EMERGING SCIENCE FOR ENVIRONMENTAL HEALTH DECISIONS

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It's Complicated

Since the completion of the Human Genome Project, there has been an explosion of biological data detailing our interactions with the environment inside and outside of our bodies. New approaches have been developed to tackle challenges like the issue discussed in this newsletter—our exposure to mixtures of environmental chemicals and other stressors—and address the impact on human health.

The Standing Committee on Emerging Science for Environmental Health Decisions creates the platform for these discussions. The Committee has brought leading scientists and policymakers together to enable a dialogue for emerging sciences, such as Epigenetics (July 2009), the Exposome (February 2010), the Microbiome (April 2011), and Mixtures (July 2011). Sophisticated tools with improved speed and sensitivity are discussed and the implications of findings are debated.

These forums are examples of how the science community is approaching human health in our complex environment—with rigorous discussion. In some cases, technology will be the limiting factor. In other situations, the scope is so massive that a discussion of what information to collect is needed, as in exposome science. This newsletter focuses on the topic of chemical mixtures and risk assessment, which brings questions from both arenas.

Cumulative Risk Assessment for Environmental Mixtures: New Approaches Based on Pathways

On July 27–28, 2011, the National Academies Standing Committee on the Use of Emerging Science for Environmental Health Decisions held a public meeting on new approaches for assessing the health risks posed by exposure to mixtures of environmental chemicals. Participants considered what criteria to use to group chemicals for evaluating the combined health effects of multiple agents and how new tools in biotechnology, computation, and exposure science can contribute to research on cumulative risk assessment of mixtures. The meeting also included discussions on the research needs and the regulatory implications of new approaches to mixture research.

Framing the Issue

At home and at work, inside and outside, we inhale, ingest, and touch hundreds or thousands of chemicals regularly. Some people may be exposed to more chemicals than others, perhaps through occupational exposure or because of their proximity to a contaminated site or an industrial area. As Linda Birnbaum, director of the National Institute of Environmental Health Sciences (NIEHS), and Linda Teuschler, of the U.S. Environmental Protection Agency (EPA), explained, we are exposed not to one chemical at a time but to multiple chemicals, and we are exposed to these mixtures repeatedly throughout our lives. Some chemicals can cause or contribute to birth defects, cancers, reproductive disorders, and other adverse health outcomes, so it is crucial to understand which chemicals are harmful, their doses and combinations, and in which circumstances they cause harm.

EPA, the U.S. Food and Drug Administration (FDA), and other federal and state agencies regulate tens of thousands of chemicals that are used in the United States. As part of its oversight, EPA conducts risk assessments to evaluate the potential health effects of these chemicals and to determine whether and how they can be used safely. Traditionally, risk assessments and regulations have focused on the effects of single chemicals, but this approach does not reflect the reality of our exposure in today's world, Birnbaum observed. Scientists and decision makers are

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This newsletter and additional information about the committee and its activities can be found at **http://dels.nas.edu/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.

THE NATIONAL ACADEMIES
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increasingly calling for *cumulative risk assessments,* which consider the combined effects on human health or the environment of exposure to two or more chemical or nonchemical stressors.

> ***One of the biggest limitations in risk assessment and to a certain degree environmental health science has been the difficulty in understanding and predicting the effects of multiple exposures.***
>
> **—Linda Birnbaum**

The risk assessment process is complicated. Risk assessors must account for multiple exposure factors, including dose, frequency, and timing of exposure, as well as other issues, such as genetics, concurrent stressors, and pre-existing health conditions of exposed individuals. Birnbaum and other meeting participants noted that risk assessments and the science that supports them often do not account for all of those variables adequately. Mixtures bring additional layers of complexity to risk assessment. Scientists and risk assessors have not reached consensus on the best criteria for selecting chemicals to evaluate together in a single cumulative risk assessment. It makes intuitive sense to group chemicals that occur together in the environment, said Ila Cote, of EPA, to address mixtures and co-exposures (exposures to multiple chemicals simultaneously). However, as several participants noted, we are exposed to numerous similarly acting chemicals from multiple sources; therefore, we need a way to group the chemicals with health effects that may add up from sequential exposures. Risk assessments are also challenged by the sheer number of chemicals that require evaluation. Because it is not possible to test all the chemicals to which we are exposed with traditional (primarily in vivo) toxicity analyses, noted Mike DeVito, of NIEHS, we must set priorities among them on the basis of high throughput (rapid) in vitro screening tests. Those assays are critical to advancing public health, DeVito said, but they also result in a wealth of data that can be hard to interpret. Birnbaum added that the threat posed by harmful chemicals depends on many variables, including those related to the exposure itself (such as dose) and those related to human biology.

Approaches to Cumulative Risk Assessment: The Journey or the Destination?

How should scientists determine which chemicals to evaluate as a group in scientific studies and in cumulative risk assessments? Typically, chemicals that occur together in the environment, are structurally similar or are known to share a *mechanism of action* or *biologic pathway* are grouped together. However, focusing on structural similarity may narrow the group of chemicals under consideration inappropriately, argued George Daston, of Procter & Gamble, in that agents that are

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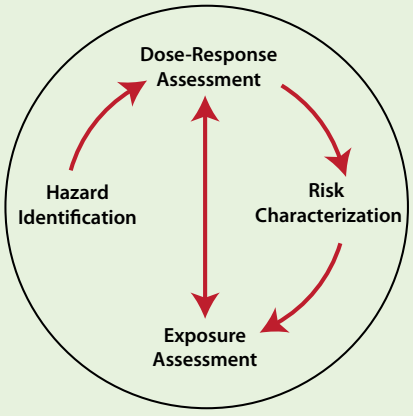
Important Factors in Cumulative Risk Assessment

Teuschler described some important considerations for the risk assessment of mixtures that are not necessary in the risk assessment of individual chemicals. She emphasized that single chemicals and mixtures share the risk assessment same paradigm, but in cumulative risk assessment, exposure and dose-response are linked. If the relative proportions of mixtures components change, the exposure and thus the dose-response will change.



Hazard Identification

- *Identify the effects from total mixture dose*
- *Consider the potential for effects resulting from joint toxic action*

Dose-Response Assessment

- *Consider mixture components potentially being below the individual thresholds*
- *Incorporate toxicologic judgment about similar toxic action of mixture components or between different toxic action of mixture components*

Risk Characterization

- *Evaluate whether data support assumptions about interactions and similarity of toxicity*
- *Consider uncertainty of changes in exposure*

Exposure Assessment

- *Consider changes in mixture compositions from chemical interactions in the environment (that is, i.e. mixtures released into the environment be altered before a person is exposed)*
- *Account for internal dose of several mixture components at the target tissue*

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structurally dissimilar may have common mechanisms of action. For example, despite having widely divergent chemical structures, ethinylestradiol, bisphenol A (BPA), and genistein have similar effects on gene regulation and thus may have cumulative effects in the body. Discerning a common pathway can be difficult. In the case of less-known chemicals, Teuschler said, we often know little more than the adverse outcomes with which they are associated. In many cases, the extent to which different components of a mixture share a common pathway may be unclear.

The 2008 National Research Council (NRC) report *Phthalates and Cumulative Risk Assessment: The Task Ahead* recommends that chemicals that cause the same or similar adverse health outcomes be included in a single cumulative risk assessment. The NRC recommendation has stimulated debate because grouping chemicals by common health outcomes is not the typical approach taken by EPA or other agencies conducting risk assessments. Chris Gennings, of Virginia Commonwealth University, L. Earl Gray, of EPA, and others agreed with the NRC recommendation, pointing out that chemicals that have distinct pathways sometimes result in the same disease, condition, or malformation. Daston, Gray, and other participants cautioned, however, that care must be taken not to define *common outcome* too broadly. Some participants advocated for the use of both common pathways and common outcomes in grouping chemicals for cumulative risk assessment.

Several approaches may be used to estimate cumulative risk. What is known about the mode of action of chemical mixtures influences which approach is chosen. As Teuschler explained, when the chemicals in a given mixture appear to have a common toxic mechanism or mode of action, *dose addition* is used to characterize the total mixture dose. A number of dose addition methods are available, Resha Putzrath, of the Navy and Marine Corps Public Health Center, and Teuschler said—and they differ in assumptions and often yield different results. When the components of a mixture appear to act in a toxicologically independent manner, *response addition* may be the best model with which to characterize the risk related to the mixture. Response addition models suggest that as long as each component in a mixture is administered below the dose at which adverse effects are seen, the mixture itself should not produce adverse effects, said Gray. In contrast, in a dose addition model, such a mixture could lead to adverse effects even if each individual chemical in the mixture is present at a "safe" concentration. *Integrated additivity* approaches are intended for mixtures that include both toxicologically similar and independent components. Teuschler suggested that one could

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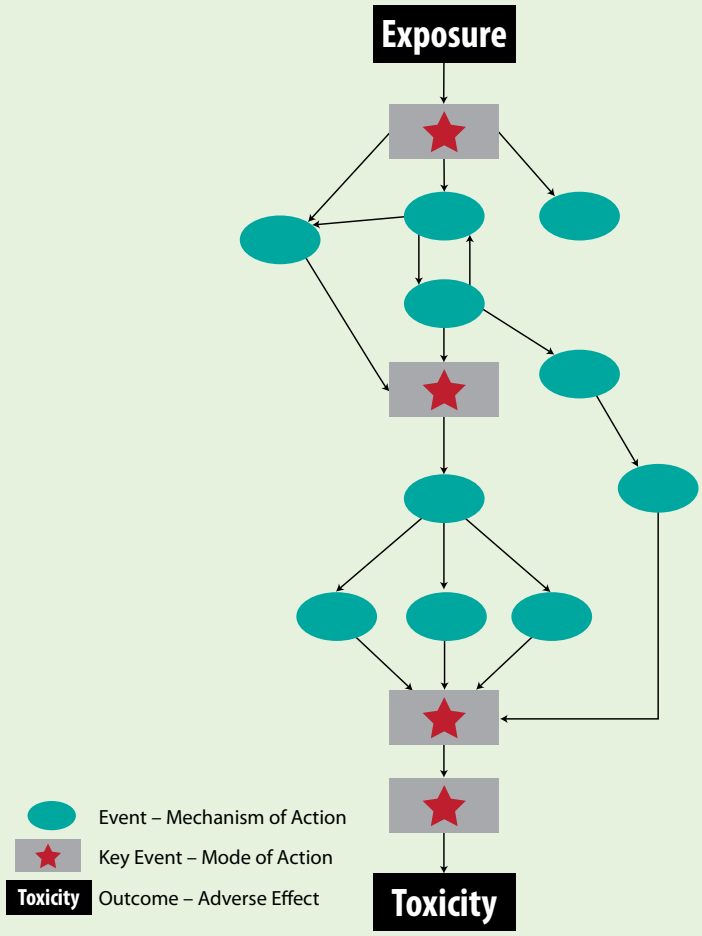
Understanding Toxicity Pathways



from U.S. EPA National Center for Environmental Assessment

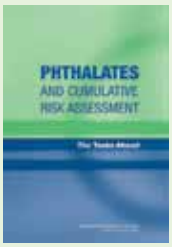
Biologic pathway: A cellular response network that leads from exposure to health outcome; in a *toxicity pathway,* the health oucome is adverse.

Mechanism of action: The molecular-level description of a *biologic pathway.*

Mode of action: Key events, typically at the organ or tissue level, along the *biologic pathway.*

Toxicity outcome: Observable adverse effect.

NRC Reports Addressing Cumulative Risk Assessment



Toxicity Testing in the 21st Century: A Vision and a Strategy (2007)
Toxicity testing should seek to identify perturbations in biologic pathways that are expected to lead to adverse effects.

Phthalates and Cumulative Risk Assessment: The Task Ahead (2008)
Cumulative risk assessment of agents that produce the same types of adverse health outcomes should be considered.

Science and Decisions: Advancing Risk Assessment (2009)
Interactions between chemical and nonchemical stressors should be incorporated into cumulative risk assessments.

Daston and other participants highlighted findings from recent National Research Council reports that contribute to new approaches to mixtures toxicology and cumulative risk assessment.

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assess cumulative risk of mixtures grouped by common adverse outcome by using an integrated additivity approach or by calculating a *hazard index* under the assumptions of a dose addition approach. A hazard index is the sum of hazard quotients (the ratio of potential exposure to a substance and the level at which no adverse effects are expected) for substances that affect the same target organ.

The evaluation of mixtures to determine the best cumulative model can be complicated. Ideally, Teuschler explained, one would assess the *whole mixture*—that is, the combination of however many chemicals are in the environment. Such an approach is prohibitively data intensive. Instead, risk assessors often must test or use toxicity data from a surrogate mixture—whether a fraction of the whole mixture, a laboratory-synthesized surrogate, or another mixture deemed *sufficiently similar*.

How similar to a mixture found in the environment must a tested mixture be? A variety of approaches may be used to define sufficient similarity objectively. Gennings described a statistical approach for defining sufficient similarity by linking occurrence and toxicologic data. Child care centers were sampled for mixtures of pyrethroid and pyrethrin pesticides. A team of researchers conducted neurotoxicity tests on a reference mixture—the pesticide mixture found in the 10% of the centers with the highest levels of detectable pesticides. On the basis of the toxicity data and expert judgment, Gennings' team defined a range of benchmark doses (essentially, the lowest dose thought capable of causing a particular adverse outcome) within which another mixture could be considered sufficiently similar. They estimated benchmark doses based on the child care center samples and found that samples from 90% of the centers were sufficiently similar to the reference mixture. The researchers were then able to weight the chemicals in each mixture on the basis of their relative potencies, which enabled estimation of each mixture's hazard index and calculation of daily intake estimates. For example, for a hypothetical 3-year-old boy, Gennings found a skewed distribution of hazard indexes among the centers: About 1% of the centers had high hazard indexes even though the median index across all the centers was low.

Emerging Science Informing Cumulative Risk Assessment

Advances in science and technology—including toxicogenomics, high throughput screening, computation, and applications of epidemiology and biomonitoring—are improving the understanding of mixtures and co-exposures, noted Daston. Meeting participants described how scientific advances expand understanding of exposures and health outcomes and facilitate the identification of toxicity pathways.

Biomonitoring

How do we know which chemicals enter our bodies? Biomonitoring—the measurement of people's exposure to chemicals by using biologic samples—can indicate the presence and concentrations of analyzed chemicals (for example, in blood or urine), trends in such concentrations, and simultaneous exposure to multiple chemicals, said Lesa Aylward, of Summit Toxicology. It can be most useful when exposure is expected to be widespread and frequent in a population, when multiple sources of exposure exist, and when studies are targeted (for example, focusing on a community near a contaminated site).

In the most comprehensive biomonitoring effort in the United States, scientists with the Centers for Disease Control and Prevention (CDC) analyze blood and urine samples collected as part of the National Health and Nutrition Examination Survey

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(NHANES) for hundreds of chemicals or their metabolites.

Biomonitoring data can be incorporated into cumulative risk assessment and mixture research in a number of ways. For example, explained Gennings, biomonitoring data may be used to estimate an individual (rather than a population) hazard index in an approach that acknowledges each person's specific exposure to chemical mixtures. She demonstrated that approach by evaluating the risk posed by mixtures of several antiandrogens (compounds that block the effects of natural androgens, such as testosterone) in pregnant women. She used urinary concentrations of the contaminants collected by NHANES—including phthalates, the plasticizer BPA, pesticides, fungicides, preservatives in pharmaceuticals and cosmetics, and a flame retardant—to estimate daily intake of each chemical per person. She found wide variation among people in the relative proportions of chemicals that made up the antiandrogen mixture. Although the median population hazard index was very low, she found that about 24% of the pregnant women had high individual hazard indexes for the mixture. Such a high individual hazard index is unacceptable, said Gennings. The findings suggest that the use of population medians for hazard indexes and similar measures may mask important individual variation in risk.

> ***We know that biologic outcomes are the results of numerous chemical and nonchemical stressors. The ability to synthesize complex information with computation is going to be a quantum leap forward.***
>
> **—George Daston**

In another application, Aylward described the use of biomonitoring equivalents, which are a translation between the units generated by tolerable external exposure level assessments (such as reference doses reported in milligrams per kilogram per day) and the units measured in biomonitoring studies (reported in nanograms per milliliter) in risk assessments. Aylward and her research team derived biomonitoring equivalent values of trihalomethanes, which are associated with fatty liver disease, and selected volatile organic chemicals, which are linked to neurotoxicity. For both classes of chemicals, such calculations provide one way to determine which chemicals contribute the most to the hazard index. Such information might allow a risk assessor to focus on the most harmful agents that should be evaluated together in a cumulative risk assessment.

> ***Instead of focusing on median effect or median daily intakes per chemical, we should focus on the fact that everybody has a different mixture [and] accommodate that in calculating the hazard index.***
>
> **—Chris Gennings**

Toxicity Models

Which chemical mixtures pose the greatest risk? Thomas Knudsen, of EPA, explained how Tox21—a multiagency collaboration to research and develop innovative chemical toxicity testing methods to characterize and predict the effects of chemical exposures—may help to provide an answer. The ToxCast program, EPA's contribution to Tox21, uses primarily in vitro tools (cell-based assays to help to understand how chemical exposures affect physiologic processes).

Knudsen and his research team are using data from ToxCast's in vitro assays and findings from published in vivo work to build models for predicting which chemicals might cause particular adverse effects. For example, they used hundreds of assays to design a model that could predict which chemicals are prenatal toxicants, affecting an array of biologic processes, in rats or rabbits. In another example, Knudsen and his team used data from ToxCast and published information to develop a dynamic, "agent-based systems model"—in which the behavior of each individual cell is modeled separately—to show perturbations of blood vessel development resulting from chemical exposures.

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National Health and Nutrition Examination Survey

NHANES is a massive, continuing public-health survey run by the CDC. Every 2 years, 10,000 people who are representative of the U.S. population are surveyed for the prevalence of several diseases and numerous other health-related factors, including a number of genes and hundreds of environmental factors in blood and urine samples. The chemicals surveyed include metals, phthalates, perchlorates, pesticides, fungicides, herbicides, flame retardants, polychlorinated biphenyls, dioxins, polycyclic aromatic hydrocarbons, and volatile organic chemicals. Aylward, Gennings, and several other meeting speakers described ways in which NHANES data can be used to study health effects of exposure to multiple chemicals.

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Such models, said Knudsen, allow finding combinations of chemicals that have synergistic effects that require further study.

Ecotoxicology

The field of ecotoxicology (the study of the effects of toxic chemicals on organisms in an ecosystem) also may offer relatively quick and inexpensive methods for determining which mixtures are harmful, explained Ed Perkins, of the U.S. Army Corps of Engineers. The use of fish, amphibians, and invertebrates traditionally used in ecotoxicology enables an integrative analysis of the effects of chemicals at various doses and in various media; on multiple tissues and organs; and on development, reproduction, and behavior—processes not easily captured by in vitro assays. The use of those animals, rather than mammals, also minimizes the time and expense of in vivo testing. In addition, *the adverse outcome pathway approach*—which begins with exposure of the individual to a chemical and incorporates cellular responses and individual-level and population-level adverse outcomes—might be useful in understanding human health risks. After comparing data on receptor function, hormone production, tissue development, and '-omics' (changes in gene expression, proteins, and cellular processes) between humans and other animals to find cross-species similarities in pathways and events, prediction of effects in humans may be possible.

Perkins provided examples of how ecotoxicologic approaches can address questions relevant to human health. Trenbolone, an androgen receptor agonist (which binds to androgen receptors and mimics the effects of natural androgens), is used in livestock—and, illicitly, by athletes—to increase muscle mass. Although they are not identical, the pathways by which trenbolone achieves its effects in humans and in fish, such as the fathead minnow, are similar, and humans and minnows have the same outcome—reduced fertility. Therefore, Perkins determined, one could use a fish model to screen for agents whose pathways converge at some point with the androgenic pathway affected by trenbolone. One chemical thought to share part of the pathway is flutamide, an antiandrogen that is used to treat some human diseases. Perkins and his research team used hundreds of DNA microarrays, which are used for rapid detection of changes in the expression of multiple genes, with multiple doses of flutamide and eight other chemicals. They used the microarray results to create a map of gene expression changes, illuminating the pathways by which the chemicals affect reproduction. The researchers found that, rather than affecting genes related to testosterone activity, as expected, flutamide primarily modulated the expression of genes related to nonandrogenic pathways. An interesting set of gene expression changes associated with flutamide exposure is linked to a

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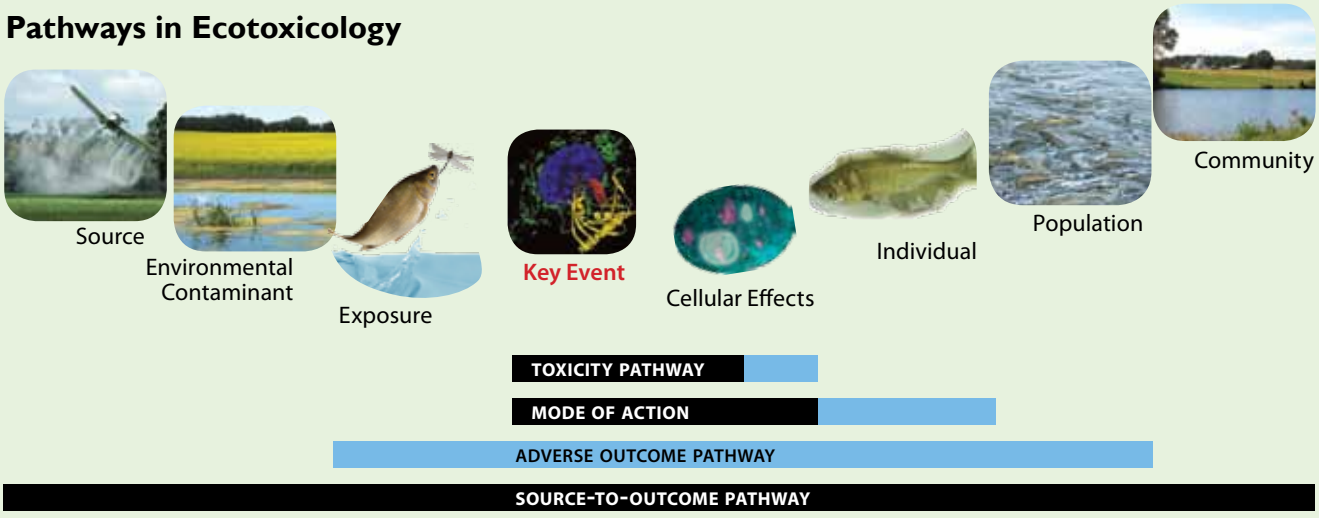
Pathways in Ecotoxicology



Perkins described the adverse-outcome pathway (AOP) as a portion of the entire source-to-outcome pathway that begins with the exposure of the animal to a chemical and a resulting molecular event that perturbs normal cellular activity. Early cellular changes are followed by adaptive responses or, at a particular dose, cell injury and an inability to regulate. The AOP ends with adverse outcomes in individuals and populations. The methods used in this kind of approach include computational chemistry to understand the structure and properties of chemicals; genomics, proteomics, metabolomics, and receptor-screening assays to understand the molecular initiating event, cellular responses, and organ responses; and whole-animal toxicology to understand individual responses.

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diminution in the number of mature oocytes (eggs) in female fish. This finding is relevant to human health because of flutamide's use in the treatment of polycystic ovary syndrome in women, a condition that can decrease fertility. It is interesting that Perkins's work on a mixture of flutamide and trenbolone in fish showed that the effects of the two chemicals essentially cancel each other out—a demonstration that chemicals need not act via the same pathway to interact with each other.

Genomics and Genetic Variation

Scientists and decision makers have shown increasing interest in using genomic data to improve risk assessment—both for single chemicals and for mixtures. For example, researchers can use toxicogenomic data in risk assessments, noted Susan Euling, of EPA, to learn how gene expression changes are linked to adverse outcomes and to discover the biologically significant level of change in pathways or gene expression. Such data can be used to identify susceptible populations or to group chemicals by mechanism of action according to a predictive set of gene expression changes associated with the chemicals. Genomic data could also become useful in accumulating pathway level responses among a group of chemicals or in determining a pathway-level dose-response relationship for a mixture and its components.

> ***One critical assumption in this [approach] is that it does not matter if a gene or a chemical affects the pathway. If the pathway is affected, you are likely to see an effect on human disease.***
>
> **—Chris Portier**

To illuminate dose–response relationships, Chris Portier, director of CDC's National Center for Environmental Health and Agency for Toxic Disease Registry, described how his team used a series of toxicity studies conducted by the National Toxicology Program (NTP) on the effects of exposure to 20 chemicals on tumor formation in mice. For each chemical, they applied genomic data from an independent study of the same chemicals to predict the results of the statistical test used by NTP to classify chemicals as liver carcinogens or noncarcinogens in mice. With a few exceptions, their predictions were accurate. They were also able to use their findings in mice to predict NTP liver carcinogen determinations in rats and in humans.

Gene × Environment Relationships

Portier, described how he and his colleagues mined published, archived genetic and environmental data to identify critical toxicity pathways associated with human disease. Portier and his colleagues identified biologic pathways that have been linked to more than 200 human diseases and to more than 2,000 alleles (alternative forms of a gene) from the National Institutes of Health Genetic Association Database. They also gathered information from the Comparative Toxicogenomic Database on gene expression patterns linked to about 1,000 different chemical exposures. To link information from the two databases, Portier's team developed statistical approaches based in part on geography to analyze pathways as *maps* rather than as sets of genes or enzymes. In fact, they developed an intricate map that links diseases and chemicals based on the strength of their association with particular biologic pathways. The calculated probabilities of particular chemicals associated with particular diseases in humans, thus identifying critical toxicity pathways, are useful for hazard assessment in cumulative risk assessment. If sufficient information is available for how the chemicals are affecting the pathways, Portier's method could also be used to determine whether dose additivity is an appropriate approach for analysis.

> ***We have set up research institutions and academic departments on the basis of an artificial separation of the study of genes and the study of the environment.***
>
> **—Atul Butte**

Atul Butte, of Stanford University, proposed taking an expanded view of mixtures in which the genome is considered as part of the mixture. Today, to most geneticists, the environment is a confounder, Butte observed; and to researchers studying environmental factors, the genome is a confounder. To move forward, we must consider both environmental and genetic factors as signals, he emphasized. In the first published environment-wide association study—a study that explored a large set of environmental exposures for potential links to disease outcomes and was analogous to a genome-wide association study—Butte and colleagues used NHANES data to explore genetic variation among people and variations in exposure to environmental chemical and biologic factors. The researchers identified the exposures associated with type 2 diabetes and looked at whether known disease-associated alleles interact with those environmental factors in causing type 2 diabetes. The environmental factors most strongly associated with type 2 diabetes included

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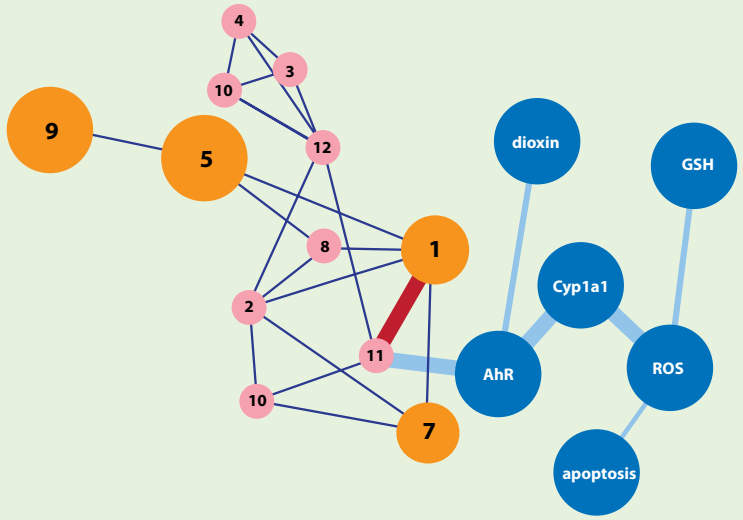
PCBs and heptachlor epoxide, a derivative of a carcinogenic pesticide that was banned in the United States in the 1980s but is still present in the environment. The researchers also confirmed previous findings of a protective effect of beta carotenes. With respect to gene–environment interactions, they found one allele that has a slight protective effect for type 2 diabetes and is modified according to the concentration of PCB in the sample. They found another allele that is known for its association with type 2 diabetes and whose harmful effect is modified by beta carotene. The bottom line is that "we should be looking at environmental and genetic factors simultaneously." Butte said.

Chemical Mixtures and Cancer

Lyle Burgoon, of EPA, presented a case study of how genomic data could be used to inform cumulative risk assessment of mixtures that cause cancer. Several mixtures that contain polycyclic aromatic hydrocarbons (PAHs)—such as tobacco smoke, soot, coal tars, coal tar pitch, and household combustion of coal—are known or suspected human carcinogens, explained Burgoon. The EPA Superfund program routinely measures 15–17 PAHs in environmental media at contaminated sites.

Burgoon's team is working on a pilot project in the multiagency collaborative program, Advancing the Next Generation of Risk Assessment. The aim of Burgoon's project is to predict tobacco smoking–related lung cancer by using molecular systems biology and high throughput assay data. Burgoon's team used two archived datasets from studies of smokers and nonsmokers who did or did not develop lung cancer. They used correlation to identify and map networks of normal, diseased (lung cancer), or exposed (smoking) states to show changes in connectivity (essentially, changes in gene expression or in cell-to-cell communication that used molecules) and to predict the probability of developing lung cancer via agglomerative biomarkers—biomarker sets that are mechanistically tied to a disease. From the two datasets, they produced a network that showed the intersection of the exposure and disease networks. They then identified several interesting network features, such as a protein expressed by an oncogene, that could be useful in predicting lung cancer in smokers whose lungs are still phenotypically normal. The approach is flexible enough to incorporate agglomerative biomarkers of nonchemical stressors and dose–response information.

Network Community



Burgoon provided a conceptual illustration of a network perturbed by a chemical exposure. Each node is a biologic component, such as a gene, protein, or metabolite. The blue lines show that the nodes are connected to one another; that is, how the biologic components communicate. The red line represents a communication link that has been turned off between node 11 and node 1 in response to dioxin exposure. Such networks can be developed for chemical and nonchemical stressors, as well as disease states, and can be used to identify agglomerative biomarkers. Burgoon posited that comparing disease and exposure–response networks may enable researchers to predict disease outcomes.

Mixtures and Reproductive Development

To illustrate the importance of common pathways vs. common adverse outcomes in grouping chemicals for cumulative risk assessment, Gray described his work on a broad group of antiandrogens—including several phthalates and pesticides—that affect male reproductive development through diverse mechanisms. For example, some pesticides act as androgen receptor antagonists that compete with natural androgens for androgen receptors and thereby inhibit androgen receptor–dependent gene expression. Phthalates and other pesticides inhibit fetal androgen synthesis but do so by slightly different mechanisms. Nevertheless, most of these chemicals eventually converge on

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the androgen signaling pathway, and they all induce malformations in the reproductive tract and cause delayed puberty in male rats.

Gray's team aimed to illuminate how a group of phthalates and antiandrogenic pesticides interact to affect reproductive development. Specifically, they determined whether they could most accurately predict effects of exposure to mixtures by using a response addition model, a dose addition model, or an integrated addition model. They hypothesized that chemicals that disrupt the development of a common reproductive tissue during sexual differentiation will produce dose additive responses regardless of the molecular mechanism or signaling pathway that is disrupted.

In studies of the male offspring of female rats exposed to mixtures of phthalates and antiandrogenic pesticides during pregnancy, Gray and his team found that dose addition is always better than or equal to response addition or integrated addition in predicting mixture effects. A reproductive malformation called hypospadias, which was not caused by either a phthalate or a known androgen antagonist pesticide alone, occurred in more than half of the male offspring exposed to the mixtures. The predictions of the dose addition model matched those observations much more closely than did the predictions of response addition model. In studies of several chemicals that have diverse mechanisms of action, Gray's team repeatedly found that dose addition predictions closely matched observations, whereas response addition underpredicted adverse effects. Gray explained that with each of these diversely acting chemicals, what the tissues "see" is a reduction in binding between androgen receptors and androgen regardless of the specific mechanism.

Gray and his team expanded their research to chemicals that disrupt male reproductive development via nonandrogenic pathways. For example, in one series of studies, they mixed dibutyl phthalate that, which disrupts the androgen signaling pathway, decreasing testosterone production and negatively affecting testicular descent, with a dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) that disrupts male reproductive development through unknown, apparently nonandrogenic pathways. Despite their different mechanisms, exposure to either of the chemicals produces malformations in some of the same reproductive tissues. Dose addition modeling was not possible in this case, but response addition again underpredicted the adverse effects of the mixture

Overall, Gray's work suggests to him that, at least in some cases, chemicals that disrupt a common tissue or system, regardless of their mechanisms of action, should be examined together in a cumulative risk assessment.

Closing the Research Gaps

Participants discussed a number of research gaps relevant to mixture science and cumulative risk assessments. In particular, numerous participants said that nonchemical stressors deserve increased research attention. Moiz Mumtaz, of CDC, John Balbus, of NIEHS, and others observed that nonchemical exposures can be at least as harmful as chemical exposures. Cote and Mumtaz added that regulators are striving to incorporate nonchemical stressors into the risk assessment equation, but the effects of these stressors often are poorly understood. Gennings pointed out that nonchemical stressors may be modifiable, whereas, even with the best remediation work, many chemicals will not go away. Therefore, we also need to improve understanding of the potential benefits of good nutrition, healthy lifestyles, and so on in partially compensating for the effects of chemical exposures.

Participants described two ways in which researchers could break out of their "silos." First, Butte argued, we can improve our understanding of the effects of mixtures on human health only if we study environmental and genetic factors and their interactions. David Balshaw, of NIEHS, noted that the National Institutes of Health's Genes, Environment, and Health Initiative—which studies the interactions between genes and environmental factors—is addressing this research need with a focus on conditions and diseases, such as congenital malformations, that have short latent periods between exposure and disease onset. Second, DeVito contended, toxicity and epidemiologic studies should be integrated better. Jane Ellen Simmons, of EPA, agreed and provided an example in which toxicology had informed epidemiology: the Spanish Bladder Cancer Study. Mechanistic toxicity data were shared with epidemiologists in the United States and Spain, where the epidemiologists determined which chemicals seemed to trigger the disease. They were then able to develop an appropriate remediation strategy.

To quantify an overall risk burden comprehensively, explained Teuschler and Lauren Zeise, of the California Environmental Protection Agency, we need a way to consider the effects of persistent chemicals already in the body (from past exposures) whose effects are similar to those of the chemicals to which we expect exposure. Researchers and regulators may also need to expand their concept of a mixture. For example, Zeise recommended that diet, including the chemicals used

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in food processing and preparation, should be considered a mixture of chemicals.

Many participants emphasized the need for improved data sharing—both to advance scientific understanding and to improve the utility of science in decision making. Butte and Gennings noted that researchers studying environmental factors should make their raw data available in repositories to enable secondary analysis, just as geneticists routinely do today.

Participants raised a number of caveats and limitations regarding the collection and application of data that can be obtained with emerging research tools. Although participants agreed that NHANES provides a rich and useful dataset, Aylward highlighted a number of limitations in the data. First, not all chemicals are measured in all people. Second, data on infants and children, who are generally the most sensitive to the harmful effects of toxic chemicals, are sparse. Third, because of their short half-lives, many chemicals measured via NHANES are highly transient, which makes it difficult to interpret results on the basis of the survey's urine samples. Blood-based chemical analyses, Aylward argued, are probably more directly related to the internal dose (the dose in the body at the affected tissue). However, Portier countered that complex physiologic interactions occur in both blood and urine, so blood-based biomarkers might be no better than urinary biomarkers in estimating an internal dose.

Teuschler and Gennings cautioned that projects that use data mining and literature mining techniques should try to ensure that they use studies that have uniformly high data quality. They should also address basic statistical issues, such as randomization and validation. Gray noted that we must acknowledge and address the limitations of high throughput screening because, for example, some modes of action are not covered. He also emphasized the need to retain older methods even as we embrace new ones.

Although molecular biology is awash with new data, Cote observed, a lot of the data have not been collected or reported in

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SCIENTIFICALLY SPEAKING

George P. Daston serves on the National Academies' Committee on Emerging Science for Environmental Health Decisions. He is Victor Mills Society Research Fellow at the Procter & Gamble Company. His current research addresses how findings in the fields of toxicogenomics and mechanistic toxicology can improve risk assessment for chemicals and the development of non-animal alternatives. He recently talked about the meeting on Cumulative Risk Assessment for Environmental Mixtures.

Q. What do you learn from Emerging Science meetings?

A. Because of the committee's breadth, I get to learn about topics that I wouldn't otherwise encounter. I have learned from our discussions of how to apply the science with government scientists who must make decisions even when the science is less than perfect. [These meetings] have also made me appreciate how we are limited by our capacity to measure. Much of the emerging science is about expanding what we can measure. For example, we can measure epigenetics changes but we don't have enough information to understand which changes are normal and which are adverse and persistent.

Q. Do you think that the Committee's meetings impact research?

A. I'm hopeful that the meetings are shaping research. For these meetings, the "sweet spot" we aim for is finding science that is new enough that it is not already being applied to decision making, but far enough along that once we make connection between the emerging science and how it might affect health, we can start asking questions about what type of research would lead to pragmatic decisions about the relevance of the information to decision making. Of course, each of us has a different view on where the sweet spot is.

Q. Why have the mixtures meeting now?

A. Risk assessment for mixtures is one of those unresolved questions that have been around forever because we don't have the tools to solve them. It's not possible to test all mixtures combinations at all concentrations, so there needs to be a scientifically rational approach to their evaluation. Chemicals that interact along a biological pathway and enhance each other's ability to produce a particular response are the ones that are of particular interest to evaluate. The emerging science that makes it interesting now is that our combination of computational methods and high information content (high throughput approaches such as

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ways that would enable the kinds of analyses that risk assessors need to do. Betsy Southerland, of EPA, and others enumerated how researchers could maximize the utility of their data for regulatory agencies. For example, researchers should test environmentally relevant doses and component proportions; chemically characterize complex mixtures; assess the bioavailability of chemicals from different media; expose and assess animals at their most sensitive developmental stages; use multiple doses and low doses in microarray studies; publish (or make available) their data on individual animals and their results on all end points evaluated, including negative findings; and take steps to ensure sufficient power in their statistical tests. Simmons also called for the development of user friendly predictive models to enable extrapolation from data rich-cases to data-poor or data-unknown cases.

Regulatory Implications and Next Steps

Among the regulatory issues associated with mixture science, participants discussed at length a question first raised by DeVito regarding the "level of protectiveness" for which regulators should aim. Are we trying to protect a tiny group of people—say, one person in a million—whose genes may confer greater vulnerability to a particular chemical exposure? Or should we use a less protective but more practical standard, such as protection of a vulnerable group in which the occurrence of exposure might be closer to one in 1,000? With the advent of "personalized medicine," noted William Farland, of Colorado State University, scientists are increasingly examining risk at the individual level. That shift is causing a disconnect between the regulatory construct, which is intended to protect populations, and the movement of the scientific community toward individual risk. Zeise argued that scientists' focus on individual risk provides an opportunity for regulators to understand the most vulnerable populations better and to set priorities among regulatory actions appropriately.

The need to break out of "silos" applies to regulators as well as

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ToxCast) produce an incredible amount of information about mode of action in a short time. The large amounts of data and the ability to analyze them will enable us to predict/identify which chemical structure elements affect which biological targets.

Q. How has the NRC's report *Phthalates and Cumulative Risk Assessment: The Tasks Ahead* (2008) influenced or impacted mixtures consideration and how was this discussed at the meeting?

A. I think the meeting got us closer to a shared understanding of what we mean by "common pathway." The phthalate report looked at two agents acting along a common pathway but with different mechanisms. The report has been interpreted by some as saying that two or more chemicals that produce the same outcome should be considered to act on the same biological pathway. Discussions at this meeting suggested that only in a subset of cases is this the right approach. It depends on whether the two mechanisms converge on a single mode of action.

Q. What insights/ideas stayed with you after the meeting?

I'm struck by the "quantum leap" in our scientific capabilities, and this is not just about mixtures and cumulative risk assessments. Our current chemical testing scheme limits us to observing what our "black box" animal models reveal—models that don't predict everything that may be important—to a more transparent system in terms of really understanding the biological effects of a given agent. The progression to a more predictive science becomes a quantum leap because of the speed enabled by today's tools. For mixtures, that speed enables us to both examine more combinations and examine fundamental mechanisms of toxicity.

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

A. I would like to see case studies and guidance. What we really need is to put together a few case studies and use these to go from new data stream to decision point. We can use the case studies to ask when it is appropriate to use which assessment approach and illustrate the decision logic.

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scientists, some participants said. Gennings, Simmons, and Gray argued that different regulatory bodies—such as EPA, FDA, and the Consumer Product Safety Commission—should work more closely together, sharing data and approaches for analyzing drugs, chemicals, and other products and collaborating more often on cumulative risk assessments.

Several participants raised the concern that cumulative risk assessments—and the new science and technology that inform them—are likely to be resisted by the regulated community. Zeise noted that when regulators base decisions on new approaches and tools, the regulated community is often quick to challenge them. Beth Doyle, of EPA, agreed, adding that regulators will need to "sell" a mixtures-based approach because the regulated community may assume that regulations based on such an approach will be less cost-effective than those based on a single-chemical approach. Richard Denison, of the Environmental Defense Fund, noted that regulators themselves may resist using the new approaches and that this could affect the threshold at which information from cumulative risk assessments triggers a regulatory action. One way to improve the application and acceptance of cumulative risk assessments, said Doyle and Zeise, would be to develop simple, nonacademic guidance documents that target both regulators and the regulated communities. Daston suggested that one or two case studies of the integration of some of the tools discussed at this meeting could inform the guidelines and facilitate their development and use.

This article was prepared by Elizabeth Stallman Brown with editing by National Research Council staff.

MEETING INFORMATION

Meeting Presentations

Would you like more details about this meeting 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 presentations. Also, we invite you to subscribe to our listserv for the latest information about meetings, newsletters, and other Emerging Science activities.

Next Meeting

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

Previous Meetings

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

- ***Systems Biology Risk Assessment*** – June 14–15, 2012
- ***Individual Variability*** – 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
- ***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
- ***Implications of the Microbiome for Environmental Health*** – April 27–28, 2011

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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