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"Going Green"

by Dennis Harris, Christine Mirzayan Science and Technology Policy Fellow

The trend to "go green" seems unstoppable. Reusable grocery bags, energy-efficient lights, refillable coffee cups, and fuel-efficient cars. One florist even promoted cactuses as a "green" product because they require less water than other plants.

The term *going green* often focuses on reducing consumption. Whether we're talking about fuel or paper bags, the goal of going green is commonly to reduce waste and reuse products. Public services and stores have jumped on the green bandwagon and offered all kinds of products that you can buy that, ironically, will reduce your consumption. And it's no surprise to see companies touting green services to appeal to their customers and tangibly increase profit. By reducing production materials and spending less energy in production and offices, industries stand to benefit financially from going green.

Business profits aside, the outcome of green services has been promising. By reducing waste, we are also reducing the chemicals and byproducts that eventually end up in our environment. Those hazardous substances in the environment—whether from production or from intended

continued on page 15

How New Toxicology Can Catalyze Green Chemistry

—by Kellyn Betts; edited by National Research Council Staff

Can chemicals be designed for reliability, cost effectiveness, *and* environmental and human health safety? Can the new, rapid toxicology tests being developed to screen chemicals also be used to inform safer chemical and material design? On September 21–22, the National Academy of Science's Standing Committee on Use of Emerging Science for Environmental Health Decisions held a public meeting to discuss those questions and to examine how breakthroughs in toxicology may promote advances in the growing field of green chemistry. Thinking up front about what substances are used to make chemicals, how a chemical may be transformed in biologic and environmental systems, and how and where a chemical is transported in those systems may help to shift society's paradigm from one of hazard or risk *response* to one of hazard and risk *prevention.*

The Gap Between Chemistry and Toxicology

Christopher Weis, National Institute of Environmental Health Sciences toxicology liaison, opened the meeting by challenging attendees "to dream a bit" about a future society that thinks up front about chemical effects on humans and the environment and moves greener chemicals into the marketplace. Richard Denison, of the Environmental Defense Fund, emphasized that the meeting's overarching objective was to "bridge the gap between chemistry and toxicology." As Denison pointed out, the process of designing chemicals has historically been divorced from considerations about toxicity. Because new tools developed in recent years allow much more rapid assessment of both toxicity and hazard, they open up the possibility of assessing these important characteristics near the beginning of the chemical design process, he said. The ability to assess the toxicity of existing chemicals also helps chemists and

continued on page 2

IN THIS ISSUE

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.

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GREEN CHEMISTRY, *cont. from page 1*

materials scientists and engineers to design greener products.

Paul Anastas of Yale University, one of the "fathers" of green chemistry and the former assistant administrator of the US Environmental Protection Agency (EPA) Office of Research and Development, defined green chemistry as "the design of chemical products and processes to reduce or eliminate the generation of hazardous substances." There are 12 principles of green chemistry for laboratory chemists, and the chemists who follow them can simultaneously "bring about environmental improvement benefiting human health and economics and profitability," Anastas stressed. He made it clear that the new approach to chemical and product design can be most effective when industrial chemists have rapid access to information about the toxicity of substances that they use.

> ***The idea of bringing together the toxicology and the green chemistry communities—having problems meet solutions—is something that is recognized as tremendously important and essential.***
>
> **– Paul Anastas**

Robert Tanguay, of Oregon State University, said that knowing why or how a chemical is toxic is particularly valuable for developing predictive models for designing inherently safer materials. However, he said, traditional whole-animal testing presents barriers to green chemical and material development in that the testing is low-throughput and expensive. He added that it is important to be able to identify hazard *and* mechanisms of toxicity more rapidly. New rapid toxicity-testing methods offer ways to do both.

Green Chemistry Needs Advanced Toxicology

Anastas pointed out that thousands of innovations have already resulted from green chemistry, including compounds used in electronics, aerospace, cosmetics, agriculture, and energy. The "clean little secret" that people are catching on to in different industries is that green chemistry leads to good business decisions, he emphasized. A market analysis report published by Pike Research in spring 2011 predicts that the green chemical industry will soar to $98.5 billion by 2020. Businesses recognize that if safety becomes an intrinsic characteristic of molecules, expenditures for safety and cleanup will be reduced. Using poisons in commerce is neither good for business nor good for human health.

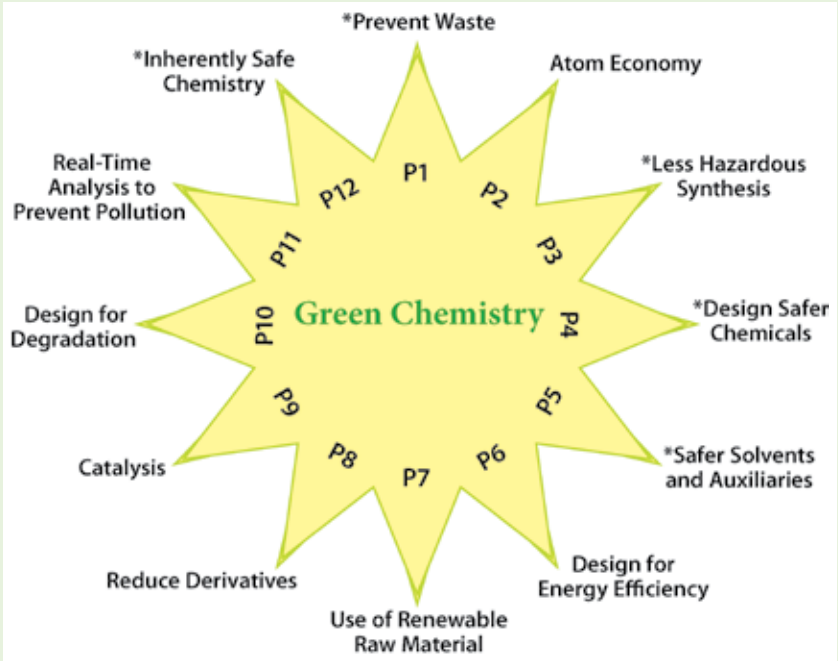
If green chemistry is good for business, why isn't it practiced more widely? Representatives of DuPont, Hewlett-Packard, and Pfizer described how green chemistry is percolating into their business models but emphasized what is still needed from toxicologists and chemists if the principles of green chemistry are to be embraced more fully.

Mark Thompson, director of DuPont Haskell Global Centers for Health and Environmental

continued on page 3

The Twelve Principles of Green Chemistry

Tanguay argued that 21st century toxicology data could be instrumental to achieving the twelve principles of green chemistry. Tanguay marked (*) five of the 12 principles where he thought 21st century toxicology could be the most helpful. In general, new toxicity testing methods can more rapidly provide data that help decision-makers differentiate between safe and non-safe chemicals throughout the design process.



GREEN CHEMISTRY, *cont. from page 2*

Sciences, underscored Anastas's points about green chemistry and business sustainability. Since 1999, DuPont has implemented a mechanism in its R&D process to eliminate "bad" products early in development. "Doing it right the first time and minimizing waste make good business sense," Thompson said.

Thompson presented a list of what companies like DuPont need to improve their ability to design greener chemicals. First, Thompson called for highly effective knowledge feedback loops which include tiered toxicity testing strategies, computational toxicology, modeling, and in silico profiling. Ideally a tiered testing strategy would couple computational toxicology with in vitro (cell-based) assays and targeted in vivo (within a living organism) testing beginning with simple animals, such as *Daphnia* water fleas. Also important is continued improvement of research tools for predicting human and ecologic toxicity, particularly tools that are rapid, inexpensive, high-throughput, and well validated. Tools and methods that require only a small amount of the new molecule or polymer being synthesized are desireable. Likewise, industry needs continued improvement of predictive tools for chemical and/or product performance and efficiency. Thompson also talked about updated hazard identification and risk assessment approaches based upon 21st century toxicology. These include the "fast fail/succeed early" corporate approach which implements decision metrics and tools for determining what to test and when to stop further development. For these multi-disciplinary approaches to succeed, well-integrated research and decision-analysis teams are important assets.

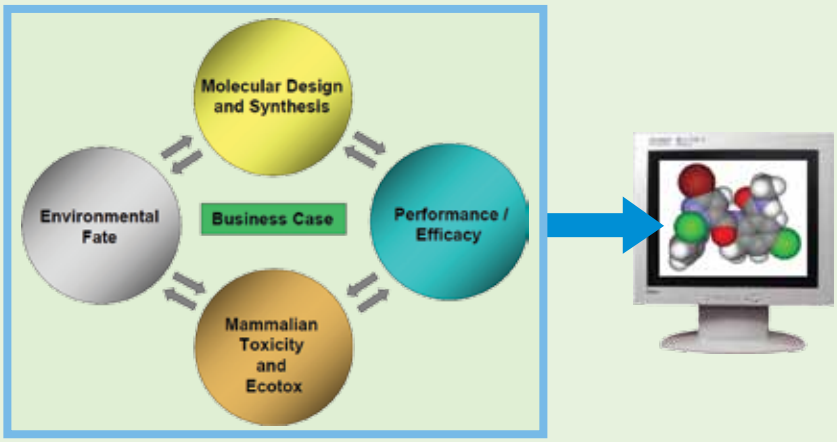
Thompson noted that DuPont's tiered approach is a major departure from the previous testing practices that focused on exposure assays with whole organisms. The current focus in toxicology on what happens at the level of genes or proteins fits well with the new approach, which relies on assays that can provide rapid results.

Helen Holder, the corporate materials-selection manager for electronics manufacturer Hewlett-Packard (HP), described her company's approach to developing safer products. HP's embrace of green chemistry was prompted in the 1990s by the European Union (EU) passage of legislation that set restrictions on materials that could be used in electronic products. By restricting previously commonly used substances, such as lead in the solder in circuit boards, the legislation—the Restriction of the Use of Certain Hazardous Substances in Electrical and Electronic Equipment (RoHS) and the Waste Electrical and Electronic Equipment (WEEE) directives—required the electronics companies to make major changes.

To meet the EU directives, HP began to scrutinize replacement materials. Replacing materials is expensive, Holder said, so HP was motivated to develop methods for choosing alternative materials that have a low risk of unintended consequences and thus a low risk of future regulation. HP adopted a hazard-reduction approach to create products like its polyvinyl chloride–free power cords. Inherent hazard is used as a proxy for reduced risk of exposure to potentially harmful materials. Holder said that the approach allows the company to screen out undesirable options before investment in further research

continued on page 4

Creating the Optimal Green Chemical?



Thompson outlined research and decision analysis needs for companies like DuPont to implement the principles of green chemistry better. He emphasized that the "holy grail" for new chemical product R&D would be the ability to sit down at a computer, enter physicochemical and other specifications for desired chemical properties, such as performance, low toxicity, and low manufacturing cost, and receive a computer-generated "optimal chemical structure" and a commercially feasible synthesis route.

GREEN CHEMISTRY, *cont. from page 3*

and development. She also noted that regulators are increasingly using hazard-based screening, so HP's approach "aligns our business process to what the regulators are doing."

To conduct its comparative chemical-hazard assessments, the company evaluates measurable hazardous end points associated with the chemicals and materials that it uses, including carcinogenicity, persistence, and bioaccumulation, Holder said. The comparative hazard assessment is followed by the Green Screen for Safer Chemicals, a software program developed by a nonprofit group, Clean Production Action (http://www.cleanproduction.org). The program assesses 17 hazard topics and scores them on a scale of high, medium, or low with some thresholds and decision logic to produce an integer score of 1–4. The resulting information, which can be generated in as little as 24 hours, is used to help engineers who are not formally trained in toxicology to make informed decisions. Part of what enables this to work is the company's reliance on a few high-quality variables, rather than a lot of lower-quality data, she said. However, Holder acknowledged that one limitation is the ability to assess a material's likelihood of endocrine disruption. There is insufficient or no information about endocrine disruption for many materials. "If we disqualify materials because of a lack of data on endocrine disruption, we would probably disqualify almost everything—and that doesn't help us," she explained.

Holder emphasized that one challenge HP and other companies face is that most chemists employed by companies in the electronics supply chain have never had a class in toxicology. She and the other mechanical engineers in her group often end up with the unlikely task of educating the chemists about toxicology. Holder urged attendees to "do whatever you can to make sure that chemists have toxicology training." She argued that professional chemists and new graduates should have a working knowledge of toxicology.

> ***Spills will happen. Accidents will happen. Exposures will happen. We want to get to a point where things are more benign already and we just don't have to worry about exposure controls.***
>
> **– Helen Holder**

Russell Naven, of Pfizer, described one way that Pfizer is approaching the use of in vitro data to predict in vivo toxicity. Pfizer's Compound Safety Prediction (CSP) group has developed a Compound Safety Evaluation tool that generates a score that product developers can use during the design phase to assess the toxicity of compounds being designed relative to known effects of existing compounds. They accomplished that by evaluating the annotated data on Pfizer drugs to look for the origins of in vivo hepatotoxicity—whether it was due to primary pharmacology, chemical structure, off-target or secondary pharmacology, or physicochemical properties. On the basis of that information, the CSP group was able to identify 15 in vitro assays known as the "promiscuity panel" that are very good predictors of in vivo hepatotoxicity. They found that in-design compounds that hit two or more targets in the promiscuity panel are about 5 times more likely to cause toxicity at a relatively low concentration (10 μM) than compounds that do not hit any targets.

The new tool relies heavily on the existence of well-characterized compound sets and collaboration among biologists, chemists, and computational scientists. It is used to initiate safety considerations early in projects, and it steers the company's scientists away from risky chemicals and improves resource use, Naven said.

However, as one meeting participant pointed out, the tool has some limitations in that the included in vitro assay panels and in silico descriptors may not encompass all mechanisms of toxicity, particular those related to minor organs. Naven's team is looking at ways to incorporate data from newer toxicology and molecular biology (-omics) data to improve sensitivity. Bill Farland, of Colorado State University, and others noted that the system that Pfizer is using highlights the importance of annotating and archiving data so that they are publicly available, which also will reduce the likelihood of "reinventing the wheel" in different industries with respect to models and processes.

The Promise of High-Throughput Toxicity Testing

Tanguay noted that much of industry today would like to use bioinformatic or biocomputational approaches as a first approach toward priority-setting among chemicals. Computational approaches are fast and much less expensive than traditional whole-animal tests in rodents. But Tanguay argued that new rapid

continued on page 5

GREEN CHEMISTRY, *cont. from page 4*

toxicity testing may be a better option as a first approach.

A major roadblock to implementing green chemistry is the fact that "there are way too many chemicals with way too little data," said David Dix, deputy director of EPA's National Computational Center for Toxicology (NCCT). Much of what we know about how chemicals behave in our bodies is courtesy of the pharmaceutical industry, but our knowledge is imperfect. And, as several meeting participants noted, industry data are often not publicly available. Moreover, there is little or no information about carcinogenic, reproductive, developmental, or genotoxic effects of most chemicals that are in use today. "We are not going to be able to test our way out of the situation by using traditional approaches," Dix said. He outlined an array of high-throughput (HTP) toxicity-testing approaches and computational tools that EPA is developing in its ToxCast and ToxPi programs. ToxCast includes various types of in vitro assays and some that use alternative organisms, such as zebrafish and *Caenorhabditis elegans*. Many of the HTP approaches were inspired by techniques developed by the pharmaceutical industry, and they use cell-based assays in combination with computational tools and take advantage of robotics technologies that automate the testing process. By comparing chemicals tested by both conventional methods and the new HTP tests, researchers are building predictive reverse-dosimetry models that connect the dots between an initial exposure to a toxic agent and the ultimate manifestation of disease.

The Drive for Predictive Toxicology

In 2007, EPA launched **ToxCast** *(*http://www.epa.gov/ncct/toxcast/*), a program focused on developing rapid and more efficient tools to predict chemical toxicity of the thousands of chemicals currently in use on which there are few or no toxicity data. ToxCast has resulted in a wealth of publicly available data on many chemicals that were tested with more than 600 types of screening assays. The agency has also tested an additional 1,000 chemicals with a subset of those assays that are relevant for endocrine activity with its Endocrine Disruptor Screening Program; the resulting data are being analyzed. EPA is now gearing up to begin testing 10,000 more chemicals in its biggest project,* **Tox21***, in collaboration with the National Institutes of Health.*

EPA has also developed **ToxPi***, the toxicological priority index (*http://www.epa.gov/ncct/ToxPi/*), to indicate visually and rank how different chemicals score on different tests. The ToxPi for endocrine-disrupting chemicals includes results from GTP assays and chemical properties and other pathways related to such phenomena as molecular interactions and reactions.*

Dix noted that the eventual goal of ToxCast, ToxPi, and other agency programs and collaborations "is to get beyond the animal toxicity data and move to understanding effects on human systems and relationships to human disease."

Dix described how data from the Endocrine Disruptor Screening Program have promise for analyzing candidates for replacing chemicals that are known to be problematic, such as plasticizers like bisphenol A and perfluorinated chemicals like perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA). One of the new assays that proved particularly valuable for data on PFOA and PFOS alternatives is a mechanistic model of pathways that are critical for blood-vessel development. ToxCast assays have also aided the selection of chemicals needed to clean up environmental contamination. In April 2010, nearly 2,000,000 gal of oil spilled in the Gulf of Mexico because of the Deepwater Horizon drilling-rig explosion. EPA researchers used a subset of ToxCast assays to assess endocrine activity and cytotoxicity of eight candidate oil dispersants within 6 weeks. "There were no glaring differences" in acute toxicity between the dispersants, Dix said.

Thaddeus Schug, of the National Institute of Environmental Health Sciences, described his agency's efforts to create a protocol for testing chemical toxicity. With a collaborative team of green chemists, biologists, and toxicologists, Schug developed a tiered protocol for endocrine disruption (TiPED). The first tier involves HTP assays, the fastest and cheapest tests, which Schug's team has also determined to be reliable and reproducible. A "hit" on any of the tier's tests requires action, Schug pointed out. The second tier includes cell-based assays, and the third and final tier involves amphibian/fish and rodent testing. The protocol will give chemists an idea of which assays they should be looking for and which procedures and testing guidelines to use. The

continued on page 6

GREEN CHEMISTRY, *cont. from page 5*

point is to go beyond what regulators do, Schug said.

Because new information is constantly becoming available, the protocol will continually be under development, Schug said. He and his colleagues hope to publish a white paper explaining their concept, but such issues as where to house the protocol and how to make it available to chemists are still to be determined.

Incentives for moving forward with rapid-testing approaches like the ones that Schug described include the potential for waivers of downstream animal testing, said Martin Stephens, of Johns Hopkins University. However, Cal Baier-Anderson, of EPA, pointed out that the use of tools like Schug's in actual decision-making is predicated on confidence in them.

Tanguay outlined why rapid toxicity testing with simple organisms—such as zebrafish, fruit flies, and roundworms—is as important as cell-based HTP assays. A major advantage, he pointed out, is that simple organisms can also be used to test thousands of chemicals a

continued on page 7

SCIENTIFICALLY SPEAKING

Nanomaterial-based technology is a new and rapidly advancing field of study that offers a unique opportunity to integrate new methods for toxicity testing with innovation and product development. Nanomaterials are inherently complex structures that can cross such sensitive biologic barriers as the blood–brain barrier, and this heightens concerns about their environmental and human health safety. Two researchers, James Hutchison, a professor of organic chemistry at the University of Oregon, and Robert Tanguay, a professor of toxicology at Oregon State University, are collaborating to develop methods and guidelines for testing and designing nanomaterials for greener products.

James Hutchinson
University of Oregon

Q: Are there safety-design rules for nanomaterials?

HUTCHISON: There are a few basic rules. The first is simply to avoid incorporating toxic elements like cadmium, zinc, and silver. Those elements leach out of nanomaterials that have high surface areas and can present a substantial hazard. A second rule is to evaluate analogous materials to pinpoint properties that you may want to avoid. We need more robust design rules. New rapid toxicity testing and computational approaches may help us to develop better rules.

Q: The traditional approach to product testing is to evaluate a material that is nearer to completion or commercialization. Why should we test nanomaterials at an early stage of design?

HUTCHISON: The characterization of materials for commercialization and their characterization for health and safety are sometimes completely different. That something meets specifications for performance does not mean that we know enough about its composition for evaluating health and safety, and this problem is amplified by the complexity of nanomaterials.

Q: What are some of the properties that make nanomaterials difficult to characterize and thus difficult to test for toxicity?

HUTCHISON: Nanomaterials differ from conventional molecular materials or larger particles in a number of key ways. They have pronounced heterogeneity in size, shape, surface coatings, and purity. They have novel three-dimensional structures and much higher surface areas per unit mass than larger particles. A direct consequence of their complexity is the inability to purify them easily for testing. As a result, nanomaterials are often analyzed in impure forms.

Q: What general approach are you are taking to understand nanomaterials' characteristics and potential for toxicity better.

HUTCHISON: Robert and I have focused on integrating toxicity and other biologic testing with emerging materials and development. We have tried to merge precision engineering with nanoparticle libraries and toxicity testing. Precision engineering allows us to create model nanomaterials that have a common metal core and size and can be "decorated" with specific surface groups, for

GREEN CHEMISTRY, *cont. from page 6*
day—far more than is possible in testing with rodents or humans. Testing models built around simple vertebrates, such as zebrafish, also overcome some of the blind spots associated with cell-based testing, Tanguay said. For example, complex interactions like those between cells or within specific systems, such as the endocrine system, cannot be easily evaluated by using cells in culture. Whole organisms, such as zebrafish, can be used to identify both hazard *and* mechanisms of toxicity, the latter of which is much more difficult to determine with cell-based assays. The genomes of zebrafish and other simple organisms have been fully sequenced, and scientists have developed validated methods for identifying changes associated with disease and altered behavior. Zebrafish share some developmental, anatomic, and physiologic characteristics with mammals, and they are more closely related to humans than are flies or worms. Newer testing tools, such as the ones he has developed, enable researchers to

continued on page 8

Robert Tanguay
Oregon State University

example, neutral, negative, and positively charged water-soluble coatings. Specifically, we use gold nanoparticles because gold is not toxic, and this allows us to focus on the nanoscale features.

TANGUAY: Using Jim's precision-engineered gold nanoparticles as models allows us to tease apart the biological effects caused by individual nanoscale properties. Using zebrafish as a systems-toxicology model organism allows us to rapidly discover and quantify adverse effects caused by these well-defined materials. The results of our toxicity tests show that even tiny differences in the structure of the materials can result in a big difference in zebrafish mortality, development, and even neuro-behavior.

Q: Have the results of your experiments changed how you conduct your research?

TANGUAY: Working with Jim has forced us to pay closer attention to material purity and characterization. The materials that we evaluate now must meet a high level of precision engineering before we use them in our zebrafish screening assays.

HUTCHISON: Purity is essential in determining the relationship between health effects and specific structures. We now take a two-tiered approach to characterization. The first tier is a shot-gun approach in which we gather as much information as possible on a nanomaterial with as many different techniques as possible. Once we understand a material class, such as the gold nanomaterials, we use a smaller set of chemical characterization approaches, including nuclear magnetic resonance, transmission electron microscopy, and UV spectra as a bare minimum. And, we develop new purification approach based on a nano filtration system.

Q: Have you come across any surprises that are important for thinking about "green" nanomaterial design?

HUTCHISON: Our work has underscored the importance of thinking about how nanomaterials may be transformed or influenced by their environment. For example, we used transmission electron microscopy to observe silver nanoparticles that were bound to a surface to mimic a fabric coating over a 5-week period. During that period, the "large" silver nanoparticles spawned many smaller particles. If the particles are actually transforming their size while they are in use, we need to consider that.

Q: Describe a key lesson learned from your collaboration on nanomaterials research.

HUTCHISON: Much of what Robert and I have discovered about how materials matter has come directly from the deep collaboration between our two research groups. Many lessons would never have been learned if one of us were a materials scientist or toxicologist going it alone. If we want to bridge the gulf between nanomaterial applications and implications, we need conversation and strong partnerships through which we can learn together, advance the science, and get the effects information back to the materials designers

Using Zebrafish for Rapid Toxicity Testing

Tanguay argued that zebrafish are an excellent model organisms for testing to the toxicity of chemicals and materials. The genomes of zebrafish and other simple organisms have been fully sequenced and scientists have developed validated methods for identifying changes associated with disease and altered behavior.

School of adult zebrafish.
Courtesy of Robert Tanguay

GREEN CHEMISTRY, *cont. from page 8*

identify early responses in molecular signaling networks that are predictive of human disease. Such toxicity pathways—or toxicity entry points, as Tanguay prefers to call them—somehow interfere with the thousands, tens of thousands, sometimes even millions of interactions involved in the normal biologic functioning of a given cell or cell network and irreparably impair its functioning.

Tanguay's testing models focus on how chemicals affect zebrafish embryonic development. The tests involve exposing embryos whose protective chorion (outermost membrane surrounding the embryo) barrier has been removed so that they are essentially bathing in the test chemicals during the period when they are developing rapidly. Most of the test assays—which evaluate everything from how the zebrafish develops and looks to how its nervous system functions and how it responds to stimuli—are completed within 5 days. Zebrafish mature within 2 months, and this enables researchers to test toxicity more rapidly during all stages of development than they can with mammalian species. Testing and understanding mechanisms of developmental toxicity are important because "most compounds are more toxic during early life stages," Tanguay said.

Another advantage of zebrafish-based toxicity testing is that molecular signaling is conserved. In other words, evolutionary mechanisms have caused the molecular signaling pathways to be similar across different animal species including humans. If exposure to a particular chemical in zebrafish results in an adverse outcome, the chemical might also be hazardous to humans. However, the consequences of disrupting cellular signaling are species-specific. "A compound that affects fin formation in a fish may have a different effect on humans, so you can't get too hung up on end points," Tanguay cautioned.

Design Guidelines for Reducing Toxicity

It is clear that new, rapid toxicity-testing methods can provide direct information about hazard and mechanisms of toxicity. Adelina Voutchkova, of George Washington University, argued that toxicity data can also be used to "rationally think about how to design hazard out of chemicals." She pointed out that the pharmaceutical industry has successfully demonstrated that molecules can be designed to hit specific biologic pathways. The challenge in industrial chemistry, Voutchkova said, is designing molecules to *avoid* hitting any critical biologic pathways. Designing safe industrial chemicals is particularly important because they are usually produced in much higher quantities than pharmaceuticals. Industrial chemicals are also often used in a much wider variety of applications than pharmaceuticals, so the potential for people and the environment to be exposed to them is greater. Research in this field, however, is slow, because biochemical pathways are "mind-bogglingly complex," and many pathways are still unknown, she said. Voutchkova emphasized that the development of design guidelines for green industrial chemicals will require the combined expertise of chemists, toxicologists, and ecologists.

Voutchkova and colleagues at Yale University developed their first design guidelines by looking at properties of epoxides and olefins, two mutagenic chemical classes with known mechanisms of action. Their initial research targeted molecular properties related to bioactivity, one of the four physiologic gates to chemical exposure. With the aid of advances in computational chemistry and toxicology, they were able to develop design guidelines that corresponded to mutagenicity.

The success of their work with epoxides led Voutchkova to ask whether it is possible to develop a combined set of design guidelines that address all four gates to toxicity of particular groups of chemical species. She and her colleagues used the mechanistic and statistical analysis approaches that they developed to produce design guidelines for reduced

continued on page 9

GREEN CHEMISTRY, *cont. from page 8*

aquatic toxicity. They compiled data on the toxicity of a variety of chemicals listed in databases of EPA and the Japanese Ministry of the Environment in three aquatic species: (fathead minnow, Japanese medaka, and *Daphnia magna*) and one algal species (*Pseudokirchneriella subcapitata*). The researchers were able to identify two chemical properties—log P (the octanol–water partition coefficient) and delta E (the difference between homo and lumo energy levels)—that in combination allowed them to distinguish roughly between toxic and nontoxic chemicals. Voutchkova and her team believe that log P is related to bioavailability and delta E to bioactivity. However, some known toxic chemicals still fall into the category of nontoxicity. Research is under way to learn why these outliers exist. Nonetheless, the right combination of log P and delta E may enable chemists designing a new chemical to improve their chances of developing a safe molecule in the design phase. Voutchkova and colleagues are continuing their work to develop design guidelines for aquatic toxicity and determining mammalian toxicity of additional groups of chemicals, including pesticides, herbicides, and fungicides.

A Chemoinformatic Approach to Greener Chemistry

Alex Tropsha, of the University of North Carolina at Chapel Hill, and his research team are developing models that use existing data on chemical safety to make predictions about the vast number of untested chemicals on which no information is publicly available. The goal of their work is to set priorities among subsets of chemicals for specific additional toxicity testing. However, Tropsha acknowledged that his models could also be used to aid in the design of chemicals. Echoing other meeting participants, Tropsha emphasized that computational modeling is crucial because such rapid testing programs as ToxCast have created "an ocean of data that cannot be used directly as deposited." However, Tropsha cautioned that the scientists who develop such models must validate them because both chemical and biologic data in the datasets used to create the models may be inaccurate. "Even small differences in structure representation can lead to important errors in the prediction accuracy of models," Tropsha stressed. He also noted that models may benefit from comparative testing with other computational models.

Tropsha participated in an international project which collaboratively evaluated 33 models, 17 techniques, and six approaches to chemoinformatics prediction models. The group found that the individual approaches could be improved by creating a consensus model. Testing demonstrated that the consensus model "could predict the highest number of compounds with the greatest accuracy."

Tropsha also helped to develop predictive quantitative structure–activity relationship (QSAR) models that were used to screen 3,000 chemicals in the Tox21 dataset virtually for binding to endocrine receptors. The models identified 135 compounds as potential estrogen-receptor binders that might induce endocrine disruption effects via an estrogen signaling pathway.

Tropsha is now researching methods for combining chemoinformatics (application of computational and informatic techniques to problems in chemistry) and short-term biologic assays. Preliminary results show that this emerging approach can improve the prediction accuracy of conventional QSAR models of chemical

continued on page 10

Physiologic Gates to Chemical Exposure

- Bioavailability: The ability of a chemical to be taken up by human or animals.
- Bioactivity: Biologic processes that activate or neutralize chemicals within the body or eliminate them.
- Distribution: Chemical dispersion to target organs and tissues in the body.
- Interaction with Biological Targets: Specific targeted (molecular) actions associated with toxicity pathways.

With colleagues at Yale University and Thomas Osimitz of Science Strategies, LCC, Voutchkova identified four physiologic gates of chemical exposure that should be considered in green chemical design. Voutchkova and her colleagues focused their research by examining such properties of molecules as size, energy state, water solubility, and vapor pressure, which may enable molecules to reach and interact with humans and other organisms through one of these four gates. For example, for a particle to be bioavailable within the human gastrointestinal tract, it must have a mass greater than 500 daltons.

GREEN CHEMISTRY, *cont. from page 9*
toxicity. In fact, such hybrid QSAR models can have higher predictive power than current commercial software. Tropsha's work is publicly available, and he argued strongly that all models and their supporting data should be public.

Ivan Rusyn, of the University of North Carolina, asked whether there is a discrepancy between Voutchkova's and Tropsha's approaches. The methods used by Voutchkova argue for developing a few chemical descriptors as potential design guidelines, but Tropsha demonstrated that hybrid predictive models based on multiple chemical descriptors are more accurate. Tropsha responded that the approaches are not contradictory but represent different levels of resolution and are used for different purposes. The ability to identify a few physicochemical characteristics that are easily understood and calculated and to relate them to biologic outcomes is an important beginning step in chemical design. The models, however, use a finer resolution to incorporate chemical properties that are not easily calculated and combine them with other characteristics to predict toxicity.

Increasing Confidence in New Toxicology

As a representative of the chemical industry, Edward Carney, of Dow Chemical, stressed the importance of finding ways to infuse green chemistry with the same attributes that give the industry confidence in traditional toxicity-testing schemes.

As an example of confidence-buttressing aspects of conventional toxicology testing, Carney described his company's search for an alternative to a teratogenic (birth-defect-causing) fungicide, Dinocap. He attributed his company's ultimate success in finding an alternative to its decades of experience with developmental toxicology, access to a reliable assay for the adverse effects (cleft palate and poor development of the inner ear, in this case), and previous whole-animal studies that provided integrated biologic data on relevant subjects, such as metabolism and pharmacokinetics. Knowledge of prior studies directed the scientists toward individual isomers as potential substitutes in the fungicide formulation.

Carney noted that it would be difficult to achieve the same end with the variety of new tools for assessing toxicity rapidly and building greener molecules discussed in this meeting. But the much greater speed of newer toxicity-testing approaches "is really essential" to the chemical industry, which must routinely evaluate groups of chemicals and consider many candidate substances. In agreement with many meeting participants, Carney stressed that businesses benefit from identifying potentially problematic compounds early in the development process.

Carney discussed Dow's new program, launched in spring 2010, that is intended to support green chemistry and the general movement toward using 21st century toxicology. Many of the testing platforms that Dow is using are similar to those discussed in the meeting, including in vitro and biochemical assays, chemoinformatics, and some tests with zebrafish embryos as model organisms. The program also focuses on identifying toxicity pathways.

Recently, Dow incorporated a new in vitro skin-sensitization assay. Skin sensitization is an important end point for consumer products, and the new assay capitalizes on the fact that relatively few key steps are required for sensitization. A study of 28 chemicals published last year showed that the sensitization assay had good correlation with human studies.

Carney also described a research program to produce a test for a prototypical toxicity pathway (vascular development) in collaboration with EPA's NCCT. Scientists are evaluating whether signals in ToxCast in vitro data correlate with functional end points. Preliminary results suggest

continued on page 11

Building Confidence in 21st Century Safety Assessment

Carney illustrated the gap between chemistry and toxicology as a canyon between two plateaus. The work of toxicologists, green chemists, computational modelers, and others to build a bridge between the two plateaus served as a metaphor for "building confidence" in 21st century toxicology. Carney emphasized that for chemists to begin incorporating new toxicity-testing approaches in product design they must have a level of confidence in the new tests that equals or exceeds their confidence in the traditional approaches. That said, he acknowledged the conventional approach's drawbacks: "It is slow, it is expensive, it's set up for one chemical at a time. And I think the most important thing is that it doesn't advance mechanistic understanding." However, Carney added, scientists must be careful not to sacrifice confidence by severing ties with traditional approaches too quickly. The bridge of confidence must be built in a "smart fashion."

GREEN CHEMISTRY, *cont. from page 10*
correspondence between platforms, for example, between computer simulations and the organs being grown in the laboratory, he said.

Finally, he detailed his company's "stepwise approach to increase confidence" in the new tools. He stressed that although green chemistry is important, it is crucial not to sacrifice current levels of confidence when trying to move from traditional chemical design approaches to new approaches involving 21st century toxicology

Identifying and Diminishing Gaps

Thomas Osimitz, of Science Strategies, pointed out that many gaps need to be filled in addition to the gap between the toxicology of this century and the toxicology of the last century. The gaps appear as challenges in the process of moving from *data* to *information* to *knowledge* to *understanding* and finally to *wisdom*. HTP toxicity testing produces a lot of *data.* Those data "can be put together properly to get some information and maybe some knowledge" in "relatively straightforward and inexpensive" approaches in comparison with whole-animal testing, Osimitz said. However, he cautioned, the shift from doing whole-animal work back to thinking about some of the basic properties of molecules is a serious challenge.

That reality makes modern toxicology more complex than historical toxicology, said George Daston, of Procter and Gamble. The first 50 years of toxicology relied on a very small set of tools that everyone agreed were useful. Daston observed that the new toxicology is and will be built around tools that are fit for specific purposes. Therefore, he stressed, discussions should not be about which techniques are promising—"they all are, but for different purposes."

Osimitz also emphasized that the scientific community needs to think about new approaches in the context of companies' resources. Time and resources should not be spent in "chasing results that have been indicated by animal studies but are not in humans." These animal data cannot be used in developing safe chemicals. And whole-animal tests should not be conducted "for nothing more than clinical chemistry," because this wastes the ability to produce important toxicologic information, Osimitz contended.

Denison argued that scientists need to move beyond simply looking for a no-observed-effect level (NOEL) in animal studies. He pointed out that the new techniques can allow scientists to investigate variability in the study populations with regard to different susceptibilities as well as exposure patterns and co-exposures.

> ***I don't believe at this point that there will be any one-size-fits-all tool.***
> **– George Daston**

Advancing the application of 21st toxicology to green chemistry does not stop with producing information. *Knowledge* and *understanding* are also needed. Osimitz defined knowledge as the ability to discern the relative effects of chemical exposures on various biologic pathways that are operating at the same time. Scientists also need to focus on determining how gene changes identified by some of the HTP screening techniques are related to specific pathways. Finding answers "is going to be a very iterative process with lots of incremental improvements," he predicted. Osimitz said that understanding the relationships between changes in gene expression in pathways and toxicity in whole animals is also important. He pointed to recent work that used a "heat map" format to show the number of genes in a pathway that were altered as a creative way to visualize such information. Understanding involves integrating all the sources of information to determine what is occurring in humans. It is a "tremendous challenge," Osimitz said.

The ultimate goal is wisdom, "the ability to make smart choices about using chemicals," said Osimitz. He pointed out that there may be a lot of uncertainty in the data and that it is easy to get wrapped up in the complexity of analysis but that at the end of the day decisions must be made. The ability to decide among myriad choices is critical. Jennifer Sass, of the Natural Resources Defense Council, opined that industry scientists should be asking questions about environmental health and safety at every step of product development. "If we know enough to produce [a chemical], we should know enough to evaluate it," Sass said, adding that finding ways to evaluate compounds for altering hormones or endocrine disruption should have high priority.

Osimitz described cultural gaps that are embedded throughout the data-to-wisdom framework. He and other meeting participants recognized that more must be

continued on page 12

GREEN CHEMISTRY, *cont. from page 11*
done to encourage collaboration between chemists and toxicologists. He emphasized the need for industry chemists to view toxicology as much more than the information provided on a chemical Material Safety and Data Sheet. People must also shift their thinking beyond regulatory frameworks.

He talked about the challenges involved in making the business case for green chemistry. Some large companies disregard green chemistry because it is hazard-based. However, risk can be used to set priorities among outcomes. As Holder, of Hewlett-Packard, noted, it is important for businesses to acknowledge that mistakes will be made rather than to succumb to the "paralysis by analysis" that has sometimes resulted from a fixation on certainty. Meanwhile, toxicologists and green chemists need to understand business considerations, such as cost, product efficacy, and other criteria involved in decisions to produce chemicals and materials. Osimitz emphasized that hazard criteria must also be balanced with other criteria for sustainability.

Osimitz concluded, and many participants agreed, that "the golden age of toxicology is now." To get where we need to be, Farland stressed, "we all need to be working together on this." Although the meeting allowed "problems to meet solutions," the continuing challenge is to scale solutions sufficiently to make a difference, he said. Many attendees left the meeting motivated to do their part to make a difference.

National Research Council Reports on . . .

–by Dennis Harris, Christine Mirzayan Science and Technology Policy Fellow

The National Research Council (NRC) has published a variety of reports related to green chemistry and sustainability. Many of them suggest invaluable benefits of conversations about sustainability, especially about topics in such relatively young fields as green chemistry. Speakers at the NRC Green Chemistry meeting in September 2011 drew from a few of the reports to highlight considerations important for advancing green chemistry. Paul Anastas, formerly of the Environmental Protection Agency, referred to ***Sustainability and the U.S. EPA,*** which proposed an EPA framework for integrating sustainability, and Robert Tanguay, of Oregon State University, mentioned ***Toxicity Testing in the 21st Century: A Vision and a Strategy,*** which delivered a variety of suggestions for improving efficiency and reducing waste and cost in agencies and organizations that characterize chemical toxicities.

Other published NRC work on sustainability addresses topics that apply to green chemistry. For the sake of clarity, sustainable was defined in Executive Order 13423 as a means "to create and maintain conditions, under which humans and nature can exist in productive harmony, that permit fulfilling the social, economic, and other requirements of present and future generations of Americans."

One challenge in designing green chemicals is to develop systems that consider the effects of the entire life cycle of a chemical—from production to disposal—on human health while also considering sustainability. The principles of enabling sustainability efforts were described in the 1999 NRC report ***Our Common Journey: A Transition Toward Sustainability.*** The report discussed the science and technology necessary for the growing global population to address increasing food, energy, and social needs in a sustainable way. More than just a technology review, the study considered implications of unsustainable human behavior and

continued on page 13

. . . Sustainability and Green Chemistry

methods for intervening. Twelve years later, the NRC revisited the topic of food sustainability in the 2011 workshop report ***A Sustainability Challenge: Food Security for All,*** which analyzed the needs for and means of fostering sustainable practices in agriculture. Those reports repeatedly discussed resource limitations as a major concern for our future.

Reducing the amount of hazardous waste introduced into the environment and enabling sustainability in the chemical industry is a focus in green chemistry. The NRC published several reports on the chemical industry's effects on environmental health and sustainable models.

The 2003 report ***The Environment: Challenges for the Chemical Sciences in the 21st Century*** reminded us that chemistry and chemical engineering are global topics and that the effects of chemical industries cannot be contained within a country's borders. This report, like others, emphasized planning by creating production models that eliminate or reduce hazardous waste. In the 2005 report ***Sustainability in the Chemical Industry: Grand Challenges and Research Needs,*** the same questions were introduced but with a guiding interest in the research that is needed to make the chemical industry more sustainable and "green."

The reports on the chemical industry and sustainability set the stage for unique challenges facing green chemistry and the chemical industry. Environmental toxicity, economic feasibility, and product life cycle all pose important challenges. As is true in other growing fields of science and technology, challenges require an education focused on matters at hand.

To support the new thinking, the NRC published a summary of discussions at a 2007 workshop ***Exploring Opportunities in Green Chemistry and Engineering Education.*** The workshop explored the importance of educating future scientists in green sciences and production. Green chemistry was introduced as a means of reducing or eliminating the generation of hazardous substances and green engineering as a way of continuing production in an economically feasible and environmental sound manner. The workshop also highlighted the necessity to prepare our future scientists to consider the already existing challenges of sustaining the chemical industry in a manner that is greener and safer than before.

The different elements brought into the conversation by past NRC reports will be useful tools in conversations on green chemistry. Sustainability has been a frequent theme of NRC reports and continues to emerge in current workshops and studies.

FURTHER INFORMATION ON THESE REPORTS

For additional information and free PDF files of these reports and workshop summaries, visit

http://www.nap.edu

Government Strategies for Going Green

—by Kellyn Betts; edited by National Research Council Staff

Much of the movement to "go green" is fueled by the public's and science community's interest in new approaches to designing safer chemicals, but governments are also exploring methods of promoting greener industrial practices. Lauren Zeise, of the California Environmental Protection Agency, noted that government frameworks to "move new toxicology tools into private design" throughout the life cycle of a product will both advance science and support a greener industrial culture.

Sharon Munn, of the European Commission Joint Research Centre's Institute for Health and Consumer Protection (IHCP), described how regulatory and research endeavors in the European Union (EU) are being used to improve chemical risk assessment and decision-making.

The EU has a number of legislative drivers moving it away from the use of animal testing and toward new toxicologic methods. Those drivers include the 2006 Registration, Evaluation, Authorisation and Restriction of Chemical substances (REACH) law, including safety of nanomaterials, the regulation on cosmetic products, and regulations that require the EU to develop criteria for identifying endocrine disruptors by the end of 2013. It is hoped that the statutory requirements will not only oblige industry to develop and use more human-relevant tests but prevent "bad" products from ever entering the environment.

The EU is also involved in research efforts to develop and validate new toxicity-testing and computational tools that are based on the mode of action of chemicals. To that end, EU scientists are working to identify "upstream" critical biochemical or cytological events that occur before empirically verifiable outcomes of exposure, such as developmental anomalies, reproductive impairment, and physical changes, including alterations in the size and histopathologic state of organs. A companion goal is to develop in vitro methods for measuring such critical events, Munn said.

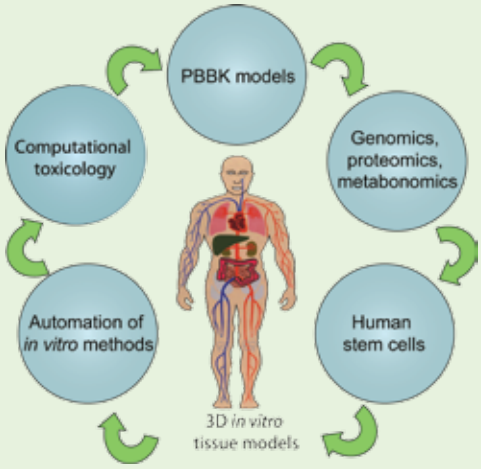
EU scientists are also investigating how to extrapolate from in vitro to in vivo dose–response relationships. They are trying to find ways of categorizing chemicals on the basis of structure–activity relationships. Another goal is to combine in vitro, in silico, and in vivo techniques. Munn pointed out that public–private partnerships and international collaborations are important for pooling knowledge and resources. For example, the IHCP is collaborating with the US Environmental Protection Agency (EPA) on the ToxCast program to find the best new tests and methods.

As Richard Denison, of the Environmental Defense Fund, noted, the EU approach to advancing development and use of the new methods is more targeted and focused than the US approach. The US government lacks regulatory incentives to encourage

continued on page 15

Benefits of Change

- Ethics—reduce the reliance on animal testing.
- Accuracy—develop more predictive risk assessments that are based on improved understanding of why chemicals are toxic.
- Regulation—use faster and less expensive methods to screen chemicals that are already in the environment and identify the "bad actors" that require risk management.



- Design—use faster, less expensive methods to screen chemicals during product development to remove bad actors and prevent their entry into the environment.

Munn highlighted multiple emerging technologies that are being explored in the EU to improve the human relevance and speed of toxicity testing. She emphasized the benefits of using 21st century toxicology for both screening existing chemicals and products and designing new ones. The new tools may be particularly helpful when it is not permitted (i.e. for safety assessment of cosmetics) or practical to use animal data to identify chemicals that have a tendency to be persistent, bioaccumulative, and toxic; to be very persistent and very bioaccumulative; to be carcinogenic, mutagenic, and reprotoxic; or to be endocrine disrupters.

GOVERNMENT, *cont. from page 14*

the application of new toxicity-testing approaches and promote greener chemistry. However, US federal agencies are developing innovative methods to encourage a shift toward greener chemical and product development. Notable among US approaches are a series of activities housed in EPA's Office of Chemical Safety and Pollution Prevention that focus on promoting green chemistry and safer product development through award programs, such as the Presidential Green Chemistry Challenge Awards, and alternative decision-support programs, such as Design for the Environment (DfE).

Cal Baier-Anderson, a toxicologist in EPA working on DfE, described the attributes of this novel, voluntary partnership program. DfE provides companies with incentives to produce safer products and has specific elements aimed at safer product labeling, life-cycle analysis, and assessment of chemical alternatives. DfE helps individual and institutional consumers to balance often competing priorities, including product reliability, cost, and environmental impact. Although all of the options that companies consider may not be truly "green," DfE does its best to direct companies to alternatives that are at least safer.

For assessing safety and hazard, EPA uses both threshold-based methods, such as acute toxicity tests, with dose thresholds that define high, moderate, and low hazard; and evidence-based methods, which involve evaluating the strength of evidence linking a chemical to an effect. However, Baier-Anderson said that hormone- and endocrine-disrupting effects are "probably the hardest for us to evaluate because there is no agreement on a hazard-ranking framework" for these effects. She also stressed that assessment of effects on the environment must take environmental degradation products into account because of the potential for human and environmental exposure to take place over a chemical's and product's lifetime. For example, some compounds, such as surfactants, can result in aquatic toxicity when they degrade in the environment.

There's no doubt that the new rapid test methods and computational toxicology can help to identify safer chemicals, Baier-Anderson said. The options for incorporating the new methods into criteria frameworks include the approach that EPA's National Center for Computational Toxicology is exploring of calculating the human-equivalent dose based on high-throughput testing, she said. The method may also be useful for making pathway-based potency comparisons within classes of chemicals to define concern thresholds of perturbations. Kathryn Guyton, a senior toxicologist in EPA's National Center for Environmental Assessment, pointed out that the new tools may aid the agency in dealing effectively with such thorny issues as variability in individual susceptibility to chemical exposures and exposures to mixtures.

However, the DfE program's current criteria are based on animal testing. "We are making decisions in real time[...] We have to use the information that is in front of us at any given moment to make decisions in a fairly short timeframe," Baier Anderson said. Incorporating data from new toxicity tests may present some challenges for programs like DfE, but interim approaches may be possible. One might involve comparing results from different test strategies and working through differences. "It's an opportunity for new thinking about criteria and evaluation," Baier-Anderson said optimistically.

"GOING GREEN," *cont. from page 15*

use—can end up harming human health. Green chemistry is considering the problem of chemical waste and byproducts in production and merchandise and in theory can reduce exposure to hazardous substances before it even starts.

The principle of green chemistry is fantastic: reduce or eliminate the amount of toxic substances that we make. The reality is that determining what is toxic and how much is acceptable is much more complicated than simply referring to a list of chemicals. It requires intimate knowledge of what chemicals are used and how, what effects they have on biologic systems, how long the effects last, and whether there are reasonable alternatives to the chemicals.

This newsletter covers a variety of topics on green chemistry. Businesses and individuals have deeply vested interests in preserving a safe environment. Green chemistry will require a new kind of debate and discussion among chemists, biologists, economists, and business leaders. In many ways, our environment and our health may depend heavily on whether we can all figure out how to "go green."

MEETING INFORMATION

Meeting Presentations

Would you like more details about the green chemistry or other Emerging Science meetings? Descriptions, agendas, and presentations for all of our meeting topics are available through our website. Also, we invite you to subscribe to our listserv for the latest information about meetings, newsletters, and other Emerging Science activities. For more information please visit

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

Upcoming Meetings

Big Data and Environmental Health: Integrating Research to Advance Discovery
Washington, DC, January 10–11, 2013

Previous Meetings

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

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

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

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

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

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

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

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

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

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

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

About the Committee

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

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