EMERGING SCIENCE FOR ENVIRONMENTAL HEALTH DECISIONS

THE NEWSLETTER OF THE STANDING COMMITTEE ON USE OF EMERGING SCIENCE FOR ENVIRONMENTAL HEALTH DECISIONS

NOVEMBER 2010 ISSUE 3 ISSN 2376-1679

Computational Toxicology: From Data to Analyses to Applications

Introduction

On September 21–22, 2009, the National Research Council Committee on Use of Emerging Science for Environmental Health Decisions held a public workshop titled "Computational Toxicology: From Data to Analyses to Applications." The workshop was convened to assess cutting-edge and practical applications of the new field of computational toxicology.

The participants discussed scientific aspects of computational toxicology, including strengths and weaknesses, the need for validation, and timelines for the use of computational toxicology in environmental science. Participants also debated immediate and long-term applications of the emerging data and knowledge, managing expectations, and maximizing returns on investment.

What Is Computational Toxicology, and Why Is It Needed?

Recent reports and discussions suggest that the field of toxicology should shift toward toxicity-pathway analysis and targeted testing. For example, the 2007 National Research Council report *Toxicity Testing in the 21st Century: A Vision and a Strategy (http://www.nap.edu/catalog.php?record_id=11970)* emphasizes the use of human cells, cell lines, and cell-component testing platforms in toxicity testing. That suggested shift emerged in response to dissatisfaction with the current approach to toxicity testing and with our inability to answer questions that scientists have regarding relevance and efficiency, according to William Farland, of Colorado State University. Questions include how to move past a focus on tests for overtly toxic responses that measure only gross changes observed in animal tests and to begin to address the safety of compounds that people are exposed to that have never undergone toxicity screening or testing.

A challenge in environmental health research is to determine the extent to which cell culture and similar non-whole animal tests reflect human outcomes. In 2009, the US Environmental Protection Agency (EPA) published a report describing how the use of new scientific tools in computational, informational, and molecular sciences can strengthen toxicity testing and risk assessment. Those tools are included in the toolbox of computational toxicology, a subdiscipline of toxicology that uses mathematical and statistical modeling and computer-science tools.

Ivan Rusyn, of the University of North Carolina (UNC), noted that many scientific and mathematical fields contribute to computational toxicology. It is important to recognize that computational toxicology requires cross-disciplinary understanding and collaboration. It is the cross-disciplinary approach that enables computational toxicology to generate knowledge from data.

> ***Computational toxicology is one of those great fields that try to take data and generate knowledge.***
>
> **—Ivan Rusyn, University of North Carolina**

THE NATIONAL ACADEMIES
Advisers to the Nation on Science, Engineering, and Medicine

What Are the Potential Uses of Computational Toxicology?

Statistician Russell Wolfinger, of the SAS Institute Inc., explained how the ability to answer questions is typically goal-dependent and noted that computational toxicology has varied goals. He asked whether the primary goal is to fulfill the lofty ideals of the 2007 National Research Council report—to be able to do quantitative risk assessment based on a set of in cell culture assays and computer models—or to be able to look at a large set of environmental stressors and understand how and under what circumstances they might cause human disease.

Christopher Portier, of NIEHS, emphasized a need for computational toxicology to help to predict how chemicals will act. Richard Judson, of EPA's National Center for Computational Toxicology, agreed, explaining that we need mechanistic predictions to say which chemicals seem to be hitting pathways that we know can lead to illness and then need to look hard at those chemicals, i.e., to set priorities for further testing rather than using computational toxicology to reveal definitively what a chemical does. Presenting a different viewpoint, Richard Superfine, of UNC, explained how we also need to determine the requirements of a predictive computational model that will eliminate animal testing and meet federal requirements.

Reflecting on the different objectives that were articulated, Abby Li, of Exponent, Inc., described the following three possible objectives of computational toxicology, with the latter two regulatory objectives needing more selective positive and negative controls:

- To develop hypotheses for further testing in a biologic system.
- To determine whether more focused testing should be added to a shorter-term study.
- To replace in vivo testing completely for regulatory decision-making.

Judson thinks that computational toxicology will, at least in the short to intermediate term, be used primarily for priority-setting, not for definitively answering questions about what a chemical does. For example, individual screens are not going to show conclusively whether a chemical is a carcinogen or an endocrine disruptor. Instead, computational toxicology will provide enough evidence for us to say which of the 10,000 compounds we look at show evidence of possibly causing particular types of toxicity and therefore need to be studied in more depth.

Emerging Data Streams

Computational toxicology provides a framework for using computational power to model key aspects of physiology and toxicant-related pathology. The data used for computational toxicology generally come from assays, such as those based on microarrays, that generate many data points in less time than traditional assays do. Rather than looking at the end result of disease or other adverse outcome, which we typically do in animal studies, we look at the beginning of the process—at changes at fundamental levels of biologic organization, some of which may be related to adverse outcomes. Judson explained how once the screening data are available, modeling can be as simple as performing basic statistical analyses that ask, for example, whether hitting a particular receptor correlates with a particular end point. This type of modeling requires that both in vitro and in vivo data on a common set of chemicals be available. Alternatively, one can construct detailed multiscale models of biologic processes and use them to run simulations to help to understand the effects of perturbing particular pathways.

Several participants focused on describing the types of data that are being generated—data that are available for use in different computational toxicology models.

See related commentary at
http://ehp03.niehs.nih.gov/article/fetchArticle.action?articleURI=info:doi/10.1289/ehp.1001925

Molecular-Scale and Cellular-Scale Data from High-Throughput Assays

Chris Austin, of the National Institutes of Health (NIH) Chemical Genomics Center (NCGC), opened this portion of the workshop with a presentation on molecular-scale and cellular-scale data. We want data on how chemicals affect humans, but what scientists have now is data on how chemicals affect rodents, he said. He described how the so-called Tox21 effort—a joint effort of the National Toxicology Program (NTP), NCGC, and EPA—involves gathering in vitro data on interactions between chemicals and target genes or proteins in primary cells or cell lines derived from humans or other species. The appropriate balance between generating data on rodents and generating data on human molecular targets is controversial because, although humans are the species of interest, most in vivo toxicology information (used for comparison with in vitro data) is derived from rodent studies.

Austin noted the large number of data points that can be generated, but pointed out that the interpretation of high-throughput screening data has limitations. High-throughput screening is useful for identifying molecular mechanisms that can be modeled, but it should be followed by correlative assays or models that have more biocomplexity before one draws conclusions about a chemical's effects. For example, metabolism needs to be accounted for, and other issues, including volatility, limit the appropriateness of using particular screening assays to test some chemicals. High-throughput screening can examine chemicals only out of context. It is important to view all results with a high degree of suspicion and to be careful to conduct follow-up assays or correlative assays before drawing conclusions about a chemical, Austin said.

Tissue-Scale Data

Linda Griffith, of the Massachusetts Institute of Technology, described her laboratory's development of approaches to research on effects of chemicals on the liver. The development of new cell-culture methods is flourishing, and her laboratory and others think a lot about how to develop methods for modeling physiologic processes. She noted that high-throughput assays have their limitations and that she and her colleagues turn to a lower-throughput assay when they need assay that addresses complexity better. Her laboratory focuses on primary cells, but the questions being asked must drive the choice of culture system.

In addition to cultured primary cells, Griffith's laboratory uses cultured liver slices, which are physiologically very responsive. However, she has questions about how accurately the liver slices represent live organism physiology after long maintenance in culture. To address that, scientists are making a large effort to develop methods for culturing cells in ways that mimic their natural environment. For example, microfabrication expert Elisabeth Verpoorte, of the University of Groningen, is containing liver slices in a microfluidic system that provides control of fluid movement around the tissue and provides a precise microenvironment. Griffith's laboratory is also working on "3D patterning," which mimics tissues by combining microfluidics with the 3-dimensional arrangement of biological material.

High-Content in Vivo Data

Robert Tanguay, of Oregon State University, explained the merits of using whole zebra fish embryos for toxicity testing and for developing predictive models. Zebra fish share many developmental, anatomic, and physiologic characteristics with mammals. Mammals do not have fins, but the molecular pathways that lead to fin-fold formation are conserved in mammals. He emphasized that scientists working with nonmammalian models need to think about which molecular pathways cause observed effects when perturbed instead of focusing on specific end points.

> ***No single model is sufficient at this time to identify hazards.***
>
> **—Robert Tanguay, Oregon State University**

Like others at the workshop, Tanguay discussed the importance of making data accessible and useful

to others. Scientists need widely used knowledge bases and open access for data-sharing.

Existing Data

Judson shifted gears from the previous presentations to discuss compiling and taking advantage of existing data. Judson is the team leader for EPA's ToxCast bioinformatics efforts, and to him an important aspect of "computational" in computational toxicology is the building and use of databases that compile data.

Data come in all forms, from numbers to images. But data are not the information on a piece of paper in your drawer. Most of the good in vivo data have not been parsed—organized in a way that they can be shared and analyzed—and are therefore still unusable. The many formats of the data can constitute an important issue. Potential data sources include the NTP, which has a lot of in vivo data (mostly from rodent studies). Judson's group has developed the Aggregated Computational Toxicology Resource (ACToR) to index all publicly available data on chemical toxicity. He also described the virtual-tissue knowledge base that the EPA NCCT is building. As for data on the ToxCast chemicals, Judson noted that it took a long time to release them because of the need to understand issues of data quality.

Carolyn Mattingly, of the Mount Desert Island Biological Laboratory, walked participants through her laboratory's Comparative Toxicogenomics Database (CTD, http://ctd.mdibl.org/), which focuses on the molecular mechanisms by which chemicals affect human disease. The team manually examines and inputs data ("curates" the data) taken from the published literature, including data on chemicals' interactions with genes and proteins and links between diseases and chemicals or genes.

For bisphenol A (BPA), for example, Mattingly and her colleagues would include which diseases are associated with the chemical, which BPA-induced genes function during development, which biologic functions and molecular pathways BPA affects, and which chemicals have interaction profiles similar to those of BPA. The database has over 230,000 chemical–gene interactions, over 7,600 chemical–disease relationships, and over 10,000 gene–disease relationships.

They use established vocabularies, but there is no vocabulary or ontology to describe molecular interactions between chemicals and genes, so CTD scientists have developed their own ontology. Because manual curation of data is so labor-intensive, they are working with experts in natural-language processing at the MITRE Corporation and the University of Colorado to develop tools to increase curation efficiency and coverage of the literature.

Different Modeling Approaches in Computational Toxicology

Combining Quantitative Structure–Activity Relationship Models and Biologic Assays

Alexander Tropsha, of UNC Chapel Hill, introduced the afternoon's discussion with a presentation on combining data from high-throughput biologic screening assays and predictions based on chemical structure (quantitative structure–activity relationship, or QSAR, models) to improve sensitivity, specificity, and overall productivity of toxicity models by using hierarchic modeling. Specifically, his laboratory translates information about molecular structures into thousands of molecular descriptors that reflect composition, charge distribution, shape, and other physical and chemical properties of a molecule. The descriptors are then analyzed with statistical models that are based on the results of the biologic assays. The models are called quantitative structure-binding relationship (QSBR) models. QSBR models can be used to generate safety alerts and set priorities among chemicals for testing, and the models can be validated experimentally. Tropsha's group is using this approach to look at how the EPA might set priorities among chemicals as part of the ToxCast program.

Statistical Comparison of Many Modeling Approaches

Like Tropsha, Wolfinger's group is using EPA's ToxCast data to test the development of chemical-toxicity signatures on the basis of in vitro data and chemical descriptors. Wolfinger's group is

also assessing QSARs but is taking a "brute force" 5-fold validation statistical approach to compare 84 modeling methods with no biases favoring particular bioassays or even predictors. No predictive modeling method is suitable for all situations, so they start with ToxCast bioassay measurements that they want to predict and then see which models predict each end point best. Comparing results from a variety of statistical models offers some assurance that they are using the best modeling method. They have also found that chemical descriptors improve predictive accuracy over bioassay data. Their results show that the models will work better once they "learn" from the additional samples available in phase 2 of ToxCast.

Tools for Network Analysis: Biologically Driven in Silico Models

H. Steven Wiley, of the Pacific Northwest National Laboratory, talked about the approaches that he and others use to make models of cell function. They reconstruct signaling networks and metabolic networks by using quantitative data on cell composition that they collect. The "compositional data" are essentially the amounts of transcript, protein, or metabolites in a cell at a given time (and, in the case of toxicology, in response to a stressor). Because it starts with a molecule rather than with a toxic end point, this is sometimes referred to as a bottom-up or data-driven approach to computational toxicology.

There is no such thing as an average cell, but the typical canonical maps used to construct signaling networks are based on the average of hundreds of cell types. Compositional-data models provide a framework for interpreting data on cellular responses by relating the responses to the actual molecular-level differences between cells.

Wiley's group combines computational modeling with high-throughput experimental measures to learn how genes and molecules give rise to higher-order networks—a "systems" approach. He described the specific series of steps involved in reconstructing a signaling network, beginning with combining data about the proteins expressed (proteomics data) and transcripts expressed (microarray data). Other researchers also use data about the metabolites (metabolomic data).

Although Wiley and other researchers focus on reconstructing the biochemical reaction networks that cells use in normal states, networks for toxic responses could be constructed by plugging in a set of data that includes alterations in cell composition that result from a toxic response

Computer-Based Modeling with Virtual Tissues

Richard Superfine, of UNC, introduced his Virtual Lung Project. He and his colleagues are building an interactive computer simulation of the human lung that represents a physical-science–based approach to biologic phenomena.

From his perspective as a physicist, toxicology or health in the lung is based on a transport problem. In people who have cystic fibrosis, chronic obstructive pulmonary disease (COPD), or asthma, the inability of the cilia to transport mucus in a normal way can result in inflammation or pathogens getting through the mucus. One goal of the Virtual Lung Project is to predict how well different treatments improve cilia clearance of mucus in the lung. For example, there are cell culture measurements and a mathematical model of a beating cilium to see if mathematic models can predict hydrodynamics that happen. How the lung interacts with the physical world around it can be looked at by adding sheer stress, water, and various mucous secretions to the model.

Superfine said that the next step is to marry the virtual tissues and biophysical modeling approaches with the databases that have been discussed at this meeting. Data on patient diagnoses, therapies, and outcomes also need to be added. His team is taking its first step into a diagnostic setting by looking at mucus and sputum flow to see whether it predicts COPD exacerbations.

Questions about Computational Toxicology

Nearly half the conference was dedicated to discussion. Invited panelists explored several matters, beginning with strengths and weaknesses of computational toxicology.

Deborah Cory-Slechta, of the University of Rochester School of Medicine and Dentistry, addressed the challenge of applying computational toxicology findings to real-world situations, warning that reliance on computational toxicology may lead to underestimation of risk. She referred to validation as the 800-lb gorilla in the room, suggesting a focus on end points for which people think validation is possible, as opposed to more complex types of toxicity. Bruce Fowler, of the Agency for Toxic Substances and Disease Registry (ATSDR), was optimistic that validation can happen by moving from in vitro or in vivo studies up through various levels of biologic organization and thus helping to make risk assessments more precise than they are now.

Cory-Slechta also noted there are other complexities. Some risks may show up only when chemicals combine with the other factors that contribute to human diseases and disorders. Chemicals also combine with other chemicals to create different risks, Susan Fisher, of the University of California, San Francisco, pointed out. Other conference attendees also expressed concern about mixtures. Fisher emphasized the importance of developing a better conceptual framework for and better testing of interactions between chemicals.

Fowler asked how computational toxicology can consider sensitive populations consisting of people whose age, sex, nutritional status, genetic susceptibility, or mixture exposures may put them at risk. Some participants favored taking advantage of existing tools and study designs, warning against "reinventing the wheel."

Referring to neurodevelopmental effects and computational toxicology, Li noted that some molecular end points are critical for the pathways that play a key role in development and are conserved across species. Computational toxicology may help us determine which end points are useful for predicting how chemicals will affect humans, she said. Most important, computational toxicology will allow us to get past the apical functional end points to those more specific to human neurodevelopment based on mechanistic understanding. However, she also noted that in order to move forward, standard criteria are needed to assure quality chemical toxicity data are used in the computational approaches.

> ***The most important thing to do right now is to ask the experts in different biologic systems to describe the 10 most important pathways that affect their biologic system when perturbed by chemicals and the cell context that we need to screen for these pathway perturbations.***
>
> **—Robert Kavlock, EPA NCCT**

Potential Research Needs and Directions

Robert Kavlock, of the EPA NCCT, said that the most important thing to do right now, to develop the needed assays, is to ask experts in different biologic systems (such as neurotoxicologists, developmental neurotoxicologists, and reproductive toxicologists) to describe the 10 most important pathways that, when perturbed by chemicals, affect their biologic system and the cell context that we need to screen for these pathway perturbations.

Li and others noted that the absence of data on dosages in computational toxicology models is a serious shortcoming: dosages are key to setting priorities among chemicals. Portier disagreed, saying that dose issues can be figured out after more pressing issues, such as commonalities in chemical effects, are addressed. Farland noted that it is quite useful that a number of the approaches described at this meeting can assess responses over a large range of doses—7.5 orders of magnitude.

Daniel Axelrad, of EPA, expressed concern that the current computational toxicology research on pesticides and pharmaceuticals may not apply to industrial chemicals. It is possible that the current approach of developing tools using the richest datasets will yield findings that are specific to pesticides and pharmaceuticals and that the behavior and activity of industrial chemicals are different.

Portier expressed concern about some of the technical details of computational toxicology. Specifically, he is concerned about whether the signals that are being reported exceed the noise; he has not seen this given serious enough consideration in the field. Replicate measurements give a sense of the signal-to-noise ratio and are important even though testing of the same chemical multiple times decreases the number of chemicals tested. Although acknowledging Portier's concerns, some other participants noted that this workshop was necessarily limited in the depth and detail of the data covered.

Shuk-mei Ho, of the University of Cincinnati, noted that there are now opportunities to incorporate human population data into computational toxicology. We can generate dose–response curves from real populations if we have accurate predictive markers, with clear cut-points, that are tied to human phenotypes or pathology. She is optimistic that computational toxicology will move directly into population biology and use human cells and perhaps clinical specimens for human body measurements. She also expects to see the use of ambient measurements (outdoor pollutant measurements) and direct measurements of individual body burdens of chemicals through rapid development of increasingly sophisticated sensors. Farland agreed that Ho's vision of incorporating human data is an ultimate goal of computational toxicology

Moving Forward

Decision-Maker Use

Participants from government agencies—including EPA, the Centers for Disease Prevention and Control, the Consumer Product Safety Commission, ATSDR, and several institutes of NIH—addressed questions about the use of computational toxicology in decision-making.

George Daston, of Procter & Gamble, said that current applications include making inferences about the toxicity of untested materials—searching chemical substructures, for example. William Sette, of EPA, said that he needs computational toxicology data because information on many chemicals that require decisions is lacking. Computational toxicology scientists have information that could help to guide thousands of decisions. For example, we need to know whether weathered toxaphene is toxicologically equivalent to the product that was originally released into the environment.

Farland reminded participants about the natural tension between wanting to involve different communities, letting people know about the advances that may be useful, and wanting to manage expectations for the use of computational toxicology in decision-making. Providing a venue for decision-makers and researchers to discuss advances and appropriate expectations is a goal of this National Research Council standing committee.

> ***The concern may be that we haven't examined the different plans together to see which goals or needs they are not addressing.***
>
> **—George Daston, Procter & Gamble**

Where Is the Field Going, and What Is Needed?

Addressing the broader question of where the field is going, Axelrad reminded participants that the major players in the field have, at this meeting, expressed uncertainty about whether we are heading in the direction of *replacing* whole-animal testing or *setting priorities for* whole-animal testing. Other conference participants and presenters also expressed concern about a lack of clear goals and direction. Daston countered that EPA's NCCT and NIEHS have had a strategy with goals for the last 5 years and that pharmaceutical companies with computational toxicology programs have clear strategies. The concern about lack of direction or clear goals may reflect either this workshop's emphasis on highlighting some interesting approaches instead of focusing on EPA's program, or it may be that different plans have not been examined together to see which goals or needs they are not addressing. Rusyn suggested that the NTP, NIEHS, and EPA outline their needs (criteria and definitions of questions to address) so that academic and industrial researchers can provide the data. That would

also help with expectation management and with making sure that limitations are recognized and communicated.

The final discussions emphasized again the value of assembling different groups to figure out how to communicate and work together. Daston emphasized that computational toxicology is a complex field that brings together a lot of people that have different languages and alphabets. They have markedly different expectations about what computational toxicology might be able to do, and it is good to share their perspectives. He emphasized the need for continual joint investment in this young field that has many potential applications. Collaboration at multiple levels is essential—among the agencies and decision-makers who may eventually use the technologies, and among researchers in academe, industry, and government.

This summary was prepared by Tina Adler and Marilee Shelton-Davenport, with editing by Norman Grossblatt.

Related News and Publications

Rusyn I and Daston GP. 2010. Computational Toxicology: Realizing the Promise of Toxicity Testing in the 21st Century. Environmental Health Perspective. Aug;118(8):1047-50.

About the Committee

At the request of the National Institute of Environmental Health Sciences, the National Academies formed the Standing Committee on the 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.

To subscribe for updates and to access presentations and audio recordings of Emerging Science meetings, please visit http://dels.nas.edu/envirohealth.shtml

The National Academies
500 Fifth Street, NW
Washington, DC 20001