for health and is affected by environmental exposures. But we have little understanding of how environmental factors, such as diet, can alter our microbiome. That makes me wonder whether nutrition plans, such as the Atkins diet, are changing metabolism or are changing the flora and fauna that are in your gut. The microbiome opens up a whole new way of looking at things.

metabolism or the microbes themselves can play roles in many cellular processes involved in the development of cancer, including carcinogen metabolism, inflammation, immune function, and hormone regulation.

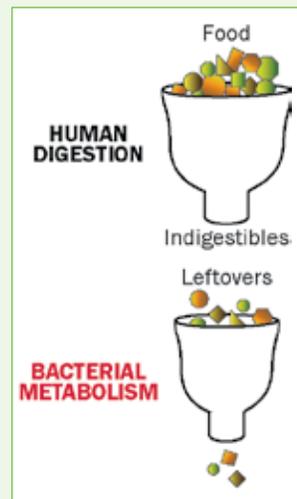
Lampe underlined the close relationship between the microbiome, diet, and cancer. Gut microbes metabolize dietary constituents that cannot be metabolized through other digestive processes, and these microbial metabolic processes generate bioactive compounds that may be either chemo-preventive or carcinogenic. For example, gut microbes are responsible for converting glucosinolates in some cooked vegetables, such as broccoli and cabbage, into isothiocyanates, compounds that are important in up-regulating enzymes to metabolize aflatoxin,

a known carcinogen. In contrast, gut microbes can produce *N*-nitroso compounds, compounds which may form cancer causing DNA adducts, from meat sources, such as hamburgers and some delicatessen meats. Either way, microbial modification of dietary exposures can affect cancer risk.

While interesting disease insights have been made, Lampe stressed that there is a great deal that scientists do not know. She and Young agreed that a major problem is that neither clinical nor in vitro studies have

Microbial Digestion: Processing Breakfast

Lampe, Van de Wiele, and other a YHb participants outlined microbes' role in digestion and metabolism. Gut microbes receive what the body is unable to digest via other processes and can metabolize these "leftovers" via reduction, hydrolysis, oxidation, and a variety of other processes.



allowed researchers to determine causality. Prospective studies that take the microbiome into consideration are sorely lacking, said Jim Goedert, of the National Cancer

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Photo: Steve McCaw

- Q:** If you could investigate any aspect of interactions between the microbiome, people, and environmental exposures, where would you start?
- A:** I would try to understand how some prototypical environmental exposures alter the composition and function of the microbiome and what the associated health effects are. I also would like to know how changes in the microbiome contribute to changes in susceptibility in differently exposed populations. Those are huge issues that can't be tackled all at once. Perhaps we first could conduct microbiome-wide association studies, a "Microbe-WAS", in the same way that we conduct genome-wide association studies. For bacteria, we could look at how 16s RNA patterns change in response to different exposures. Perhaps that could also be done for viruses.
- Q:** How do you think some of the new findings about the microbiome could influence environmental-health research at NIEHS?
- A:** Studying the interactions between the microbiome and our external environment is the tip of the iceberg for understanding human susceptibility. The use of genetic-mapping techniques to catalog the different microorganisms in the body is a particularly exciting frontier where we need to think about how to move forward and study relationships. NIEHS is thinking about a research initiative to look at interactions between environmental exposures and the microbiome.

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Institute. Goedert said that his agency is “working very hard” to devise ways of collecting fecal samples that are a low burden on human volunteers so that material can be collected for functional studies and for classifying microbiota and microbial gene expression levels. He counseled finding ways to measure the microbiome locally and to measure the metagenome (genetic material obtained from environmental samples), the diet, and the human genes systemically. “Metagenomics is going to help to identify the microbial species that are missing from the microbiome or are related to what is going on,” Goedert said. A growing body of research implicates microbiome perturbations in obesity and metabolic syndrome, a group of disorders that increases the risk of developing cardiovascular disease and diabetes. Matam Vijay Kumar, of Emory University described how several of his experiments on microbes’ effects on innate immune deficiency have uncovered a major link to metabolic disease. The gut’s specialized mucosal immune system detects and clears pathogens and keeps opportunists in check without harming beneficial microbes and host tissues. Kumar’s work involves toll-like receptors (TLRs), which can sense the presence of microbes and activate the innate immune system. For example, TLR-5 recognizes the flagellin protein that is associated with the presence of the whip-like flagella that allow many bacteria to move. Research has shown that TLR-5-flagellin interaction is crucial for the body to mount an immune response to some pathogens.

Locating the Resistome

Ellen Silbergeld, of Johns Hopkins University, argued that the expansive use of antibiotics in the United States is generating substantial evolutionary pressure on microbiota. Silbergeld stated that nearly 80% of the antibiotics produced in the United States are used as additives for livestock and poultry feed. As a result, we are beginning to see “extraordinarily complex patterns of multi-drug resistance” and “extraordinary distributions of resistant phenotypes” in microorganisms inhabiting domesticated animals and the environment. That is, the widespread use of antibiotics is creating a resistome, the collection of all resistance genes in pathogenic and commensal (nonpathogenic) bacteria.

Silbergeld equated the resistome to cloud computing in that it is a resource of genetic knowledge that can be stored anywhere—“even as naked DNA in soil.” So antibiotic resistance could be stored in a farm animal, in farm-animal waste, or wherever the farm-animal waste is distributed (such as soil and water).

The ability of microbes to incorporate external DNA into their own genetic backbone through horizontal gene transfer allows resistance to spread quickly. That has caused some researchers to propose that “both antibiotics and antibiotic-resistance genes should be considered environmental pollutants,” Silbergeld said. Expanding the concept of environmental agents to include the antibiotic-resistance genes spread by microbes could provide another route for environmental scientists to attack the problem of antibiotic resistance and the enormous public-health burden that that puts on our society, said John Balbus, of the National Institute of Environmental Health Sciences.

Kumar’s research with TLR-5-deficient mice exposed to the flagellin protein showed that the 10% of the mice spontaneously developed rectal prolapse, a severe form of colitis (inflammation of the colon), and 25% of the mice exhibited moderate to robust colitis. Further investigation revealed that the mice that developed colitis also harbored greater numbers of bacteria. He hypothesized that the loss of TLR-5 leads to failure to manage the microbiota, which in turn leads to persistent inflammation of the gut and colitis.

Kumar’s group also observed that the TLR-5-knockout mice that did not develop colitis consistently ate more and gained more weight than unaltered “wild-type” mice fed the same diet. Both the male and female mice become overweight.

Are these findings relevant to humans?

Preliminary work by Kumar and collaborators show that a small fraction of human patients exhibiting characteristics of metabolic syndrome are TLR-5-deficient. Like the TLR-knockout mice, these people had symptoms associated with the metabolic syndrome, including higher levels of blood glucose, elevated serum triglycerides, greater body-mass indexes, and high blood pressure.

Steve Rappaport, of the University of California, Berkeley School of Public Health, noted that discussions of environmental exposures that might affect human health should include “all kinds of endogenous processes that have so far escaped our attention.” Rappaport and Lampe said that

integrating -omics technologies into research will help to elucidate interactions across these processes. Rappaport emphasized that -omics will allow scientists to move away from strict hypothesis-based experimentation and reductionist thinking and toward discovery-based research.

Antibiotics—Good or Bad?

Meeting participants repeatedly emphasized the growing body of evidence that microbiome perturbations, particularly by antibiotics, can have unintended consequences. Les Dethlefsen, of Stanford University, talked about his recent work on how antibiotics affect the human gut microbiome. Healthy human volunteers signed up for a 10-month study that included taking two 5-day courses of ciprofloxacin, an antibiotic used to treat a wide array of microbial infections.

Although ciprofloxacin is considered to be a relatively mild antibiotic, it had a dramatic effect on the composition of Dethlefsen's study subjects' microbiomes. The microbiomes rebounded fairly quickly after two courses of antibiotics, but by the end of the study their composition was changed. What Dethlefsen found intriguing is that his study subjects reported no gastrointestinal or other symptoms despite perturbation of their microbiomes. He said this suggests that "whatever is causing a return largely to the initial community, it is not being driven by some gross functional deficiency."

On the other hand, animal studies by Huffnagle have shown that perturbations of the microbiome by antibiotics can render some laboratory mice more susceptible to allergic reactions. His group's work involved treating two inbred strains with

cefoperazone, a broad-spectrum antibiotic. The mice became more susceptible to the presence of mold spores in their nasal passages—to the point where they developed a striking allergic phenotype with changes in their airways and mucus production.

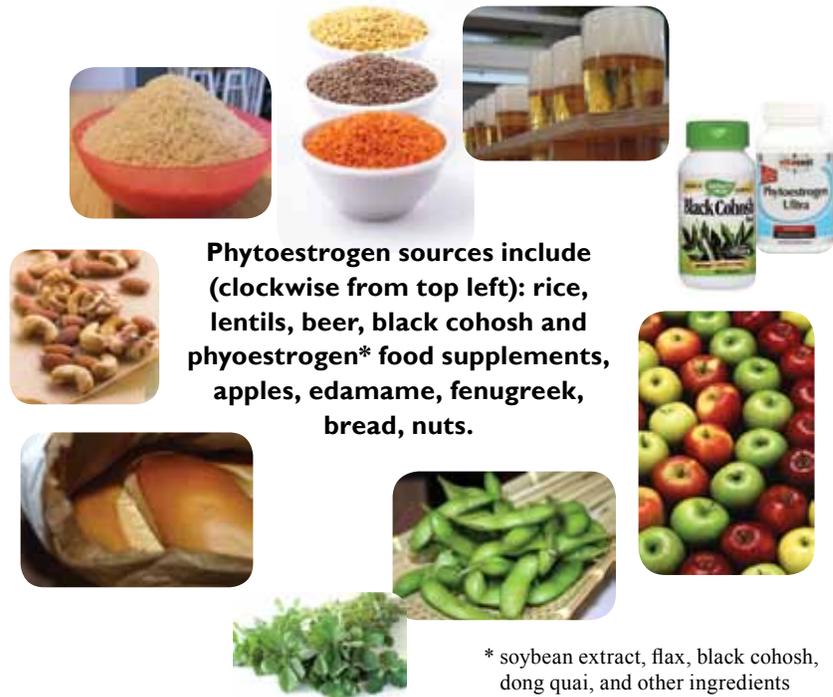
More recently, Huffnagle's group confirmed that cefoperazone restricts its activity to the gastrointestinal tract. That suggests that the gut microbiome is responsible for the new lung allergies in the

mice. He noted that although people typically think of these allergic responses as happening in the lungs, we swallow virtually everything that we inhale, especially particles.

Antibiotic perturbation of the microbiome may also affect the mammalian sleep cycle. McFall-Ngai mentioned recent research on rats focusing on the microbiome and the third wave of mammalian sleep. When the

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Phytoestrogens—An Environmental Contaminant?



Phytoestrogen sources include (clockwise from top left): rice, lentils, beer, black cohosh and phytoestrogen* food supplements, apples, edamame, fenugreek, bread, nuts.

* soybean extract, flax, black cohosh, dong quai, and other ingredients

Van de Wiele briefly discussed phytoestrogens, plant-derived compounds that may act like estrogens or block the activity of estrogens. Phytoestrogens are sometimes used to prevent hormone-related diseases and to balance hormone disruption during menopause. All kinds of functional and beneficial foods contain phytoestrogen precursors, including soy, hops, fruits, and vegetables. Activation of phytoestrogens into biologically active metabolites in most cases relies on gut microbes. However, there is huge individual variability between people in producing phytoestrogens. But whether phytoestrogens can be considered an environmental contaminant is under debate. Because the level of exposure to the bioactive compound is variable "caution is warranted when applying phytoestrogens as functional food or a nutraceutical," Van de Wiele said.

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microbiome was perturbed by antibiotics, the rats' sleep cycle was disrupted. McFall-Ngai said that this finding demonstrates that the microbiome is "tremendously integrated" into mammalian system and "is a network, just like the circulatory system." Including the microbiome in research on human health and disease will be an appropriately holistic approach, she emphasized.

Cheater Microbes

Dethlefsen defined our relationship with our microbiomes as more complicated than a "no-cost" mutualism whereby we provide the microbes a place to live and they metabolize foodstuffs that we cannot metabolize. He pointed out that we can have "costly mutualisms" with our microbes. For example, some *Lactobacillus* strains help to rid our systems of pathogenic bacteria, such as *Listeria monocytogenes*, without a direct benefit. In fact, the *Lactobacillus* cell dies as a result of attacking the pathogen. Evolutionary biologists say such costly mutualisms are explained by the shared fate of the host and microorganism, Dethlefsen said. That is, the microbes that live with us, by making us more fit derive the benefit of increased likelihood their descendants will be transmitted to the next generation.

The costly mutualism, however, can be exploited by a cheater. In addition "within a host generation, these cheaters have an advantage. They will be increasing in population," Dethlefsen said. A ramification of perturbing microbiota by, say, the use of antibiotics might be some loss of mutualist functions by the cheater microorganisms. The only thing that

opposes the spread of a cheating phenotype is the slow process of natural selection which occurs across multiple host generations. In a related discussion, Young noted that most microbial generation times are minutes to hours. When microbes are pressured by antibiotics or other environmental exposures, "they are not necessarily going to wait to see how it affects us during our generation time," Young said.

The Microbiome and Environmental Contaminants

Meeting participants discussed the microbiome's interactions with several "classic" environmental exposures, including metals and air pollutants. Van de Wiele presented research that showed that microbes can affect the bioavailability of toxic compounds. He described experiments conducted with equipment known as the Simulator of the Human Intestinal Microbial Ecosystem (SHIME), which mimics different digestive processes in the gut. His team used SHIME to investigate the extent to which colon microbes contribute to contaminant toxicity by altering the bioavailability of arsenic.

They found that the microbes could convert arsenate to arsenite, which is 10 times more toxic. The microbes actively methylated (added a methyl group, CH₃) to 1–10% of the arsenate and thereby produced monomethyl arsenate and monomethyl arsenite. Monomethyl arsenate is less toxic than arsenate, but monomethyl arsenite is as toxic as arsenite. The researchers were surprised to discover that the microbes also catalyzed the formation of monomethyl monothio arsonic acid, a

compound that is as toxic as or even more toxic than arsenite. Van de Wiele noted that the new data could help to explain findings of methylated and thiomethylated arsenic compounds in the urine of people who were exposed to sodium arsenate in drinking water in Bangladesh. Van de Wiele has also done work with arsenic-contaminated rice from China and shown that the bioavailability of the arsenic varies dramatically as the contaminated rice goes through the digestive system.

Silbergeld noted that documentation that the human microbiome can both methylate and demethylate arsenic is enormously important because of the implications for the toxicity and, by extension, chronic diseases associated with exposure to arsenic – and potentially other metals such as methyl mercury. New evidence that the human microbiome can both methylate inorganic mercury and demethylate methylmercury "challenges a great deal of our exposure assessments about mercury," she said. That our microbiomes may enable exposure to inorganic mercury, which is more toxic than methylmercury in its ability to disrupt the immune system has serious implications for regulation. Historically, regulators have focused mainly on human exposure to methylmercury, Silbergeld emphasized.

That raises questions about how other metals known to be bioactive may be affecting our microbiomes, said Bruce Fowler, of the Centers for Disease Control and Prevention National Center for Environmental Health. For example, some packaged salads are sold in plastic bags into which silver nanomaterials are incorporated as a preservative. Bacteria have had

silver-resistance genes for a long time, commented Anne Summers, of the University of Georgia. They cause problems in burn units, where silver and antibiotics are used as treatments, she said. Some of the metal-containing compounds are effective when they are used on a small scale in hospitals, but when manufacturers want to put them in every sheet and all the laboratory coats, and so forth, “that is inviting disaster,” Summers emphasized.

Metals are not the only environmental contaminants that may perturb the microbiome. In another experiment, Van de Wiele and his group incubated polycyclic aromatic hydrocarbons (PAHs)—including naphthalene, phenanthrene, pyrene and benzo[*a*]pyrene—into the simulated colon environment with active gut microbes. They found that although the PAHs are normally not estrogenic, incubating them with colon microbes produces compounds that have an increased affinity for the estrogen receptor.

Huffnagle’s research group is actively investigating the effects of cigarette smoke—which contains PAHs, such as benzo[*a*]pyrene—on the lung and gut microbiome in animals. Preliminary results are showing small changes in the gut microbiome and a change in immunity against enteropathogens, but implications of these changes are not yet clear. Dan Sharp, of the National Institute for Occupational Safety and Health, pointed out that an approach similar to Huffnagle’s might also be useful for evaluating how the microbiome of the lung responds to occupational exposures to noxious agents, such as isocyanates, chlorine gas, and ammonia gas.

Akbar Khan, of the DOD Defense Threat Reduction Agency, said that his agency might benefit from having a record of what troops’ microbiomes look like before and after they are deployed to different parts of the world. Khan said that military records show that different soldiers respond differently to vaccinations, and the microbiome may be playing a role. The microbiome may also influence how soldiers respond to medical countermeasures designed to protect soldiers against chemical and biologic weapons.

The Food and Drug Administration (FDA) is planning to evaluate the effects of medical devices—particularly implanted medical devices, such as cardiovascular implants and hip replacements—on the microbiome and vice versa, said Marilyn Lightfoote, who is a risk assessor for FDA.

Questions, Implications, and Challenges

Is how environmental stressors affect the microbiome a real concern? In reflecting on the shared evolutionary history of microbes and humans, Trey Thomas, of the Consumer Product Safety Commission, asked whether humans can adapt to increasing numbers of antimicrobial agents in consumer products. In response, Silbergeld pointed out that a growing body of evidence links the use of antimicrobials, such as antibiotics and metals, to the spread of microbes that are resistant to multiple substances. Dethlefsen said that microbial communities will probably continue to successfully adapt to the presence of antimicrobial agents in their environment but that the consequences of the adaptation for

humans are unclear. “We cannot assume that the beneficial effects that we are accustomed to getting from our microbes are favored in any selection that is based directly on the microbes or microbial communities themselves,” he said. Once microbial communities are modified, they may or may not continue to provide the benefits that we now enjoy.

The absence of microbiome information may affect the interpretation of toxicity-testing results, said Vince Cogliano, head of the US Environmental Protection Agency (EPA) Integrated Risk Information System (IRIS). He noted that federal regulatory agencies are putting a lot of effort into understanding the sequence of mechanistic events that lead to disease but have been overlooking many events that could be taking place. This, however, raises questions about the promise of using in vitro and predictive toxicity testing systems. George Daston of Procter & Gamble noted that the absence of the microbiome cell-based systems is no different from the absence of a liver or the endocrine system. He underscored the importance of learning enough about the missing aspects to create opportunities to reduce the noise and include the appropriate elements.

Daston emphasized that the future of toxicology will be a tiered approach that builds on 50 years of primarily animal toxicology studies. In the last few years, scientists have begun to assess the microbiome’s total functional capabilities on an -omics basis, which is where reasonable comparisons can be made between animals and humans. However, Martin Stephens, of the Humane

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Society of the United States, contended that using microbiome research to interpret results of historical animal studies should not become a major research effort. Stephens was skeptical about the extent to which scientists will be able to improve interpretation of animal toxicity results from past decades. He reasoned that microbiome research should instead aim at refining human-focused, pathway-based toxicity testing—the future as envisioned by the 2007 National Academies report *Toxicity Testing in the 21st Century: A Vision and a Strategy*. Lauren Zeise, of the California Environmental Protection Agency, noted that for some exposures, such as exposures to metals, there are many inconsistencies among animal species and in human–animal comparisons. These types of inconsistencies may be indicators of which exposures microbiome activity may be important.

Kerry Dearfield, of the US Department of Agriculture (USDA) Food Safety and Inspection Service, said that one issue that bears consideration is whether the interplay between microbial and chemical exposures could be considered an aspect of cumulative risk. He speculated that a chemical exposure that alters a person’s microbiome could also render the person more susceptible to a pathogen that would not normally be able to make a healthy person ill. Similarly, Dearfield raised questions about whether the continuous use of antibiotics in food animals could result in exposures of people that shift their susceptibility. The effects of eating food from animals that have been on chronic low-dose antibiotics is a topic that might be

worthy of further investigation, he said. Dearfield also pointed out that the microbiome is going to be an important component of the rapid–risk-assessment exposure-scenario models that his agency is developing. USDA has long focused on exposures to pathogens and is trying to address the risks posed by chemical residues in food products more effectively. Food animals may be exposed to chemicals through water, feed, air, and soil (particularly grazers). Dearfield said that one question they have is “after exposure, how much is actually getting to the meat product that humans consume?” This meeting made it clear that the microbiome can affect chemical uptake by both food animals and the people who eat products from exposed animals, he said.

Fowler posited that it may be possible to “re-craft” or tailor risk assessments by taking the microbiome into consideration. “We recognize that some members of the population are especially sensitive to chemicals as a result of such factors as age, life stage, diet, and nutrition,” he said. Research suggests that it is possible for people (or their microbiomes) to have an adaptive response to chemicals that could become adverse, he stressed. “I think the microbiome simply hasn’t been plugged into this, and it should be,” he concluded. Thomas agreed that the potential for sensitive populations to be disproportionately affected is a concern. Rita Schoeny of the US EPA Office of Water called attention to the finding that the microbiomes’ composition changes over a person’s lifetime.

I think what is badly needed is integration, and microbiome research is where NIEHS may show leadership and integrate the intramural and extramural communities.

—Ivan Rusyn

“Life-stage susceptibility is a major function of how we do risk assessment in EPA” she said. And she noted that the agency has given little consideration to life stages other than the neonatal period and childhood.

William Farland, of Colorado State University, agreed that the microbiome is fundamental to the assessment of risk. However, “we can’t turn our toxicology over on its head thinking that we have missed a lot of things,” he said. The microbiome may have important implications, but we have a lot of information on how animals respond to chemicals and other toxicants that should not be ignored or tossed to the side in light of new information on the microbiome, Farland cautioned.

Ivan Rusyn, of the University of North Carolina at Chapel Hill, pointed out that a funding is a major challenge, and the research studies discussed during the meeting are being conducted in a specific field, such as immunology or ecology, but efforts need to be made to integrate the disciplines. Lisa Chadwick, of NIEHS, noted that NIEHS would accept grant applications in this field of research. She said that NIEHS is interested in “understanding what makes some people more susceptible to adverse health outcomes from an exposure than others.” Schoeny pointed out, however, that the “microbiome is not on the radar in terms of policy-making,” so research is likely to get short

Microbiome and Risk Perceptions

Mildred Cho, of Stanford University, an invited speaker at the NAS microbiome meeting held April 27-28, 2011, discussed preliminary research on how the risks and benefits associated with research practices and findings about the microbiome are portrayed in scientific literature and by the mass media. She shared with meeting attendees the idea that risk and benefit “are essentially

values questions.” In a study based in part on an assessment of 270 scientific research articles from the peer-reviewed literature, “one of the things that was striking . . . was the near absence of any statements about the risks posed by microbiome research or its applications,” Cho said.

The statements that Cho’s group found centered around three types of risk: 1) epistemic

risks—risks that inhibit the production of knowledge, such as limitations of tools and technologies or uncertainty about how to conduct analysis; 2) risks to humans, such as cancer, which can be addressed by human microbiome research; and 3) risks posed by manipulating the microbiome. The latter, however, was discussed very little.

The general lack of discussion about risk is peculiar to this discipline, Cho said. It is probably “an indication of the emerging nature of microbiome research as a field,” she explained.

The analysis also showed that the papers contained many “statements that indicated that there were very fluid boundaries between us and the environment”—and a fluid definition of what the term “environment” means.

The articles that Cho and her group analyzed—and the meeting discussions—made it clear that the microbiome has several roles, she pointed out. They include being the source of beneficial microbes and being a repository for genes that confer resistance against antibiotics. Cho’s group also looked at articles written for the general public, which focused mainly on supplements of probiotics (that is, supplements that contain microbes thought to be beneficial to health) and prebiotics (indigestible food ingredients intended to stimulate the growth or activity of bacteria in the digestive system in ways claimed to be beneficial). The articles that the researchers

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shrift in budget decisions. She emphasized that researchers will need to be able to communicate effectively why the microbiome is important and how it can be used.

Farland said that the microbiome is an ideal subject for interdisciplinary problem-solving that may not be funded by individual institutes or agencies. He pointed out that federal agencies have a role in encouraging interdisciplinary research on topics like the microbiome through how they characterize their requests for proposals. He also noted that legislative decision-makers associated with some important subjects—including the Toxic Substances Control Act, the farm bill, and health-care legislation—could benefit by having an understanding of the microbiome and its effects.

Concluding Remarks

In summarizing the meeting, Silbergeld noted that the microbiome raises additional complexity in research and may cause us to question whether research in this field is going to be worthwhile.

“Is it going to enlighten us?” she asked. But the discussions during this meeting suggest that additional research is merited. The microbiome could very well be affecting the fate and transformation of chemicals in the environment, Silbergeld argued. She said that incorporating the microbiome into toxicology could help scientists to “rethink toxicokinetics and some of the biomarkers used to deduce the transfer of material from outside the body into the body.” And it might aid in understanding and predicting individual susceptibility, she said. Finally, Silbergeld emphasized that considering the microbiome in toxicity testing would restore the importance of immunology and evolution in environmental health sciences. Uniting scientific disciplines—environmental health, microbiology, immunology, and others—will help scientists to form better conceptual frameworks for understanding health and the etiology of disease.

—Prepared by Kellyn Betts
and Keegan Sawyer,
edited by Norman Grossblatt

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Learning More about the Microbiome

Research into the microbiome is advancing rapidly, and it benefits from contributions by scientists in many fields. The microbiome experts at the Emerging Science meeting highlighted a number of new and important findings. Below are a few of the exciting new findings on the microbiome and what meeting participants had to say about them:

People's microbiomes appear to fall into three general groups, according to a paper published in *Nature* a few weeks before the meeting [available on line at <http://www.nature.com/nature/journal/v473/n7346/full/nature09944.html>]. The work by the European Commission's MetaHIT consortium, which is investigating associations between human intestinal microbiota, human health, and disease, presents an analysis of 39 gut microbiome metagenomes—22 of which were newly sequenced—of adults in four European countries, the United States, and Japan. The researchers called the microbial clusters enterotypes, and they cross international and continental borders, races, ethnicities, sexes, and ages. Lita Proctor, program coordinator of the National Institutes of Health Human Microbiome Project, deemed enterotypes “a good structuring or organizing principle for looking at environmental health.”

The role that the microbiome may play in arterial inflammation was the subject of an article published in *Nature* in early April [available on line at <http://www.nature.com/nature/journal/v472/n7341/full/nature09922.html>]. A group led by Stanley Hazen, of the Cleveland Clinic Department of Cell Biology, reported identifying trimethylamine oxide (TMAO)

as a biomarker that is highly predictive of cardiovascular disease in Americans, and they provide evidence that the gut microbiome plays a role in generating it. Gary Huffnagle and Steve Rappaport, of UC Berkeley, termed this an important paper, although Rappaport noted that “exactly what TMAO is a biomarker of—the presence of choline, particular gut bacteria, metabolism of trimethylamine in the liver, factors related to arterial inflammation, or some combination of these—is unclear.”

Maternal antimicrobial peptides help hydra embryos to control bacterial colonization, according to work published in *Proceedings of the National Academy of Sciences of the United States of America* on October 19, 2010 [available on line at <http://www.pnas.org/content/107/42/18067.full>]. The hydra is a simple freshwater animal that is in many ways similar to a human intestine, and early hydra embryos develop outside their mothers' bodies. A team led by Thomas Bosch, of the University of Kiel, Germany, showed that antimicrobial peptides of the periculin family can affect the structure of the hydra's microbial community by selecting particular bacterial “partners” as the organism develops and eliminating undesirable microbes. Margaret McFall-Ngai, of Yale University, told meeting attendees that other work has shown that the hydra's microbiome is stable over time and across generations.

How diets shape the microbiomes of people in the developed and developing worlds is the subject of a 2010 article published in *Proceedings of the National Academy of Sciences of the United States of America* [available on line at <http://www.pnas.org/content/107/33/14691.long>]. The research by a team of scientists at the University of Florence, Italy, compared the gut microbiomes of children in the European Union (EU) and a rural African village. Most notably, the African children had a “unique abundance” of bacteria of the genera *Prevotella* and *Xylanibacter*, which are known to contain a set of bacterial genes for breaking down such fibers as cellulose and xylan and were lacking in the EU children. Les Dethlefsen, of Stanford University, called the study a “must read” for people interested in interhuman variability and international differences in the microbiome.

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analyzed included no discussion of risks posed by probiotics and prebiotics, she said.

Regulations and policy require precise definitions of such terms as *risk* and *environment*, Cho pointed out. She suggested that researchers in this field would do well to try to answer fundamental questions about the microbiome, including “What is health? What is disease? What does a healthy microbiome look like” before “jumping ahead to applications to specific diseases.”

*Prepared by Kellyn Betts and Keegan Sawyer,
edited by Norman Grossblatt*

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MEETING INFORMATION

Meeting Presentations

Would you like more details about the microbiome or other Emerging Science meetings? Archived presentations are available through our website. Please visit <http://nas-sites.org/emergingscience/> to access audio-synched PowerPoint or PDFs of the meeting presentations. Archived webcasts are available for the green chemistry and individual exposome meeting. Also, we invite you to subscribe to our listserv for the latest information about our forums, newsletters, and other Emerging Science activities.

Next Meetings

Individual Variability (Washington, DC), April 18–19, 2012

Omics Informed Risk Assessment (Washington, DC), June 14–15, 2012

Previous Meetings

Newsletter highlights of the meetings, archived presentations, and discussions are available online for the following Emerging Science meetings:

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

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 Application — 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.

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