Toxicity-Pathway-Based Risk Assessment: Preparing for Paradigm Change: A Symposium Summary

DETAILS

134 pages | 8.5 x 11 | PAPERBACK
ISBN 978-0-309-15422-2 | DOI 10.17226/12913

AUTHORS

Ellen Mantus, Rapporteur; Standing Committee on Risk Analysis Issues and Reviews; Board on Environmental Studies and Toxicology; Division on Earth and Life Studies; National Research Council

TOXICITY-PATHWAY-BASED RISK ASSESSMENT

PREPARING FOR PARADIGM CHANGE

A Symposium Summary

Ellen Mantus, Rapporteur

Standing Committee on Risk Analysis Issues and Reviews

Board on Environmental Studies and Toxicology

Division on Earth and Life Studies

NATIONAL RESEARCH COUNCIL
OF THE NATIONAL ACADEMIES

THE NATIONAL ACADEMIES PRESS
Washington, D.C.
www.nap.edu

THE NATIONAL ACADEMIES PRESS 500 Fifth Street, NW Washington, DC 20001

NOTICE: The project that is the subject of this report was approved by the Governing Board of the National Research Council, whose members are drawn from the councils of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. The members of the committee responsible for the report were chosen for their special competences and with regard for appropriate balance.

This project was supported by Contract No. EP-C-06-057 between the National Academy of Sciences and the U.S. Environmental Protection Agency. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the organizations or agencies that provided support for this project.

International Standard Book Number-13: 978-0-309-15422-2
International Standard Book Number-10: 0-309-15422-7

Additional copies of this report are available from

The National Academies Press
500 Fifth Street, NW
Box 285
Washington, DC 20055

800-624-6242
202-334-3313 (in the Washington metropolitan area)
http://www.nap.edu

Printed in the United States of America

THE NATIONAL ACADEMIES

Advisers to the Nation on Science, Engineering, and Medicine

The **National Academy of Sciences** is a private, nonprofit, self-perpetuating society of distinguished scholars engaged in scientific and engineering research, dedicated to the furtherance of science and technology and to their use for the general welfare. Upon the authority of the charter granted to it by the Congress in 1863, the Academy has a mandate that requires it to advise the federal government on scientific and technical matters. Dr. Ralph J. Cicerone is president of the National Academy of Sciences.

The **National Academy of Engineering** was established in 1964, under the charter of the National Academy of Sciences, as a parallel organization of outstanding engineers. It is autonomous in its administration and in the selection of its members, sharing with the National Academy of Sciences the responsibility for advising the federal government. The National Academy of Engineering also sponsors engineering programs aimed at meeting national needs, encourages education and research, and recognizes the superior achievements of engineers. Dr. Charles M. Vest is president of the National Academy of Engineering.

The **Institute of Medicine** was established in 1970 by the National Academy of Sciences to secure the services of eminent members of appropriate professions in the examination of policy matters pertaining to the health of the public. The Institute acts under the responsibility given to the National Academy of Sciences by its congressional charter to be an adviser to the federal government and, upon its own initiative, to identify issues of medical care, research, and education. Dr. Harvey V. Fineberg is president of the Institute of Medicine.

The **National Research Council** was organized by the National Academy of Sciences in 1916 to associate the broad community of science and technology with the Academy's purposes of furthering knowledge and advising the federal government. Functioning in accordance with general policies determined by the Academy, the Council has become the principal operating agency of both the National Academy of Sciences and the National Academy of Engineering in providing services to the government, the public, and the scientific and engineering communities. The Council is administered jointly by both Academies and the Institute of Medicine. Dr. Ralph J. Cicerone and Dr. Charles M. Vest are chair and vice chair, respectively, of the National Research Council.

www.national-academies.org

PLANNING COMMITTEE FOR A SYMPOSIUM ON TOXICITY-PATHWAY-BASED RISK ASSESSMENT

Members

LORENZ RHOMBERG (*Chair*), Gradient Corporation, Cambridge, MA
ELAINE FAUSTMAN, University of Washington, Seattle
LYNN GOLDMAN, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
MICHAEL LAWTON, Pfizer Global Research and Development, Groton, CT
GEORGE LEIKAUF, University of Pittsburgh, Pittsburgh, PA
JOEL POUNDS, Pacific Northwest National Laboratory, Richland, WA
JOYCE TSUJI, Exponent, Inc., Bellevue, WA
LAUREN ZEISE, California Environmental Protection Agency, Oakland

Staff

ELLEN MANTUS, Project Director
NORMAN GROSSBLATT, Senior Editor
HEIDI MURRAY-SMITH, Associate Program Officer
KEEGAN SAWYER, Associate Program Officer
JOHN BROWN, Program Associate

Sponsor

U.S. ENVIRONMENTAL PROTECTION AGENCY

STANDING COMMITTEE ON RISK ANALYSIS ISSUES AND REVIEWS

Members

BERNARD GOLDSTEIN *(Chair),* University of Pittsburgh, Pittsburgh, PA
FREDERÍC BOIS, Institut National de l'Environnement Industriel et des Risques, France
MICHAEL BRAUER, University of British Columbia, Vancouver, Canada
RICHARD CORLEY, Pacific Northwest National Laboratory, Richland, WA
LINDA COWAN, University of Oklahoma, Oklahoma City
KENNETH CRUMP, Environ, Ruston, LA
LYNN GOLDMAN, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
PHILIP LANDRIGAN, Mount Sinai School of Medicine, New York, NY
THOMAS LOUIS, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
NU-MAY RUBY REED, California Environmental Protection Agency, Sacramento
LORENZ RHOMBERG, Gradient Corporation, Cambridge, MA
JOYCE TSUJI, Exponent, Inc., Bellevue, WA

Staff

ELLEN MANTUS, Project Director
HEIDI MURRAY-SMITH, Associate Program Officer
KEEGAN SAWYER, Associate Program Officer
JOHN BROWN, Program Associate

Sponsor

U.S. ENVIRONMENTAL PROTECTION AGENCY

[1]This study was planned, overseen, and supported by the Board on Environmental Studies and Toxicology.

OTHER REPORTS OF THE
BOARD ON ENVIRONMENTAL STUDIES AND TOXICOLOGY

The Use of Title 42 Authority at the U.S. Environmental Protection Agency (2010)
Review of the Environmental Protection Agency's Draft IRIS Assessment of Tetrachloroethylene (2010)
Hidden Costs of Energy: Unpriced Consequences of Energy Production and Use (2009)
Contaminated Water Supplies at Camp Lejeune—Assessing Potential Health Effects (2009)
Review of the Federal Strategy for Nanotechnology-Related Environmental, Health, and Safety Research (2009)
Science and Decisions: Advancing Risk Assessment (2009)
Phthalates and Cumulative Risk Assessment: The Tasks Ahead (2008)
Estimating Mortality Risk Reduction and Economic Benefits from Controlling Ozone Air Pollution (2008)
Respiratory Diseases Research at NIOSH (2008)
Evaluating Research Efficiency in the U.S. Environmental Protection Agency (2008)
Hydrology, Ecology, and Fishes of the Klamath River Basin (2008)
Applications of Toxicogenomic Technologies to Predictive Toxicology and Risk Assessment (2007)
Models in Environmental Regulatory Decision Making (2007)
Toxicity Testing in the Twenty-first Century: A Vision and a Strategy (2007)
Sediment Dredging at Superfund Megasites: Assessing the Effectiveness (2007)
Environmental Impacts of Wind-Energy Projects (2007)
Scientific Review of the Proposed Risk Assessment Bulletin from the Office of Management and Budget (2007)
Assessing the Human Health Risks of Trichloroethylene: Key Scientific Issues (2006)
New Source Review for Stationary Sources of Air Pollution (2006)
Human Biomonitoring for Environmental Chemicals (2006)
Health Risks from Dioxin and Related Compounds: Evaluation of the EPA Reassessment (2006)
Fluoride in Drinking Water: A Scientific Review of EPA's Standards (2006)
State and Federal Standards for Mobile-Source Emissions (2006)
Superfund and Mining Megasites—Lessons from the Coeur d'Alene River Basin (2005)
Health Implications of Perchlorate Ingestion (2005)
Air Quality Management in the United States (2004)
Endangered and Threatened Species of the Platte River (2004)
Atlantic Salmon in Maine (2004)
Endangered and Threatened Fishes in the Klamath River Basin (2004)

Cumulative Environmental Effects of Alaska North Slope Oil and Gas Development (2003)
Estimating the Public Health Benefits of Proposed Air Pollution Regulations (2002)
Biosolids Applied to Land: Advancing Standards and Practices (2002)
The Airliner Cabin Environment and Health of Passengers and Crew (2002)
Arsenic in Drinking Water: 2001 Update (2001)
Evaluating Vehicle Emissions Inspection and Maintenance Programs (2001)
Compensating for Wetland Losses Under the Clean Water Act (2001)
A Risk-Management Strategy for PCB-Contaminated Sediments (2001)
Acute Exposure Guideline Levels for Selected Airborne Chemicals (seven volumes, 2000-2009)
Toxicological Effects of Methylmercury (2000)
Strengthening Science at the U.S. Environmental Protection Agency (2000)
Scientific Frontiers in Developmental Toxicology and Risk Assessment (2000)
Ecological Indicators for the Nation (2000)
Waste Incineration and Public Health (2000)
Hormonally Active Agents in the Environment (1999)
Research Priorities for Airborne Particulate Matter (four volumes, 1998-2004)
The National Research Council's Committee on Toxicology: The First 50 Years (1997)
Carcinogens and Anticarcinogens in the Human Diet (1996)
Upstream: Salmon and Society in the Pacific Northwest (1996)
Science and the Endangered Species Act (1995)
Wetlands: Characteristics and Boundaries (1995)
Biologic Markers (five volumes, 1989-1995)
Science and Judgment in Risk Assessment (1994)
Pesticides in the Diets of Infants and Children (1993)
Dolphins and the Tuna Industry (1992)
Science and the National Parks (1992)
Human Exposure Assessment for Airborne Pollutants (1991)
Rethinking the Ozone Problem in Urban and Regional Air Pollution (1991)
Decline of the Sea Turtles (1990)

Copies of these reports may be ordered from the National Academies Press
(800) 624-6242 or (202) 334-3313
www.nap.edu

Preface

In 2007, the National Research Council (NRC) released a report titled *Toxicity Testing in the 21st Century: A Vision and a Strategy*. That report proposed a new paradigm for toxicity testing that envisioned evaluation of biologically significant perturbations in key toxicity pathways by using new methods in molecular biology, bioinformatics, and computational toxicology and a comprehensive array of in vitro tests based primarily on human biology. The revolution in toxicity testing is under way, and a large influx of new data is anticipated. The U.S. Environmental Protection Agency will need to be able to interpret the new data and therefore asked the Standing Committee on Risk Analysis Issues and Reviews to convene a symposium to stimulate discussion on the application of the new approaches and data in risk assessment. This summary provides an overview of the presentations and discussions that took place at that symposium.

This summary has been reviewed in draft form by persons chosen for their diverse perspectives and technical expertise in accordance with procedures approved by the NRC's Report Review Committee. The purpose of the independent review is to provide candid and critical comments that will assist the institution in making its published summary as sound as possible and to ensure that the summary meets institutional standards of objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We thank the following for their review of this summary: Cynthia A. Afshari, Amgen, Inc.; Jonathan H. Freedman, Duke University; William B. Mattes, PharmPoint Consulting; and Joyce S. Tsuji, Exponent, Inc.

Although the reviewers listed above have provided many constructive comments and suggestions, they did not see the final draft of the summary before its release. The review of the summary was overseen by David L. Eaton, University of Washington. Appointed by the NRC, he was responsible for making certain that an independent examination of the summary was carried out in accordance with institutional procedures and that all review comments were

carefully considered. Responsibility for the final content of the summary rests entirely with the author and the institution.

The committee gratefully acknowledges those who made presentations or served on discussion panels at the symposium (see Appendix C for a list of speakers and affiliations). The committee is also grateful for the assistance of the NRC staff in preparing this summary. Staff members who contributed to the effort are Ellen Mantus, project director; Norman Grossblatt, senior editor; Heidi Murray-Smith, associate program officer; Keegan Sawyer, associate program officer; and Radiah Rose, editorial projects manager. I thank especially all the members of the planning committee for their efforts in the development of the program and the conduct of the symposium.

Lorenz Rhomberg, *Chair*
Planning Committee for a Symposium on
Toxicity-Pathway-Based Risk Assessment

Contents

SUMMARY OF THE SYMPOSIUM **3**

APPENDIXES

A Biographic Information on the Standing Committee on Risk Analysis Issues and Reviews **53**

B Biographic Information on the Planning Committee for a Symposium on Toxicity-Pathway-Based Risk Assessment **59**

C Symposium Agenda **63**

D Biographic Information on the Speakers and Panelists for a Symposium on Toxicity-Pathway-Based Risk Assessment **70**

E Symposium Presentations **83**

F Poster Abstracts. **84**

TABLES AND FIGURES

TABLES

1 Options for Future Toxicity-Testing Strategies Considered by the NRC Committee on Toxicity Testing and Assessment of Environmental Agents, 7
2 Phased Development of ToxCast Program, 16
3 Types of ToxCast Assays, 17

FIGURES

1 Components of the vision described in the report, *Toxicity Testing in the 21st Century: A Vision and a Strategy*, 8

2 Perturbation of cellular response pathway, leading to adverse effects, 8
3 DNA sequencing output, 11
4 Throughput potential for data acquisition as related to levels of biologic organization, 13
5 Illustration of bioactivity profiling using high-throughput technologies to screen chemicals, 14
6 Overview of chemical registration for REACH, 21
7 Integration of new approaches for toxicology, 22
8 Dosimetry considerations in cell systems, 28
9 What do cells see? Protein adsorption by nanomaterials is a universal phenomenon in biologic systems, 28
10 Example of gene ontology for DNA metabolism, a biologic process, 31
11 Framework for interpretation of dose- and time-dependent genomic data, 32
12 Integrated data provide more comprehensive and accurate network reconstruction, 36
13 Illustration of the development of modular network models, 37
14 Interaction network that can be used to associate environmental factors with toxicity pathways and associated human diseases, 43

TOXICITY-PATHWAY-BASED RISK ASSESSMENT

PREPARING FOR PARADIGM CHANGE

A Symposium Summary

Summary of the Symposium

In 2007, a committee of the National Research Council (NRC) proposed a vision that embraced recent scientific advances and set a new course for toxicity testing (NRC 2007a). The committee envisioned a new paradigm in which biologically important perturbations in key toxicity pathways would be evaluated with new methods in molecular biology, bioinformatics, computational toxicology, and a comprehensive array of in vitro tests based primarily on human biology. Although some view the vision as too optimistic with respect to the promise of the new science and debate the time required to implement the vision, no one can deny that a revolution in toxicity testing is under way. New approaches are being developed, and data are being generated. As a result, the U.S. Environmental Protection Agency (EPA) expects a large influx of data that will need to be evaluated. EPA also is faced with tens of thousands of chemicals on which toxicity information is incomplete and emerging chemicals and substances that will need risk assessment and possible regulation. Therefore, the agency asked the NRC Standing Committee on Risk Analysis Issues and Reviews to convene a symposium to stimulate discussion on the application of the new approaches and data in risk assessment.

The standing committee was established in 2006 at the request of EPA to plan and conduct a series of public workshops that could serve as a venue for discussion of issues critical for the development and review of objective, realistic, and scientifically based human health risk assessment. An ad hoc planning committee was formally appointed under the oversight of the standing committee to organize and conduct the symposium. The biographies of the standing committee and planning committee members are provided in Appendixes A and B, respectively.

The symposium was held on May 11-13, 2009, in Washington, DC, and included presentations and discussion sessions on pathway-based approaches for hazard identification, applications of new approaches to mode-of-action analyses, the challenges to and opportunities for risk assessment in the changing paradigm, and future directions. The symposium agenda, speaker and panelist biographies, and presentations are provided in Appendixes C, D, and E, respectively. The symposium also included a poster session to showcase examples of

how new technologies might be applied to quantitative and qualitative aspects of risk assessment. The poster abstracts are provided in Appendix F. This summary provides the highlights of the presentations and discussions at the symposium. Any views expressed here are those of the individual committee members, presenters, or other symposium participants and do not reflect any findings or conclusions of the National Academies.

A PARADIGM CHANGE ON THE HORIZON

Warren Muir, of the National Academies, welcomed the audience to the symposium and stated that the environmental-management paradigm of the 1970s is starting to break down with recent scientific advances and the exponential growth of information and that the symposium should be seen as the first of many discussions on the impact of advances in toxicology on risk assessment. He introduced Bernard Goldstein, of the University of Pittsburgh, chair of the Standing Committee on Risk Analysis Issues and Reviews, who stated that although the standing committee does not make recommendations, symposium participants should feel free to suggest how to move the field forward and to make research recommendations. Peter Preuss, of EPA, concluded the opening remarks and emphasized that substantial changes are on the horizon for risk assessment. The agency will soon be confronted with enormous quantities of data from high-throughput testing and as a result of the regulatory requirements of the REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) program in Europe that requires testing of thousands of chemicals. He urged the audience to consider the question, What is the future of risk assessment?

Making Risk Assessment More Useful in an Era of Paradigm Change

E. Donald Elliott, of Yale Law School and Willkie Farr & Gallagher LLP, addressed issues associated with acceptance and implementation of the new pathway approaches that will usher in the paradigm change. He emphasized that simply building a better mousetrap does not ensure its use, and he provided several examples in which innovations, such as movable type and the wheel, were not adopted until centuries later. He felt that ultimately innovations must win the support of a user community to be successful, so the new tools and approaches should be applied to problems that the current paradigm has difficulty in addressing. Elliott stated that the advocates of pathway-based toxicity testing should illustrate how it can address the needs of a user community, such as satisfying data requirements for REACH; providing valuable information on sensitive populations; evaluating materials, such as nanomaterials, that are not easily evaluated in typical animal models; and demonstrating that fewer animal tests are needed if the approaches are applied. He warned, however, that the new ap-

proaches will not be as influential if they are defined as merely less expensive screening techniques.

Elliott continued by saying that the next steps needed to effect the paradigm change will be model evaluation and judicial acceptance. NRC (2007b) and Beck (2002) set forth a number of questions to consider in evaluating a model, such as whether the results are accurate and represent the system being modeled? The standards for judicial acceptance in agency reviews and private damage cases are different. The standards for agency reviews are much more lenient than those in private cases in which a judge must determine whether an expert's testimony is scientifically valid and applicable. Accordingly, the best approach for judicial acceptance would be to have a record established on the basis of judicial review of agency decisions, in which a court generally defers to the agency when decisions involve determinations at the frontiers of science. Elliott stated that the key issue is to create a record showing that the new approach works as well as or better than existing methods in a particular regulatory application. He concluded, however, that the best way to establish acceptance might be for EPA to use its broad rule-making authority under Section 4 of the Toxic Substances Control Act to establish what constitutes a valid testing method in particular applications.

Emerging Science and Public Health

Lynn Goldman, of Johns Hopkins Bloomberg School of Public Health, a member of the standing committee and the planning committee, discussed the public-health aspects of the emerging science and potential challenges. She agreed with Elliott that a crisis is looming, given the number of chemicals that need to be evaluated and the perception that the process for ensuring that commercial chemicals are safe is broken and needs to be re-evaluated. The emerging public-health issues are compounding the sense of urgency in that society will not be able to take 20 years to make decisions. Given the uncertainties surrounding species extrapolation, dose extrapolation, and evaluation of sensitive populations today, the vision provided in the NRC report *Toxicity Testing in the 21st Century: A Vision and a Strategy* offers tremendous promise. However, Goldman used the example of EPA's Endocrine Disruptor Screening Program as a cautionary tale. In 1996, Congress passed two laws, the Food Quality Protection Act and the Safe Drinking Water Act, that directed EPA to develop a process for screening and testing chemicals for endocrine-disruptor potential. Over 13 years, while three advisory committees have been formed, six policy statements have been issued, and screening tests have been modified four times, no tier 2 protocols have been approved, and only one list of 67 pesticides to be screened has been generated. One of the most troubling aspects is that most of the science is now more than 15 years old. EPA lacked adequate funding, appropriate expertise, enforceable expectations by Congress, and the political will to push the

program forward. The fear that a chemical would be blacklisted on the basis of a screening test and the "fatigue factor," in which supporters eventually tire and move on to other issues, compounded the problems. Goldman suggested that the following lessons should be learned from the foregoing example: support is needed from stakeholders, administration, and Congress for long-term investments in people, time, and resources to develop and implement new toxicity-testing approaches and technologies; strong partnerships within the agency and with other agencies, such as the National Institutes of Health (NIH), are valuable; new paradigms will not be supported unless there are convincing proof-of-concept and verification studies; and new processes are needed to move science into regulatory science more rapidly. Goldman concluded that the new approaches and technologies have many potential benefits, including improvement in the ability to identify chemicals that have the greatest potential for risk, the generation of more scientifically relevant data on which to base decisions, and improved strategies of hazard and risk management. However, she warned that resources are required to implement the changes: not only funding but highly trained scientists will be needed, and the pipeline of scientists who will be qualified and capable of doing the work needs to be addressed.

Toxicity Testing in the 21st Century

Kim Boekelheide, of Brown University, who was a member of the committee responsible for the report *Toxicity Testing in the 21st Century: a Vision and a Strategy* reviewed the report and posed several questions to consider throughout the discussion in the present symposium. The committee was formed when frustration with toxicity-testing approaches was increasing. Boekelheide cited various problems with toxicity-testing approaches, including low throughput, high cost, questionable relevance to actual human risks, use of conservative defaults, and reliance on animals. Thus, the committee was motivated by the following design criteria for its vision: to provide the broadest possible coverage of chemicals, end points, and life stages; to reduce the cost and time of testing; to minimize animal use and suffering; and to develop detailed mechanistic and dose-response information for human health risk assessment. The committee considered several options, which are summarized in Table 1. Option I was essentially the status quo, option II was a tiered approach, and options III and IV were fundamental shifts in the current approaches. Although the committee acknowledged option IV as the ultimate goal for toxicity testing, it chose option III to represent the vision for the next 10-20 years. That approach is a fundamental shift—one that is based primarily on human biology, covers a broad range of doses, is mostly high-throughput, is less expensive and time-consuming, uses substantially fewer animals, and focuses on perturbations of critical cellular responses.

TABLE 1 Options for Future Toxicity-Testing Strategies Considered by the NRC Committee on Toxicity Testing and Assessment of Environmental Agents

Option I In Vivo	Option II Tiered In Vivo	Option III In Vitro and In Vivo	Option IV In Vitro
Animal biology	Animal biology	Primarily human biology	Primarily human biology
High doses	High doses	Broad range of doses	Broad range of doses
Low throughput	Improved throughput	High and medium throughput	High throughput
Expensive	Les expensive	Less expensive	Less expensive
Time-consuming	Less time-consuming	Less time-consuming	Less time-consuming
Relatively large number of animals	Fewer animals	Substantially fewer animals	Virtually no animals
Apical end points	Apical end points	Perturbations of toxicity pathways	Perturbations of toxicity pathways
	Some in silico and in vitro screens	In silico screens possible	In silico screens

Source: Modified from NRC 2007a. K. Boekelheide, Brown University, presented at the symposium.

Boekelheide described the components of the vision, which are illustrated in Figure 1. The core component is toxicity-testing, in which toxicity-pathway assays play a dominant role. The committee defined a toxicity pathway as a cellular-response pathway that, when sufficiently perturbed, is expected to result in an adverse health effect (see Figure 2), and it envisioned a toxicity-testing system that evaluates biologically important perturbations in key toxicity pathways by using new methods in computational biology and a comprehensive array of in vitro tests based on human biology. Boekelheide noted that since release of the report, rapid progress in human stem-cell biology, better accessibility to human cells, and development of bioengineered tissues have made the committee's vision more attainable. He also noted that the toxicity-pathway approach moves away from extrapolation from high dose to low dose and from animals to humans but involves extrapolation from in vitro to in vivo and between levels of biologic organization. Thus, there will be a need to build computational systems-biology models of toxicity-pathway circuitry and pharmacokinetic models that can predict human blood and tissue concentrations under specific exposure conditions.

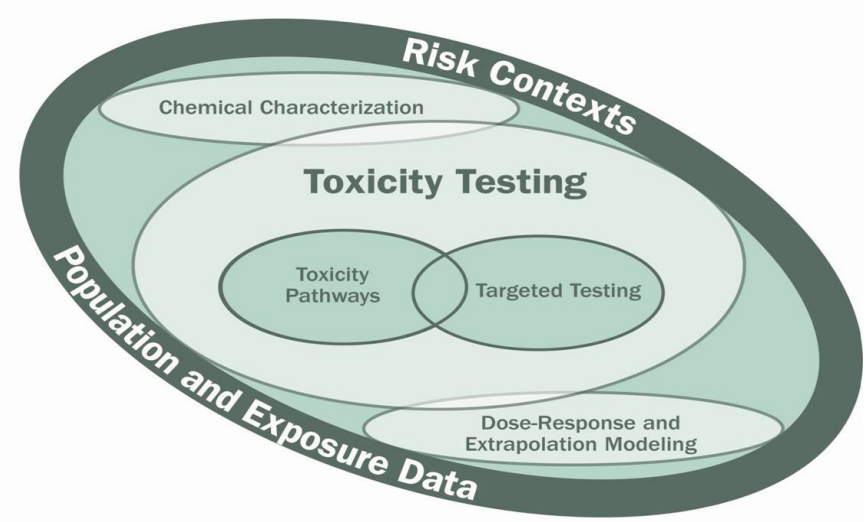

FIGURE 1 Components of the vision described in the report, *Toxicity Testing in the 21st Century: A Vision and a Strategy*. Source: NRC 2007a. K. Boekelheide, Brown University, presented at the symposium.

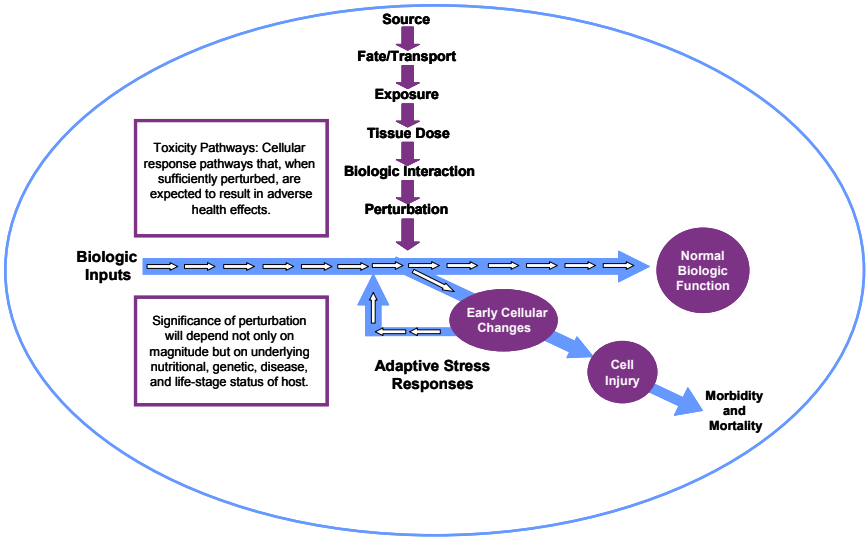

FIGURE 2 Perturbation of cellular response pathway, leading to adverse effects. Source: Modified from NRC 2007a. K. Boekelheide, Brown University, modified from symposium presentation.

Boekelheide stated that the vision offers a toxicity-testing system more focused on human biology with more dose-relevant testing and the possibility of addressing many of the frustrating problems in the current system. He listed some challenges with the proposed vision, including development of assays for the toxicity pathways, identification and testing of metabolites, use of the results to establish safe levels of exposure, and training of scientists and regulators to use the new science. Boekelheide concluded by asking several questions for consideration throughout the symposium program: How long will it take to implement the new toxicity-testing paradigm? How will adaptive responses be distinguished from adverse responses? Is the proposed approach a screening tool or a stand-alone system? How will the new paradigm be validated? How will new science be incorporated? How will regulators handle the transition in testing?

Symposium Issues and Questions

Lorenz Rhomberg, of Gradient Corporation, a member of the standing committee and chair of the planning committee, closed the first session by providing an overview of issues and questions to consider throughout the symposium. Rhomberg stated that the new tools will enable and require new approaches. Massive quantities of multivariate data are being generated, and this poses challenges for data handling and interpretation. The focus is on "normal" biologic control and processes and the effects of perturbations on those processes, and a substantial investment will be required to improve understanding in fundamental biology. More important, our frame of reference has shifted dramatically: traditional toxicology starts with the whole organism, observes apical effects, and then tries to explain the effects by looking at changes at lower levels of biologic organization, whereas the new paradigm looks at molecular and cellular processes and tries to explain what the effects on the whole organism will be if the processes are perturbed.

People have different views on the purposes and applications of the new tools. For example, some want to use them to screen out problematic chemicals in drug, pesticide, or product development; to identify chemicals for testing and the in vivo testing that needs to be conducted; to establish testing priorities for data-poor chemicals; to identify biomarkers or early indicators of exposure or toxicity in the traditional paradigm; or to conduct pathway-based evaluations of causal processes of toxicity. Using the new tools will pose challenges, such as distinguishing between causes and effects, dissecting complicated networks of pathways to determine how they interact, and determining which changes are adverse effects rather than adaptive responses. However, the new tools hold great promise, particularly for examining how variations in the population affect how people react to various exposures.

Rhomberg concluded with some overarching questions to be considered throughout the symposium: What are the possibilities of the new tools, and how do we realize them? What are the pitfalls, and how can we avoid them? How is the short-term use of the new tools different from the ultimate vision? When should the focus be on particular pathways rather than on interactions, variability, and complexity? How is regulatory and public acceptance of the new paradigm to be accomplished?

THE NEW SCIENCE

An Overview

John Groopman, of Johns Hopkins Bloomberg School of Public Health, began the discussion of the new science by providing several examples of how it has been used. He first discussed the Keap1-Nrf2 signaling pathway, which is sensitive to a variety of environmental stressors. Keap1-Nrf2 signaling pathways have been investigated by using knockout animal models, and the investigations have provided insight into how the pathways modulate disease outcomes. Research has shown that different stressors in Nrf2 knockout mice affect different organs; that is, one stressor might lead to a liver effect, and another to a lung effect. Use of knockout animals has allowed scientists to tease apart some of the pathway integration and has shown that the signaling pathways can have large dose-response curves—in the 20,000-fold range—in response to activation.

Groopman stated, however, that some of the research has provided cautionary tales. For example, when scientists evaluated the value of an aflatoxin-albumin biomarker to predict which rats were at risk for hepatocellular carcinoma, they found that the biomarker concentration tracked with the disease at the population level but not in the individual animals. Thus, one may need to be wary of the predictive value of a single biomarker for a complex disease. In another case, scientists thought that overexpression of a particular enzyme in a signaling pathway would lead to risk reduction, but they found that transgene overexpression had no effect on tumor burden. Overall, the research suggests that a reductionist approach might not work for complex diseases. Groopman acknowledged substantial increases in the sensitivity of mass spectrometry over the last 10 years but noted that the throughput in many cases has not increased, and this is often an underappreciated and underdiscussed aspect of the new paradigm.

Groopman concluded by discussing the recent data on cancer genomes. Sequence analysis of cancer genomes has shown that different types of cancer, such as breast cancer and colon cancer, are not the same disease, and although there are common mutations within the same cancer type, the disease differs among individuals. Through sequence analysis, the number of confirmed genetic contributors to common human diseases has increased dramatically since 2000. Genome-wide association studies have shown that many alleles have modest

effects in disease outcomes, that many genes are involved in each disease, that most genes that have been shown to be involved in human disease were not predicted on the basis of current biologic understanding, and that many risk factors are in noncoding regions of the genome. Sequencing methods and technology have improved dramatically, and researchers who once dreamed of sequencing the human genome in a matter of years can now complete the task in a matter of days (see Figure 3). Groopman concluded by stating that the sequencing technology needs to be extended to experimental models so that questions about the concordance between effects observed in people and those observed in experimental models can be answered.

Gene-Environment Interactions

George Leikauf, of the University of Pittsburgh, discussed the current understanding of gene-environment interactions. In risk assessment, human variability and susceptibility are considered, and an uncertainty factor of 10 has traditionally been used to account for these factors. However, new tools available today are helping scientists to elucidate gene-environment interactions, and this research may provide a more scientific basis for evaluating human variability and susceptibility in the context of risk assessment. Leikauf noted that genetic disorders, such as sickle-cell anemia and cystic fibrosis, and environmental disorders, such as asbestosis and pneumoconiosis, cause relatively few deaths compared with complex diseases that are influenced by many genetic and environmental factors. Accordingly, it is the interaction between genome and environment that needs to be elucidated in the case of complex diseases.

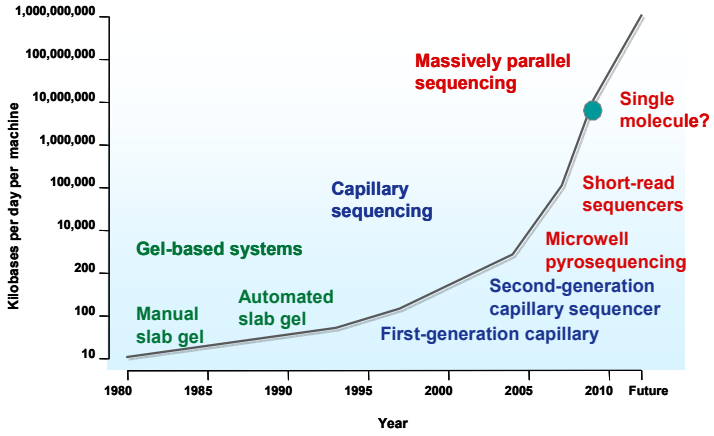

FIGURE 3 DNA sequencing output. Current output is 1-2 billion bases per machine per day. The human genome contains 3 billion bases. Source: Stratton et al. 2009. Reprinted with permission; copyright 2009, *Nature*. J. Groopman, Johns Hopkins Bloomberg School of Public Health, presented at the symposium.

Leikauf continued, saying that evaluating genetic factors that affect pharmacokinetics or pharmacodynamics can provide valuable information for risk assessment. For example, genetic variations that lead to differences in carrier or transporter proteins can affect chemical absorption rates and determine the magnitude of a chemical's effect on the body. Genetic variations that lead to differences in metabolism may also alter a chemical's effect on the body. For example, if someone's metabolism is such that a chemical is quickly converted to a reactive intermediate and then slowly eliminated, the person may be at greater risk because of the longer residence time of the reactive intermediate in the body. Thus, the relative rates of absorption and metabolism can be used to evaluate variability and susceptibility and can provide some scientific basis for selection of uncertainty factors. Leikauf noted, however, that determining the physiologic and pharmacologic consequences of the many genetic polymorphisms is difficult. He discussed several challenges to using genetic information to predict outcome. For example, not all genes are expressed or cause a given phenotype even if they are expressed. Thus, knowing one particular polymorphism does not mean knowing the likelihood of an outcome. Leikauf concluded, however, that the next step in genetics is to use the powerful new tools to understand the complexity and how it leads to diversity.

Tools and Technologies for Pathway-Based Research

Ivan Rusyn, of the University of North Carolina at Chapel Hill, discussed various tools and technologies that are now available for pathway-based research. He noted that the genomes of more than 180 organisms have been sequenced since 1995 and that although determining genetic sequence is important, understanding how we are different from one another may be more important. High-throughput sequencing—some of which can provide information on gene regulation and control by incorporating transcriptome analysis—has enabled the genome-wide association studies already discussed at the symposium and has provided valuable information on experimental models, both whole-animal and in vitro systems.

Rusyn described various tools and technologies available at the different levels of biologic organization and noted that the throughput potential for data acquisition diminishes as data relevance increases (see Figure 4). Single-molecule-based screening can involve cell-free systems or cell-based systems. In the case of cell-free systems, many of the concepts have been known for decades, but technologic advances have enabled researchers to evaluate classes of proteins, transporters, nuclear receptors, and other molecules and to screen hundreds of chemicals in a relatively short time. Miniaturization of cell-based systems has allowed researchers to create high-throughput formats that allow evaluation of P450 inhibition, metabolic stability, cellular toxicity, and enzyme induction. Screening with cell cultures has advanced rapidly as a result of robotic technologies and high-content plate design, and concentration-response

profiles on multiple phenotypes can now be generated quickly. Much effort is being invested in developing engineered tissue assays, some of which are being used by the pharmaceutical industry as screening tools. Finally, screening that uses invertebrates, such as *Caenorhabditis elegans,* and lower vertebrates, such as zebrafish, has been used for years, but scientists now have the ability to generate transgenic animals and to screen environmental chemicals in high-throughput or medium-throughput formats to evaluate phenotypes.

Rusyn described seminal work with knock-out strains of yeast that advanced pathway-based analysis (see, for example, Begley et al. 2002; Fry et al. 2005). High-throughput screens were used to identify pathways involved in response to chemicals that damaged DNA. Since then, multiple transcription-factor analyses have further advanced our knowledge of important pathways and have allowed scientists to rediscover "old" biology with new tools and technology. Rusyn noted, however, that it is difficult to go from gene expression to a whole pathway. A substantial volume of data is being generated, and the major challenge is to integrate all the data—chemical, traditional toxicologic, –omics, and high-throughput screening data—to advance our biologic understanding. Rusyn concluded that the complexity of science today creates an urgent need to train new scientists and develop new interdisciplinary graduate programs.

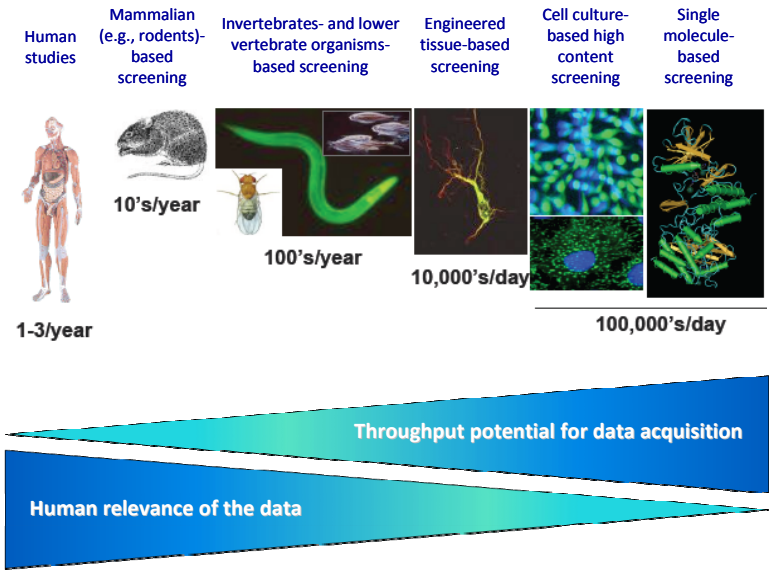

FIGURE 4 Throughput potential for data acquisition as related to levels of biologic organization. As the human relevance increases the throughput potential decreases. Source: NIEHS, unpublished data. I. Rusyn, University of North Carolina at Chapel Hill, presented at the symposium.

PATHWAY-BASED APPROACHES FOR HAZARD IDENTIFICATION

ToxCast: Redefining Hazard Identification

Robert Kavlock, of EPA, opened the afternoon session by discussing ToxCast, an EPA research program. He stated that a substantial problem is the lack of data on chemicals. In a recent survey (Judson et al. 2009), EPA identified about 10,000 high-priority chemicals in EPA's program offices; found huge gaps in data on cancer, reproductive toxicity, and developmental toxicity; and found no evidence in the public domain of safety or hazard data on more than 70% of the identified chemicals. Kavlock noted that this problem is not restricted to the United States; a better job must also be done internationally to eliminate the chemical information gap. He emphasized that at this stage, priorities must be set for testing of the chemicals. The options include conducting more animal studies, using exposure as a priority-setting metric, using structure-activity models, and using bioactivity profiling, which would screen chemicals by using high-throughput technologies (see Figure 5). The ToxCast program was designed to implement the fourth option, and the name attempts to capture the key goals of the program: to *cast* a broad net to capture the bioactivity of the chemicals and to try to forecast the *toxicity* of the chemicals. The ToxCast program is part of EPA's contribution to the Tox21 Consortium (Collins et al. 2008), a partnership of the NIH Chemical Genomics Center (NCGC), EPA's Office of Research and Development, and the National Toxicology Program (NTP) to advance the vision proposed in the NRC report (NRC 2007a). Kavlock also noted that EPA responded to that report by issuing a strategic plan for evaluating the toxicity of chemicals that included three goals: identifying toxicity pathways and using them in screening, using toxicity pathways in risk assessment, and making an institutional transition to incorporate the new science.

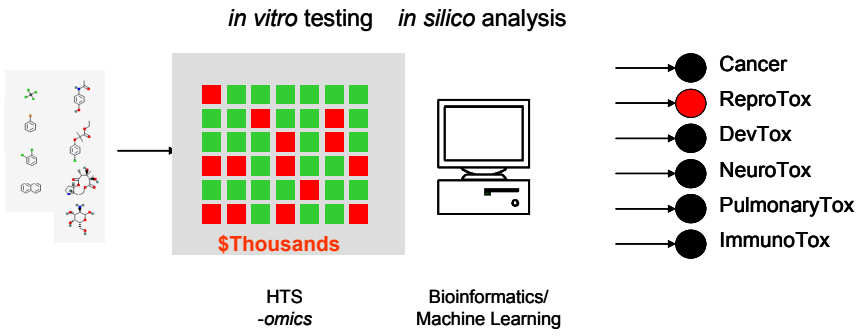

FIGURE 5 Illustration of bioactivity profiling using high-throughput technologies to screen chemicals. Source: EPA 2009. R. Kavlock, U.S. Environmental Protection Agency, presented at the symposium.

Kavlock provided further details on the ToxCast program. It is a research program that was started by the National Center for Computational Toxicology and was developed to address the chemical screening and priority-setting needs for inert pesticide components, antimicrobial agents, drinking-water contaminants, and high- and medium-production-volume chemicals. The ToxCast program is currently the most comprehensive use of high-throughput technologies, at least in the public domain, to elucidate predictive chemical signatures. It is committed to stakeholder involvement and public release of the data generated. The program components are identifying toxicity pathways, developing high-throughput assays for them, screening chemical libraries, and linking the results to in vivo effects. Each component involves challenges, such as incorporating metabolic capabilities into the assays, determining whether to link assay results to effects found in rodent toxicity studies or to human toxicity, and predicting effective in vivo concentrations from effective in vitro concentrations. Kavlock described the three phases of the program (see Table 2) and noted that it is completing the first phase, proof-of-concept, and preparing for the second phase, which involves validation. He mentioned that it has developed a relational database, ToxRefDB, that contains animal toxicology data that will serve as the in vivo "anchor" for the ToxCast predictions.

Kavlock stated that 467 biochemical and cellular assays (see Table 3) are being used to evaluate chemicals, but the expectation is that a larger number of assays will eventually be used. Multiple assays and technologies are used to evaluate each end point, and initial results have been positive in that the results agree with what is known about the chemicals being testing. Kavlock concluded that the future of screening is here, and the challenge is to interpret all the data being generated. He predicted that the first application will be use of the data to set priorities among chemicals for targeted testing and that application to risk assessment will follow as more knowledge is gained from its initial use.

Practical Applications: Pharmaceuticals

William Pennie, of Pfizer, discussed screening approaches in the pharmaceutical industry and provided several examples of their use. Pennie noted that implementing new screening paradigms and using in silico and in vitro approaches may be easier in the pharmaceutical industry because of the ultimate purpose—screening out unpromising drug candidates early in the research phase as opposed to screening in environmental chemicals whose toxicity needs to be evaluated. Huge challenges are still associated with using these approaches in the pharmaceutical industry, and Pennie emphasized that academe, regulatory agencies, and industries need to collaborate to build the infrastructure needed. Otherwise, only incremental change will be made in developing and implementing pathway-based approaches.

TABLE 2 Phased Development of ToxCast Program

Phase[a]	Number of Chemicals	Chemical Criteria	Purpose	Number of Assays	Cost per Chemical	Target Date
Ia	320	Data-rich (pesticides)	Signature development	>500	$20,000	FY 2007-2008
Ib	15	Nanomaterials	Pilot	166	$10,000	FY 2009
IIa	>300	Data-rich chemicals	Validation	>400	~$20,000-25,000	FY 2009
IIb	>100	Known human toxicants	Extrapolation	>400	~$20,000-25,000	FY 2009
IIc	>300	Expanded structure and use diversity	Extension	>400	~$20,000-25,000	FY 2010
IId	>12	Nanomaterials	PMN	>200	~$15,000-20,000	FY 2009-2010
III	Thousands	Data-poor	Prediction and priority-setting	>300	~$15,000-20,000	FY 2011-2012

[a]Since the symposium, phases IIa, IIb, and IIc have been merged into a single endeavor. Source: R. Kavlock, U.S. Environmental Protection Agency, presented at the symposium.

TABLE 3 Types of ToxCast Assays

Biochemical Assays	Cellular Assays
• Protein families GPCR NR Kinase Phosphatase Protease Other enzyme Ion channel Transporter • Assay formats Radioligand binding Enzyme activity Coactivator recruitment	• Cell lines HepG2 human hepatoblastoma A549 human lung carcinoma HEK 293 human embryonic kidney • Primary cells Human endothelial cells Human monocytes Human keratinocytes Human fibroblasts Human renal proximal tubule cells Human small-airway epithelial cells • Biotransformation-competent cells Primary rat hepatocytes Primary human hepatocytes • Assay formats Cytotoxicity Reporter gene Gene expression Biomarker production High-content imaging for cellular phenotype

Source: R. Kavlock, U.S. Environmental Protection Agency, presented at the symposium.

Pennie stated that some of the pathway-based approaches have been applied more successfully in the later stages of drug development than in the early, drug-discovery phase. One problem in developing the new approaches is that scientists often focus on activation of one pathway rather than considering the complexity of the system. Pennie stated that pathway knowledge should be added to a broader understanding of the biology; thus, the focus should be on a combination of properties rather than on one specific feature. Although the pharmaceutical industry is currently using in vitro assays that are typically functional end-point assays, Pennie noted that there is no reason why those assays could not be supplemented or replaced with pathway-based assays, given a substantial investment in validation. He said that the industry is focusing on using batteries of in vitro assays to predict in vivo outcomes, similar to the ToxCast program, and described an effort at Pfizer to develop a single-assay platform that would evaluate multiple end points simultaneously and provide a single predictive score for hepatic injury. Seven assays were evaluated by using 500

compounds that spanned the classes of hepatic toxicity. Researchers were able to develop a multiparameter optimization model that determined the combination of assays that would yield the most predictive power. On the basis of that analysis, they identified a combination of assays—a general cell-viability assay followed by an optimized imaging-based hepatic platform that measured several end points—that resulted in over 60% sensitivity and about 90% specificity. Pennie emphasized the value of integrating the pathway information into the testing cascade. If an issue is identified with a chemical, that knowledge can guide the in vivo testing and, instead of a fishing expedition, scientists can test a hypothesis. Pfizer has also developed a multiparameter optimization model that uses six physicochemical properties to characterize permeability, clearance, and safety and that helps to predict the success of a drug candidate. Pennie concluded by saying that the future challenge is to develop prediction models that combine data from multiple sources (that is, structural-alert data, physicochemical data, in vitro test data, and in vivo study data) to provide a holistic view of compound safety.

Practical Applications: Consumer Products

George Daston, of Procter and Gamble, discussed harnessing the available computational power to support new approaches to toxicology to solve some problems in the consumer-products industry. He noted that the new paradigm is a shift from outcome-driven toxicology, in which models are selected to evaluate a particular disease state without knowledge about the events from exposure to outcome, to mechanism-driven toxicology, in which scientists seek answers to several questions: How does the chemical interact with the system? What is the mechanism of action? How can we predict what the outcome would be on the basis of the mechanism? The transition to mechanism-driven toxicology will be enabled by the 50 years of data from traditional toxicology, the ability to do high-throughput and high-content biology, and the huge computational power currently available.

Daston provided two examples of taking advantage of today's computational power. First, his company needed a system to evaluate chemicals without testing every new chemical entity to make initial predictions about safety. A chemical database was developed to search chemical substructures to identify analogues that might help to predict the toxicity of untested chemicals. A process was then developed in which first a chemist reviews a new compound and designs a reasonable search strategy, then the computer is used to search enormous volumes of data for specific patterns, and finally the output is evaluated according to expert rules based on physical chemistry, metabolism, reactivity, and toxicity to support testing decisions. Daston mentioned several public databases (DSSTox, ACTOR, and ToxRefdB) that are available for conducting similar searches and emphasized the importance of public data-sharing.

His second example involved analysis of high-content datasets from microarrays in which all potential mechanisms of action of a particular chemical are evaluated as changes in gene expression. That approach complements the one discussed by Kavlock for the ToxCast program in that it is a detailed analysis of one assay rather than a scan of multiple types of assays. Daston and others focused on using steroid-hormone mechanisms to evaluate whether gene-expression analysis (that is, genomics) could predict those mechanisms. Steroid hormone mechanisms were chosen because research has shown that effects regulated by estrogen, androgen, and other steroid hormones depend on gene expression. That is, a chemical binds to a receptor; the receptor complex migrates to the nucleus, binds to specific sites on the DNA, and causes upregulation or downregulation of specific genes; and this change in gene expression causes the observed cellular and tissue response. They found not only that chemicals that act by the same mechanism of action affect the same genes in the same direction but that the magnitude of the changes is the same as long as the chemicals are matched for pharmacologic activity. Thus, they found that genomics could be used quantitatively to improve dose-response assessments. Daston stated that genomics can be used to accelerate the mechanistic understanding, and the information gained can be used to determine whether similar kinds of effects can be modeled in an in vitro system. One surprising discovery was how extrapolatable the results were, not only from in vivo to in vitro but from species to species. Daston concluded by saying that once the critical steps in a toxicologic process are known, quantitative models can be built to predict behavior at various levels of organization.

Practical Applications: Mixtures

John Groten, of Schering-Plough, discussed current approaches and possible applications of the new science to mixtures risk assessment. He noted that especially in toxicology research in the pharmaceutical industry (but also in the food and chemical industry) there is an increasing need for parallel and efficient processes to assess compound classes, more alternatives to animal testing, tiered approaches that link toxicokinetics and toxicodynamics, enhanced use of systems biology in toxicology, and an emphasis on understanding interactions, combined action, and mixtures risk assessment. Today, risk assessments in the food, drug, and chemical industries attempt to evaluate and incorporate mixture effects, but the processes for doing so are case-driven and relatively simplistic. For example, adding hazard quotients to ensure that a sum does not exceed a threshold might be a beginning, but the likelihood of joint exposure and the possibility that compounds affect the same target system need to be assessed qualitatively and, preferably, quantitatively. Although research has been conducted on toxicokinetics and toxicodynamics of mixtures, most publications have dealt with toxicokinetic interactions. Groten stated that toxicokinetics should be used to correct for differences in exposure to mixture components but, because of a

lack of mechanistic understanding in the toxicodynamic phase, not to predict toxic interactions. He noted that empirical approaches are adequate as a starting point but that in many cases these models depend on mathematical laws rather than biologic laws, and he recommended that mechanistic understanding be used to fine tune experiments and to test or support empirical findings.

Groten listed several challenges for mixtures research, including the difficulty of using empirical models and conventional toxicology to show the underlying sequence of events in joint action, the adequacy (or inadequacy) of conventional toxicity end points to provide a wide array of testable responses at the no-observed-adverse-effect level, and the inability of current models to distinguish kinetic and dynamic interactions. He concluded by noting that the health effects of chemical mixtures are mostly related to specific interactions at the molecular level and that the application of functional genomics (sequencing, genotyping, transcriptomics, proteomics, and metabolomics) will provide new insights and advance the risk assessment of mixtures. He echoed the need that previous speakers raised for the use of multidisciplinary teams with statisticians, bioinformaticians, molecular biologists, and others to conduct future research in this field.

Pathway-Based Approaches: A European Perspective

Thomas Hartung, of the Center for Alternatives to Animal Testing, provided a European perspective on pathway-based approaches and reviewed the status of the European REACH program. He noted that regulatory toxicology is a business; toxicity testing with animals in the European Union is an $800 million/year business that employs about 15,000 people. The data generated, however, are not always helpful for reaching conclusions about toxicity. For example, one study examined 29 risk assessments of trichloroethylene and found that four concluded that it was a carcinogen, 19 were equivocal, and six concluded that it was not a carcinogen (Rudén 2001). Hartung stated that one problem is that the system today is a patchwork to which every health scare over decades has added a patch. For example, the thalidomide disaster resulted in a requirement for reproductive-toxicity testing. Many patches are 50-80 years old, and there is no way to remove a patch because international guidelines have been created and are extremely difficult to change once they have been agreed on. Another problem is that animal models are limited—humans are not 70-kg rats. However, cell cultures are also limited; metabolism and defense mechanisms are lacking, the fate of test compounds is unknown, and dedifferentiation is favored by the growth conditions. Thus, the current system is far from perfect.

Hartung discussed the REACH initiative and noted that it constitutes the biggest investment in consumer safety ever undertaken. The original projection was that REACH would involve 27,000 companies in Europe (one-third of the world market) but affect the entire global market in that it also affects imported chemicals and that it would result in the assessment of at least 30,000 chemicals

(see Figure 6 for an overview of the chemical registration process). Given that about 5,000 chemicals have been assessed in 25 years, the program goal is quite ambitious. REACH, however, is much bigger than originally expected. By December 2008, 65,000 companies have submitted over 2.7 million preregistrations on 144,000 substances. The feasibility of REACH is now being reassessed.

Alternative methods clearly will be needed to provide the necessary data. REACH, however, requires companies to review all existing information on a chemical and to make optimal use of in vitro systems and in silico modeling. Animal testing is considered a last resort and can be conducted only with authorization by the European Chemical Agency. Hartung stated that one problem is to determine how to validate new testing approaches. It is not known how predictive the existing animal tests are for human health effects, so it does not make sense to validate truly novel approaches against animal tests. Hartung said that the key problem for REACH will be the need for reproductive-toxicity testing, which will represent 70% of the costs of REACH and involve more than 80% of the animals that will be used. That problem will mushroom because few facilities are capable of conducting the testing. The bigger challenges, however, are the number of false positives that will result from the testing and the need to determine which chemicals truly represent a threat to humans. Hartung concluded that a revolution, construction of something new, is needed rather than an evolution—replacement of parts or pieces one by one. The worst mistake would be to integrate small advances into the existing system. The new technologies offer tremendous opportunities for a new system (see Figure 7) that can overcome the problems that we face today.

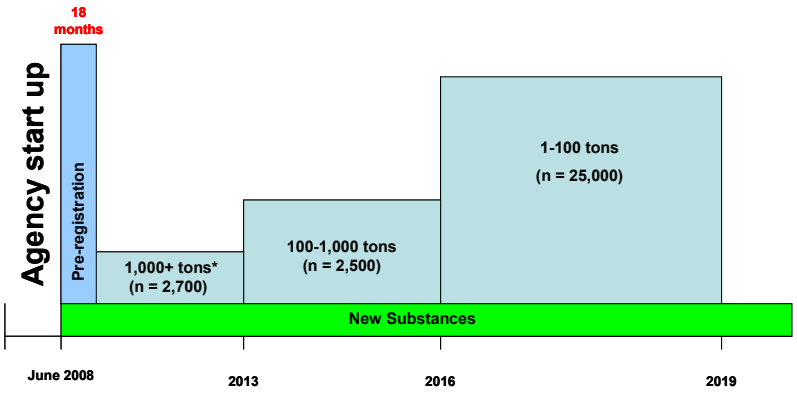

FIGURE 6 Overview of chemical registration for REACH. *Registration includes chemicals that are suspected of being carcinogens, mutagens, or reproductive toxicants and have production volumes of at least 1 ton and chemicals that are considered persistent and have production volumes of at least 100 tons. Source: Modified from EC 2007. Reprinted with permission; copyright 2007, European Union. T. Hartung, Johns Hopkins University, modified from symposium presentation.

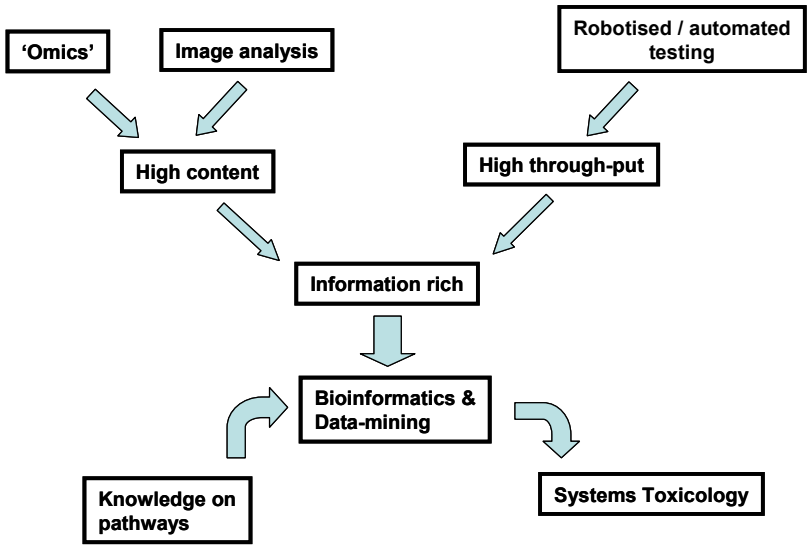

FIGURE 7 Integration of new approaches for toxicology. Source: Hartung and Leist 2008. Reprinted with permission; copyright 2008, *ALTEX*. T. Hartung, Johns Hopkins University, presented at the symposium.

Panel Discussion

The afternoon session closed with a panel discussion that focused on data gaps, pitfalls, and research needs. Kavlock commented that scientists need data to validate the systems, such as data from the pharmaceutical industry, which has extensive human and animal toxicology data on pharmaceutical agents. David Jacobson-Kram, of the Food and Drug Administration (FDA), stated that FDA will soon be joining the efforts of other federal agencies on high-throughput screening and pathway profiling and may be able to provide extensive data. He continued by saying that developing a battery of short-term tests that uses changes in gene-expression patterns to predict human carcinogenic potential will revolutionize toxicology. He cautioned, however, that the tests need to be validated; negative results may simply represent the lack of metabolic activation in a system or the inability of water-insoluble compounds to reach their target. Charles Auer, retired from EPA, emphasized the substantial resources needed for such an effort.

Leikauf stated that a critical problem will be interpretation of the data. Kavlock noted that the goal of ToxCast is to determine the probability that a chemical will cause a particular adverse health effect. The data must be generated and provided to scientists so that they can evaluate them and determine whether the system is working. Hartung agreed with Kavlock that scientists will

deal with probabilities rather than bright lines (that is, point estimates). Frederic Bois, a member of the standing committee, reminded the audience that determining the probability of whether a chemical causes an effect is hazard assessment, not risk assessment; risk assessment requires a dose-response component, which is where the issue of metabolism becomes critically important. Goldstein noted that although probabilities might be generated, regulators will draw bright lines.

Kavlock stated that a key difference between the current system and the new approaches is the scale of information. Substantially more information will be generated with the new approaches, and it is hoped that that information will drive intelligent targeted testing that allows interpretation of data for risk assessment. Several symposium participants emphasized that the discussion on data interpretation and probability highlighted the need to educate the public on the new science and its implications. Linking upstream biomarkers or effects with downstream effects will be critical.

APPLICATION TO MODE-OF-ACTION ANALYSIS

What Is Required for Acceptance?

John Bucher, of the NTP, opened the morning session of the second day by exploring the relationship between toxicity pathways and modes of action and questions surrounding validation. He noted that the concept of mode of action arose from frustration over the inability to describe the biologic pathway of an outcome at the molecular level. Instead, mode of action describes a series of "key events" that lead to an outcome; key events are measurable effects in experimental studies and can be compared among studies. Bucher stated that toxicity pathways are the contents of the "black boxes" described by the modes of action and that key toxicity pathways will be identified with the help of toxicogenomic data and genetic-association studies that examine relationships between genetic alterations and human diseases. He contrasted toxicity pathways and mode of action: mode of action accommodates a less-than-complete mechanistic understanding, allows and requires considerable human judgment, and provides for conceptual cross-species extrapolation; toxicity pathways accommodate unbiased discovery, can provide integrated dose-response information, may allow more precise mechanistic "binning," and can reveal a spectrum of responses. Bucher stated that acceptance of modes of action and toxicity pathways is complicated by various "inconvenient truths." For mode of action, it is not a trivial task to lay out the key events for an outcome, and inconsistencies sometimes plague associations, for example, in the case of hepatic tumors in PPAR-alpha knockout mice that have been exposed to peroxisome-proliferating agents. For toxicity pathways, scientists are evaluating the worst-case scenario; chemicals are applied to cells that have lost their protective mechanism, so the chances of positive results are substantially increased. Furthermore, cells begin to deterio-

rate quickly; if an effect requires time to be observed, a cellular assay may not be conducive to detecting it.

Bucher stated that Tox21—a collaboration of EPA, NTP, and NCGC—has been remarkably successful, with each group bringing its own strengths to the effort; this collaboration should make important contributions to the advancement of the new science. However, many goals will need to be met for acceptance of the toxicity-pathway approach as the new paradigm, and until some of the goals have been reached, the scientific community cannot adequately know what will be needed for acceptance. Bucher noted that the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the Interagency Coordinating Committee on Validation of Alternative Methods (ICCVAM) were established in 2000 to facilitate development, scientific review, and validation of alternative toxicologic test methods and were charged to ensure that new and revised test methods are validated to meet the needs of federal agencies. The law that created NICEATM and ICCVAM set a high hurdle for validation of new or revised methods, but NICEATM and ICCVAM have put forth a 5-year plan to evaluate high-throughput approaches and facilitate development of alternative test methods. Bucher concluded, saying that "at some point toxicologists will have to decide when our collective understanding of adverse biological responses in…in vitro assays…has advanced to the point that data from these assays would support decisions that are as protective of the public health as are current approaches relying on the results of the two-year rodent bioassay" (Bucher and Portier 2004).

Environmental Disease: Evaluation at the Molecular Level

Kenneth Ramos, of University of Louisville, described a functional-genomics approach to unraveling the molecular mechanisms of environmental disease and used his research on polycyclic aromatic hydrocarbons (PAHs), specifically benzo[a]pyrene (BaP), as a case study. Ramos noted that a challenge for elucidating chemical toxicity is that chemicals can interact in multiple ways to cause toxicity, so the task is not defining key events but understanding the inter-relationships and interactions of all the key events. BaP is no exception in causing toxicity potentially through multiple mechanisms; it is a prototypical PAH that binds to the aryl hydrocarbon receptor (AHR), deregulates gene expression, and is metabolized by CYP450 to intermediates that cause DNA damage and oxidative stress. Ramos stated that his laboratory has focused on using computational approaches to understand genomic data and construct biologic networks, which will provide clues to BaP toxicity. He interjected that the notion of pathway-based toxicity may be problematic because intersecting pathways all contribute to the ultimate biologic outcome, so the focus should be on understanding networks.

He said that taking advantage of genomics allowed his laboratory to identify three major molecular events: reactivation of L1 retroelement (Lu et al.

2000), activation of inflammatory signaling (Johnson et al. 2003), and inhibition of genes involved in the immune response (Johnson et al. 2004). The researchers then began to investigate the observation that BaP activated repetitive genetic sequences known as retrotransposons. Retrotransposons are mobile elements in the genome, propagate through a copy-and-paste mechanism, and use reverse transcriptase and RNA intermediates. L1s are the most characterized and abundant retrotransposons, make up about 17% of mammalian DNA by mass, and mediate genome-wide changes via insertional and noninsertional mechanisms. They may cause a host of adverse effects in humans and animals because their ability to copy themselves allows them to insert themselves randomly throughout the genome.

Ramos described the work on elucidating L1 regulatory networks by using genomics and stated that the key was identifying nodes where multiple pathways appeared to overlap. He and his co-workers used silencing RNA approaches to knock down specific proteins, such as the AHR, so that they could investigate the effect on the biologic network, and they concluded that the repetitive sequences are important molecular target for PAHs. His laboratory has now turned to trying to understand the epigenetic basis of regulation of repetitive sequences and how PAHs might affect those regulatory control mechanisms in cells. The idea that biologic outcomes are affected by disruption of epigenetic events adds another layer of complexity to the story of environmental disease. It means that in addition to the direct actions of the chemical, the state of regulation of endogenous systems is important for understanding the biologic response. Ramos concluded that L1 is linked to many human diseases—such as chronic myeloid leukemia, Duchenne muscular dystrophy, colon cancer, and atherosclerosis—and that research has shown that environmental agents, such as BaP, regulate the cellular expression of L1 by transcriptional mechanisms, DNA methylation, and histone covalent modifications. Thus, the molecular machinery involved in silencing and reactivating retroelements not only is important in environmental responses but might be playing a prominent role in defining disease outcomes.

Dioxin: Evaluation of Pathways at the Molecular Level

Alvaro Puga, of the University of Cincinnati College of Medicine, used dioxin as an example to discuss molecular pathways in disease outcomes. Dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin [TCDD]) is a contaminant of Agent Orange—a herbicide used during the Vietnam War—that has been linked with numerous health effects. Some effects are characterized as antiproliferative, such as the antiestrogenic, antiandrogenic, and immunosuppressive effects; others are proliferative, such as cancer; and the remainder are characterized as effects on differentiation and development, such as birth defects. The effects of dioxin are primarily receptor-dependent. Dioxin binds to the AHR, a ligand-activated transcription factor. The resulting complex translocates to the nucleus and binds with the AHR nuclear translocator to form a complex that then binds to DNA-responsive ele-

ments on the genome; that binding induces gene expression. Dioxin is not the only ligand to bind to the AHR, and that raises the question of whether results from one ligand can be extrapolated to all ligands. That has essentially been done for several classes of halogenated aromatic hydrocarbons—polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans, and polychlorinated biphenyls—with dioxin as the reference compound. AHR ligands have substantially different potencies to activate AHR-dependent gene expression, and their ability to produce toxicity appears to depend on their metabolic stability.

Puga stated that most toxic effects of dioxin are mediated by the AHR. For example, research has shown that AHR-deficient mice are resistant to dioxin-induced cytotoxicity and teratogenicity and that AHR-deficient zebrafish are resistant to dioxin-induced cardiac edema. The AHR has been implicated in many signaling pathways, and thus dioxin has the potential for disrupting other signaling pathways, such as MAP-kinase pathways and pathways associated with the cell cycle. Research indicates that cellular conditions determine whether the effects will be proliferative or antiproliferative. Puga stated that his laboratory is working to map the AHR regulatory network by varying the genotype of the cells (that is, using cells that have a wild-type receptor and cells that have a point mutation in the receptor that prevents binding to DNA) and asking the question, What is the target of the AHR at the whole-genome level? He said that recent research indicates that dioxin causes massive deregulation of homeobox and differentiation genes, so scientists should be critically investigating the developmental outcomes associated with dioxin exposure.

Systems-Level Approaches for Understanding Nanomaterial Biocompatibility

Brian Thrall, of the Pacific Northwest National Laboratory, discussed the challenges in evaluating mode of action and conducting hazard assessment of nanomaterials, and he provided examples of approaches from his laboratory to address the challenges. Nanotechnology will soon affect all aspects of society; current estimates are that sales from products that incorporate nanotechnology will reach $3 trillion by 2015. Although there are no documented cases of human toxicity of or disease caused by nanomaterials, concern has arisen because other types of particles and fibers have been linked to human disease, and comparisons have recently been made between asbestos fibers and carbon nanotubes. Thrall noted that if hazard assessments of the nanoproducts *currently* on the market were conducted using chronic bioassays, it could cost over $1 billion and take 30-50 years. Clearly, rapid screening approaches that lead to a small number of chronic bioassays or other in vivo testing would dramatically reduce the cost and time required to test the products.

Thrall stated that nanomaterials are difficult to evaluate because they are engineered materials that are made on the scale of biologic molecules and could

therefore interact with biologic pathways in many complex ways. Critical challenges in nanotoxicology revolve around addressing fundamental questions of exposure, dose, and mode of action. Focusing on dose, Thrall stated that a number of reports have indicated that the toxicity of particles depends on size: smaller particles tend to be more toxic on an equal-mass basis. However, Oberdorster et al. (2005) showed that using mass as the basis of comparison may not reveal much about chemical potency and biologic reactivity. The question then becomes what dose metric—mass, particle number, or surface area—is the most informative. Thrall stated that research with amorphous silica in his laboratory showed that surface area was the most appropriate dose metric in experiments evaluating cytotoxicity (Waters et al. 2009). Using genomics, he and co-workers also showed that the predominant dose-response pattern for gene expression depends on surface area and is independent of particle size. Furthermore, reverse transcription polymerase chain reaction (RT-PCR) showed that for more than 75% of the genes identified, the magnitude of expression correlated better with nominal surface area than with mass or particle number.

Thrall then asked whether any biologic processes can be attributed to size dependence; that is, do the chemical and physical properties that make nanomaterials commercially attractive cause unique biologic responses? His laboratory investigated that question by conducting gene-set enrichment analyses—a statistical approach in which the ontologic attributes of a gene set are compared. He and co-workers found that the major cellular processes affected by 10-nm and 500-nm silica were identical; none of over 1,000 biologic processes identified was statistically different as a function of particle size. So for amorphous silica, there was no compelling evidence that new biologic processes arise as a function of size at the nanoscale. His research on amorphous silica also showed how high-content data can be used to address some fundamental questions concerning nanomaterials.

Thrall concluded his presentation by noting a few other challenges that arise with nanomaterials. First, engineered particles are not as simple as soluble chemicals because such physical forces as gravity, diffusion, and convection act on them, particularly in cell-culture systems, and can influence dose and changes in dose (see Figure 8). For example, the dose measured for a particle that settles slowly or hardly at all could differ significantly from the dose measured for a dense particle that settles quickly in the culture. Dosimetry models for the in vitro systems need to be validated so that mode-of-action studies can be anchored by biologic dose. Second, nanomaterials adsorb proteins in biologic systems (see Figure 9), and this could have an important effect on disposition of nanoparticles and biologic response to the nanoparticles. Structure-based modeling might provide insight on principles that guide protein interaction with nanomaterials and might serve in the future as a screening tool for evaluating relationships between alterations in surface chemistry and toxicity. Thrall noted the importance of developing hybrid quantitative structure-activity relationship models that integrate structural, chemical, and biologic data and stated that re-

cent work has shown that specific aspects of surface area, such as the presence of polar functional groups, rather than total surface area alone are important for toxicity. He said that although this symposium is not focused on exposure, there is a need for more information on potential exposure to set priorities among and validate nanotoxicity studies.

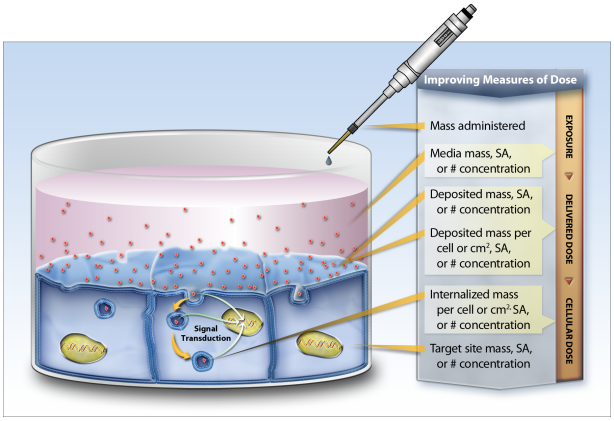

FIGURE 8 Dosimetry considerations in cell systems. Source: Teeguarden et al. 2007. Reprinted with permission; copyright 2007, Society of Toxicology. B. Thrall, Pacific Northwest National Laboratory, presented at the symposium.

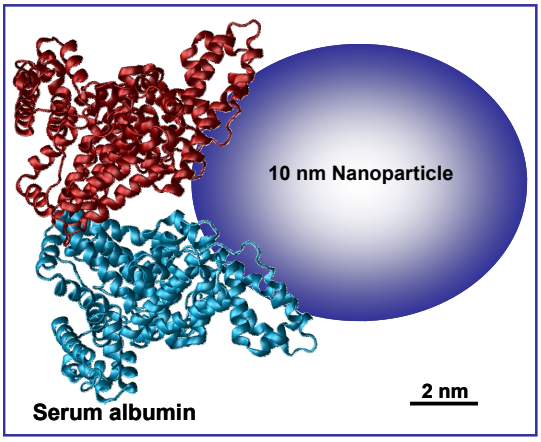

FIGURE 9 What do cells see? Protein adsorption by nanomaterials is a universal phenomenon in biologic systems. Source: B. Thrall, unpublished data, Pacific Northwest National Laboratory, presented at the symposium. Reprinted with permission; copyright 2007, Pacific Northwest National Laboratory.

Panel Discussion

The morning session closed with a panel discussion that focused on the use of the new science in regulatory decision-making. Thrall commented that nanomaterials may not be a good case study because there are still relatively few data on them, and he emphasized the need to collect exposure data particularly to determine the magnitude of exposure and the materials to which people are being exposed. That information is needed to allow priority-setting for research. Bucher stated that dioxin and dioxin-like compounds may be good examples for incorporating the new science and noted that recent research has supported the toxic equivalency factors established by the World Health Organization for predicting carcinogenic outcome. He added that research has shown that it will not be sufficient simply to evaluate pathways and that other information on timing and persistence will need to be integrated with pathway data.

Jonathan Wiener, of Duke University, addressed policy and legal aspects of incorporating the new science in agency decision-making. He considered the scenario in which EPA based a decision, such as a decision to regulate a chemical, on the new toxicity-pathway-based science or on a combination of traditional and new testing methods. He first noted that the agency would face internal review in the executive branch in the Office of Management and Budget Office of Information and Regulatory Affairs (OIRA) and possibly in the Office of Science and Technology Policy (OSTP). Wiener suggested that the field may be open for some new and interesting approaches to guide agency science and decision-making, in light of recent actions of President Obama's administration, such as his call for a new executive order on regulatory oversight by OIRA and his memorandum on improving agency science and strengthening OSTP's role.

After internal review within the executive branch, Wiener observed, the next hurdle would be judicial review. He argued that, although courts can be skeptical of new scientific methods in civil tort liability lawsuits, judicial review of agency science may be more deferential, especially when agencies are acting "at the frontiers of science." Several regulatory statutes now call for agencies to use the "best available science" or the "latest scientific knowledge," and a court could be convinced that toxicity-pathway approaches constitute the best and latest science. Furthermore, the Supreme Court has recently held that if the agency provides a persuasive reason for changing its policy or its basis of decision-making, the courts will be receptive to the change even if the reason is not the one that a court would have given. Thus, an agency seeking to rely in whole or in part on toxicity-pathway-based approaches for making regulatory decisions ought to give a good explanation of why these new methods are valuable.

Finally, Wiener pointed out that the question of what constitutes an "adverse effect" may be pivotal. Yet, as others have discussed during this symposium, responses observed in toxicity-pathway studies may not always indicate an adverse effect as opposed to, for example, an adaptive effect. Wiener's research indicates that the term *adverse effect* has been used in hundreds of federal statutes and thousands of judicial opinions since 1970, but it is almost never

defined. Wiener suggested that EPA try to provide a thorough and tractable interpretation of what an adverse effect is and how the new toxicity-pathway testing methods can demonstrate such an effect.

The question of whether a framework that would facilitate the use of new data could be developed or whether it was too soon to use the new data was discussed. Thrall noted that the development of a framework and advancing the new science would depend on fields outside toxicology, such as improving computational abilities to handle various approaches and assumptions. Ramos, however, stated that there is now technology that allows a pathway-based approach to classification, to increase understanding of modes of action, to gain insight into biologic outcome, and ultimately to predict safety. He warned, however, that one has to temper that optimism with reality and recognize that a system that has checks and balances to minimize error must be built because we do not yet know whether we can make predictions on the basis of the new science with a given level of certainty. Elliott agreed, emphasizing that the issue should not be framed as an all-or-nothing decision, and suggested that the agency should take a relatively simple, well-understood system, establish the pathway-based approach for it, and then build on that precedent. Ramos stated that there are some chemicals, such as arsenic and PAHs, with which that approach could be taken. Wiener underscored Elliott's point and stated that in the near term toxicity-pathway-based approaches should be combined with whole-animal tests and human epidemiology so that reviewing bodies, such as OIRA and the courts, become comfortable with the information as providing a fuller picture rather than as a replacement at this stage. Other symposium participants echoed the idea of pushing forward and using and applying the data that have been collected, and one noted that no single assay is going to give a yes or no answer for a risk assessment or substantive decision. We will need to integrate all the information and use the best interpretive skills and scientific judgment to answer the important questions.

CHALLENGES AND OPPORTUNITIES FOR RISK ASSESSMENT IN THE CHANGING PARADIGM

Dose and Temporal Response

Elaine Faustman, of the University of Washington, opened the afternoon session by discussing datasets and tools available to examine dose and temporal response and what is needed to move forward. Faustman discussed the creation of gene ontologies and noted the paper by Ashburner et al. (2000) as critical in advancing the field. Three categories—biologic process (goal or objective), molecular function (elemental activity or task), and cellular component (location or complex)—have been defined, and each category has a structured, controlled vocabulary. Figure 10 provides an example of a gene ontology and shows the

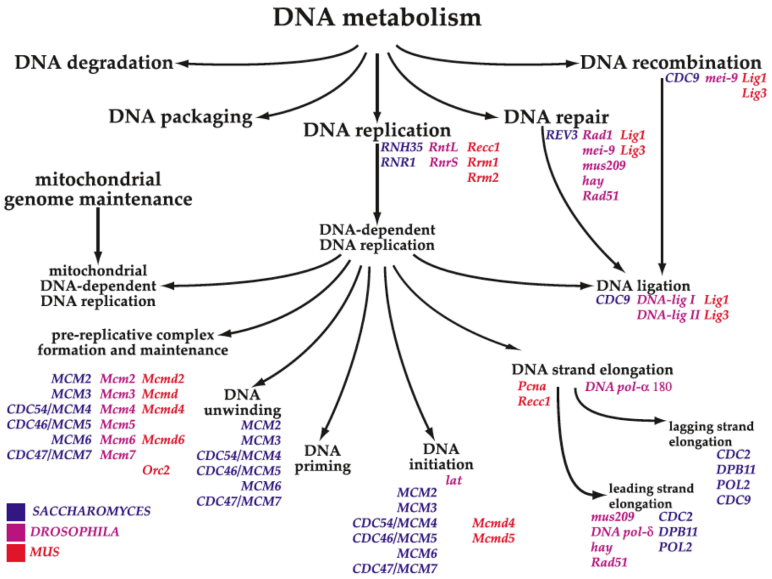

FIGURE 10 Example of gene ontology for DNA metabolism, a biologic process. Similar ontologies can be built for molecular function and cellular component. Source: Ashburner et al. 2000. Reprinted with permission; copyright 2000, *Nature Genetics*. E. Faustman, University of Washington, presented at the symposium.

equivalent genes for three species for a specific biologic process. For risk assessment, gene ontologies provide an outstanding opportunity to use genomic information for cross-species comparisons.

Several years ago, Faustman and co-workers recognized that decision rules were needed to evaluate dose- and time-dependent genomic data. Consequently, a system-based framework to interpret those data was developed (Yu et al. 2006). That framework (see Figure 11) has been used to identify potential signaling pathways versus single genes significantly changed after exposure. Once biologic processes are linked to pathway changes, one can begin to evaluate deviations from the normal patterns of gene expression that result from chemical exposure (Yu et al. 2006). That approach has been applied to metals, phthalates, and sulfur mustard (Robinson et al. 2010; Yu et al. 2006, 2009, 2010).

Faustman noted the report *Scientific Frontiers in Developmental Toxicology and Risk Assessment* (NRC 2000) and summarized the three main points of the report: signaling is used in almost every developmental event, about 17 pathways of cell-cell signaling are responsible for all of development, and the 17

pathways are highly conserved among metazoa. The report was valuable because it identified pathways involved in early, middle, and late development and laid the foundation for future work. Faustman commented that when evaluating pathways, one needs to consider not only whether the pathway is present but what the signal means (that is, a signaling pathway in one organism may have roles different from those in another organism). Furthermore, not only whether a pathway is expressed but when it is expressed and how it is expressed makes a difference. Like other speakers, Faustman emphasized the inter-relatedness of some of the pathways and the relationship of the network to general function.

Returning to the topic of risk assessment, Faustman noted observations from studies in her laboratory on the effects of metal exposure on mouse development. The studies found that metals affect genes involved in the Wnt signaling pathway, that multiple transcription-factor families are affected by metals, and that more than 50% of the genes affected were uncharacterized at the pathway level; the latter finding indicates that much work still needs to be done to determine the link between gene changes and pathways and the importance of the gene changes. Faustman closed by listing several needs, including new tools for evaluating quantitative genomic response at multiple levels of biologic organization, kinetic and dynamic models that can allow for integration at various organization levels, better characterization of variability in genomic data, discussion of and consensus on how responses and changes in responses should be considered for effect-level assessment, and discussion of approaches to evaluate responses to early low-dose exposures vs responses at increasing complexity and decreased specificity.

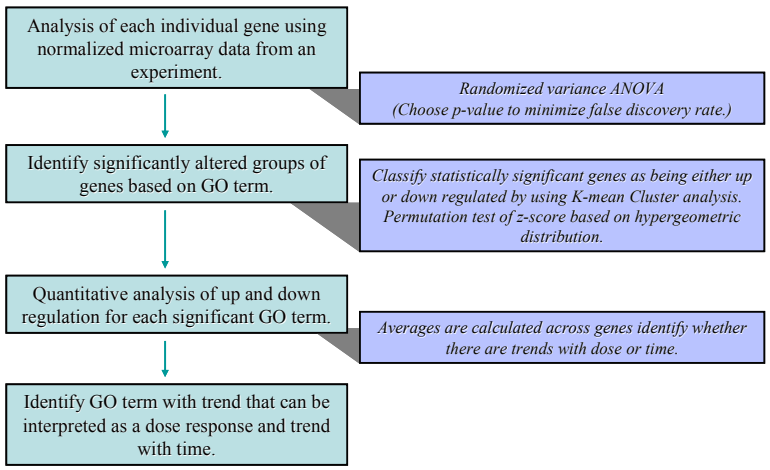

FIGURE 11 Framework for interpretation of dose- and time-dependent genomic data. Source: Yu et al. 2006. Reprinted with permission; copyright 2006, *Toxicological Sciences*. E. Faustman, University of Washington, presented at the symposium.

Application of Genomic Dose-Response Data to Define Mode of Action and Low-Dose Behavior of Chemical Toxicants

Russell Thomas, of the Hamner Institutes for Health Sciences, provided a series of practical applications of genomic data to risk assessment. He noted several aspects of genomics that make it applicable to risk assessment. First, gene-expression microarray technology is more than a decade old, and multiple studies have demonstrated the sensitivity and reproducibility of the current generation of microarrays. Second, genomic technology is capable of broadly evaluating transcriptional changes (genome-wide) and of focusing on changes in individual genes and pathways. Third, gene-expression microarray analysis can provide insight into the dose required to affect cellular processes and the underlying biology of dose-dependent transitions. Thomas commented, however, that there is still no consensus on how to use genomic information in risk assessment.

In his first example, Thomas described an experiment from his laboratory in which the dose-response changes in gene expression of several chemical carcinogens, previously tested by NTP, were evaluated with transcriptomic data, and the results were compared with tumor-incidence data. For the experiment, groups of female B6C3F1 mice were exposed to one of five doses of a given carcinogen for 90 days, the transcriptional changes in target tissues were evaluated with whole-genome microarrays from Affymetrix, and the dose-response changes in gene expression were examined with a pathway-based approach. Specifically, the researchers calculated benchmark doses (BMDs) for individual genes on the basis of their inherent variability; grouped genes on the basis of biologic function, such as their role in proliferation, apoptosis, and metabolism; and finally calculated a summary value for the particular pathway. They found good correlation not only between the BMDs for the pathway-based transcriptional responses, such as cell proliferation and DNA damage response, and tumor incidence but between overall changes in gene expression and tumor incidence. They also found that the BMD for the most sensitive biologic process was always less than the BMD for the tumor response. Thomas concluded that transcriptomic dose-response alterations correlate with tumor incidence and that BMD values for the most sensitive pathways are protective.

In the second example, Thomas described how to use cross-species differences in transcriptional dose-response data to evaluate mode of action. Thomas and co-workers exposed groups of rats and mice to chloroprene for 5 or 15 days. Chloroprene is metabolized to epoxide metabolites, and the rate of metabolism and of generation of the epoxide metabolites is about 10 times higher in mice than in rats. Accordingly, they used a physiologically based pharmacokinetic (PBPK) model to try to normalize the doses so that mice and rats received about the same internal dose. The BMD analysis of the genomic data indicated that at 5 days glutathione metabolism was perturbed and at 15 days DNA repair genes

were affected and that the mouse was substantially more sensitive than the rat, although some differences disappeared when the comparison was based on internal dose. Overall, the results indicated the importance of the generation of the reactive epoxide metabolites in the proposed mode of action of chloroprene. Thomas concluded that pathway-based transcriptomic dose-response data can provide insights into the mode of action.

In the third example, Thomas provided suggestions for using genomics data to conduct risk assessments. If the mode of action is known, transcriptional benchmark dose lower confidence limit (BMDL) values could be derived on the basis of responses in key pathways and the values could be used in the risk assessment. If the mode of action is not known, the challenge is to discriminate between "adverse" and "adaptive" changes. However, one could proceed by evaluating all responses, deriving transcriptional BMDL values for the most sensitive pathways, and using them in risk assessments. Thomas compared reference doses and risk-specific doses derived from transcriptional BMDL values with comparable risk-assessment values from EPA's Integrated Risk Information System. On the basis of those comparisons, Thomas concluded that pathway-based transcriptomic dose-response data can provide reasonable reference doses or points of departure for performing cancer and noncancer risk assessments. He added the hope is that genomics data can be used in the future to inform the shape of the dose-response curve in the low-dose region and allow some assessment of whether a linear or nonlinear approach should be taken.

Using Physiologically Based Pharmacokinetic Models to Interpret –Omics Dose-Response Data

Gregory Kedderis, of Chapel Hill, NC, reviewed the use of PBPK models in risk assessment and discussed how PBPK models could be used to inform in vitro experiments. He stated that PBPK models are mathematical descriptions of anatomy, physiology, and biochemistry in which model compartments represent organs or organ groups that are linked by blood flow. There are two types of data needed for PBPK models: physiologic measures, such as body weight, alveolar ventilation rates, blood-flow distribution to tissues, and organ volumes; and chemical-specific parameters, such as partition coefficients, biotransformation kinetic values, and values related to protein binding and chemical reactivity. Generally, the information is available in the literature, but Kedderis cautioned that some data require technical expertise to evaluate (for example, distinguishing relative measurements from objective measurements). PBPK models are used to relate external exposures to an internal dose—ultimately, the target-organ dose. Kedderis stated that the major contribution of PBPK models to risk assessment is that they allow one to reconcile various exposure routes and species differences. PBPK models can also be used to extrapolate human in vitro data to the in vivo system if data are available on the composition of the tissue.

In the new paradigm, Kedderis stated that PBPK models could be used prospectively to inform in vitro experiments. For example, one could run a PBPK model for a given chemical by using a realistic exposure scenario and obtain a target-organ dose that could then be used to set concentration ranges for an in vitro experiment. Using that approach would ensure that cells are exposed to concentrations that are realistic for in vivo conditions. The problem is that cell cultures are closed systems, and biotransformation is an extremely important consideration. Many toxic chemicals require biotransformation to produce toxicity, and metabolism of a direct-acting chemical is often a detoxification event. Furthermore, many metabolites are transitory or chemically reactive. Kedderis emphasized that metabolically competent cells are needed but stated that for chemicals metabolized exclusively or primarily by the liver, there are commercially available tissues or cellular preparations that have integrated metabolism similar to that of the whole liver. He noted several other concerns regarding cell cultures, including issues about chemical solubility and volatility, the use of carrier solvents that can act as potent inhibitors, and changes in gene expression of immortalized cell lines. Kedderis was optimistic, however, about the promises of the new science and technologies and concluded that PBPK models will be able to augment the interpretation of –omic dose-response data and ultimately to provide information on physiologic variability, bioactivation variability, and response variability in the population.

Modular Network Modeling of Toxicity Pathways for Extrapolation

Katrina Waters, of Pacific Northwest National Laboratory, described her laboratory research on –omics approaches to modeling of human disease states. She began by noting several challenges in using a pathway-based approach for risk assessment, including elucidating accurate mechanistic response models from global response data, given that most global response data capture only one regulatory mechanism at a single time; distinguishing reversible, adaptive processes from true toxicity pathways; validating dose-response models that capture single biologic pathways and assume isolation from other systems; and extrapolating from in vitro to in vivo systems. Biologic systems are inherently complex and have many redundant interdependent signaling networks, and cell-response modeling will require incorporation of complex feedback and compensatory mechanisms to be predictive at a tissue level.

Waters stated that global technologies—such as microarrays to measure global transcriptional responses, tandem mass-spectrometry proteomics to measure global protein changes, and parallel Western blot technology to measure protein abundance and protein phosphorylation states—are biased in their measurement of cellular processes. However, integrating the multiple data types provides many advantages, including more comprehensive coverage of network and pathway processes. Waters illustrated her point by showing a network re-

construction from her laboratory based on microarray, proteomic, and parallel Western blot data (see Figure 12). The researchers found that individual technologies were not measuring the same thing multiple times, but rather measuring different parts of a network. Ultimately, no single technology could have led to the construction of a signaling network as comprehensive as the one developed when data of all three types were used.

Waters continued with another example from her laboratory in which macrophages and type 2 epithelial cells were exposed to silica nanoparticles, carbon nanotubes, or non-particle lipopolysaccharides. The cellular responses were evaluated by using whole-genome microarrays and global proteomic analysis. Using the microarray data, they could distinguish between lipopolysaccharide-induced inflammation and silica-induced inflammation in the macrophage cells, but no distinction could be made between silica, crystalline silica, and carbon nanotubes on the basis of those data. The proteomic data, however, allowed them to distinguish the different particles from each other by using protein profiles. From those studies, Waters concluded that integrated heterogeneous data provide more comprehensive cell-response networks than any single data type alone.

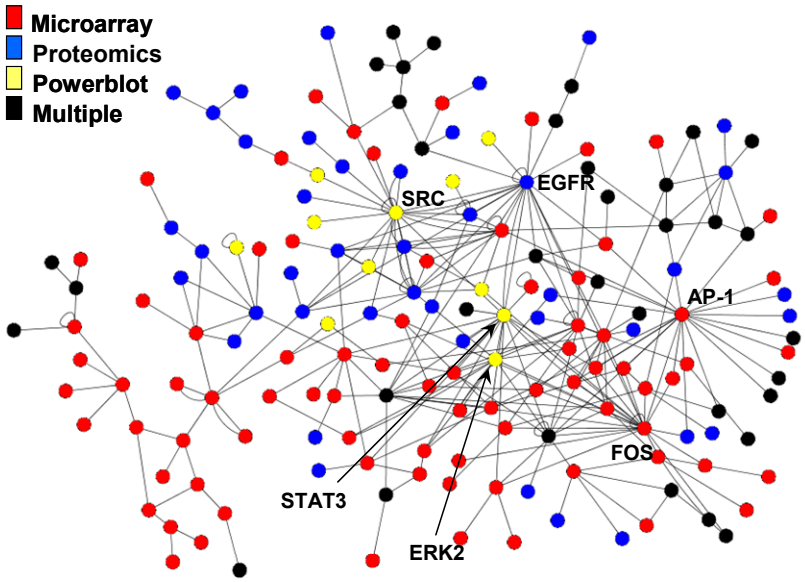

FIGURE 12 Integrated data provide more comprehensive and accurate network reconstruction. Black nodes represent genes or proteins that were measured with more than one technology. Red, blue, and yellow nodes represent genes or proteins measured with individual technology indicated in the figure. Source: Waters and Thrall, unpublished data, presented at the symposium. Reprinted with permission; copyright 2010, Pacific Northwest National Laboratory.

Waters next described the advantages of developing modular network models to describe disease states. She stated that biologic systems are too complex for mechanistic models and that simpler abstractions are needed. So one can use the –omics data to define a set of inputs and a set of outputs and then represent the network clusters as functional modules (see Figure 13). The system is defined in terms of functional modules; the focus is on information flow (cause-effect relationships) rather than on molecular mechanisms. Thus, the modular network models can capture biologic complexity while reducing computational load to a finite number of variables that can be validated experimentally. Inferred network relationships can be verified by using molecular-intervention techniques, such as inhibiting a key enzyme in a biologic process and observing the cellular response. Waters stated that eventually it would be desirable to use the model to ask which functional module is responsible for a transition from an adaptive response to an adverse response. Once that module has been identified, mechanistic detail can be added to allow the model to predict an outcome better given the input dose. The next step is to scale the process to multicellular systems. The hope is to identify the mediators of cell-cell communication and the regulatory network underlying synthesis and secretion of the mediators.

Waters emphasized that biology is a dynamic process, that multiple levels of regulatory processes are necessary to capture biologic complexity, and that dose-response and temporal data will be required to capture the transition from adaptive processes to adverse outcomes. Furthermore, cells do not respond in isolation in tissues; thus, Waters stated, models must account for paracrine, neurologic, and other physiologic interactions to extrapolate from in vitro to in vivo systems accurately. She concluded by noting several needs to advance the toxicity-pathway approach for risk assessment, including biologically based models that incorporate epigenetic, proteomic, metabolomic, and post-translational modification data better; improved understanding of the relationships between toxicity pathways and toxicity outcomes; criteria for defining appropriate in vitro systems that represent in vivo toxicity sufficiently; and improved understanding of dosimetry and temporal differences between in vitro and in vivo systems.

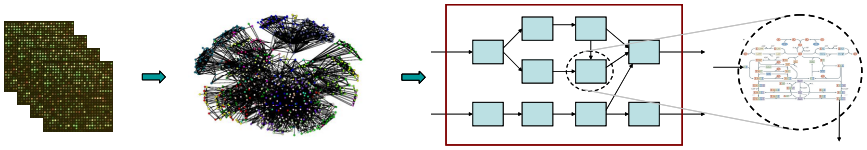

FIGURE 13 Illustration of the development of modular network models. A system consisting of functional modules can be developed from –omics data, and mechanistic detail can be added once the module that is primarily responsible for the toxic response is identified. Source: K. Waters, unpublished data, Pacific Northwest National Laboratory, modified from symposium presentation. Reprinted with permission; copyright 2010, Pacific Northwest National Laboratory.

Integrating Global Gene Expression and Survival of 60 Cell Lines: Applications for Risk Assessment

Albert J. Fornace, Jr., of Georgetown University, discussed the use of –omics and other approaches to investigate individual sensitivity to ionizing radiation and possible applications to risk assessment. His research focus is on understanding variations in individual injury by using genomics rather than dose alone. Research implications include the elucidation of markers for screening radiologic workers or first responders after a radiation emergency, identification of persons with higher risks of adverse outcomes from exposure and possibly risks of late effects and second cancers, and development of a model for testing other toxicants on the basis of research on radiation effects. For his research, his laboratory uses the NCI60 cell lines as a model for population datasets. NCI60 cell lines consist of 60 human tumor-cell lines that have been used to test more than 100,000 compounds for chemosensitivity and on which numerous assays have been performed to identify molecular markers. Fornace noted several advantages to using ionizing radiation as the agent for studying individual sensitivity, including its well-characterized stress response, the absence of drug uptake or metabolism issues, and the linear response of stress genes (that is, a roughly proportional increase in the production of mRNA to dose).

Fornace stated that the approach has been to activate pathways as robustly as possible (that is, to cause an acute response) and evaluate increases in gene expression by using hybridized microarrays. A recent study (Amundson et al. 2008) found great heterogeneity of gene expression. On further analysis, 25 genes that were p53-responsive were identified; several had not been known previously as p53-regulated genes. The researchers were also able to identify genes related to radiation sensitivity and found that basal gene expression provided better predictions of radiation sensitivity than radiation-induced gene expression. Focusing next on pathways, they found that a set of genes—many of which regulate cell-cycle progression—was robustly and clearly repressed. They built a network by using protein-interaction databases and overlaid gene-expression data from the cell-cycle cluster. Ultimately, they identified a variety of genes known to be repressed by E2F4 and found that this transcription factor moves into the nucleus on exposure of the cell to ionizing radiation, where it presumably represses the genes. Fornace concluded that the approach developed to identify stress-response signatures of radiation could be adapted to non-genotoxic agents. The key is to select doses that will yield appreciable stress-pathway activation with reliable changes in gene expression. Initial studies in his laboratory have shown potential for using that approach to identify signatures of activation of various pathways for direct-acting agents.

The Food and Drug Administration Experience in Analyzing Genomic Data

Federico Goodsaid, of the FDA Office of Clinical Pharmacology, described

genomic data submissions to FDA and its approach to their review. He stated that FDA receives genomic data through voluntary exploratory data submissions (VXDS), which involve meetings with sponsors in an open nonregulatory environment. Since 2004, FDA has had 35 data submissions from a wide variety of clinical divisions. The majority of platforms from 2004-2006 have been for microarray differential gene expression, and the majority from 2006-2008 for candidate gene identification. Most of the data have been from clinical studies, and the number of submissions concerning efficacy has been about twice the number concerning safety. Goodsaid noted that the data can be used in several ways: as part of a submission for drug approval in which the data are interpreted in the context of any specific claims made by the sponsor, in the co-development of drugs and tests in which a drug and a genetic test are validated as part of a phase III trial, for labeling updates in which genomic information is added to pre-existing labels, or in the biomarker-qualification process in which the data can enter a formal evaluation and review process with the purpose of qualifying a biomarker for a specific context of use (for example, as a preclinical marker of nephrotoxicity, hepatotoxicity, or vascular injury).

Next, Goodsaid noted the work of the Microarray Quality Control Consortium, which was initiated and is coordinated by the FDA National Center for Toxicological Research and was tasked with addressing issues of reliability, performance, quality, and analysis of microarray data. The consortium found that microarray data are repeatable within a laboratory, reproducible among laboratories, concordant among platforms, and comparable with alternative technologies. The consortium then began to address technical issues related to the development and validation of predictive signatures and classifiers on the basis of gene-expression data from microarrays and to assess the capabilities and limitations of microarray technology. Goodsaid noted that publications on that work would be released soon.

Goodsaid returned to the VXDS program at FDA and stated that one aspect involves the biologic interpretation of the lists of differentially expressed genes. He noted several questions that FDA considers, such as, What functions or pathways are associated with substantially over-represented genes in the list? How many pathways are affected? What types of pathways are affected? What is the inferred mechanism of action and toxicity of the gene-expression changes? What is the tissue specificity of the pathways and gene function? Goodsaid stated that FDA reviews a sponsor's interpretation, tries to reconstruct it, and attempts to provide alternative biologic interpretations. He mentioned that FDA has entered into a cooperative research and development agreement with one company to provide software that provides several methods for evaluating the lists of differentially expressed genes. Goodsaid concluded by saying that FDA has been able to draft guidance for pharmacogenomic-data submissions on the basis of its experience with the voluntary data submissions and recommendations from sponsors, the Microarray Quality Control Consortium, other interested parties, and public forums.

Panel Discussion

The afternoon session closed with a panel discussion that focused on the definition of *toxicity pathway*, an issue raised during several presentations. Faustman noted that defining a toxicity pathway is context-dependent. For example, apoptosis is generally seen as an adverse effect, but if apoptosis did not occur during development, humans would have fins. So time, place, and response make a difference. Goodsaid added that he would be hesitant to label a pathway as a toxicity pathway in isolation. For FDA, pathway information helps the agency to make regulatory decisions; mechanistic data allow the agency to interpret other test data.

Waters stated that ideally what should be identified are pathways that are consistently and measurably changed within 2 weeks—or possibly even 4 weeks—of exposure and that are indicative and predictive of some outcome downstream that is recognized as a toxicity end point. She noted, however, that her definition raises the controversy about distinguishing an adverse response from an adaptive response; until gene expression, protein abundance, or some other measure has been evaluated over the dynamic cycle, one cannot distinguish whether it is a time-dependent expression of some adaptive response or truly a dose-dependent change indicative of toxicity.

Thomas stated that his work has not focused on defining a toxicity pathway itself, but on grouping pathways according to common biologic function to make predictions. Faustman noted that there are differences between the various analytic tools and approaches that are being used to define pathways and that the differences could affect how one defines a pathway. The tools and approaches are only as good as the data that go into them, and the scientific community has not yet developed an unbiased approach for integrating pathway information.

Rhomberg stated that *toxicity pathway* might not be the best descriptive term. Normal biologic processes need to be understood, as does how the processes are changed by various agents and result in toxicity. Furthermore, the processes are not linear sequences of events but networks of interactions. Thus, Rhomberg concluded that the focus should be on discovering the key pathway components and how they are integrated to make the functional modules discussed by Waters. One participant questioned, however, whether one needed to understand what a toxicity pathway was; perhaps one could use the models described during the symposium to derive a benchmark dose for a response, calculate a tissue dose, and then extrapolate to a human exposure that would drive the given response. Thomas and Kedderis agreed, but Kedderis noted that it is important to evaluate mechanisms when dealing with unknowns, agents on which few, if any, data are available. Thomas countered that one can provide guidance on reference doses or risk-specific doses by using genomic approaches and thus bridge the gap between making decisions on the basis of reasonable scientific data and not making decisions because of lack of data and simply ignoring the possible risks posed by exposure to the unknowns. He added that if genomic

profiles are obtained on six specific tissues, one can predict about 90% of all positive responses by using rat and mouse bioassays and thus obtain valuable information for risk assessment.

WHAT THE FUTURE HOLDS

Where Are We Going? or, Are We There Yet, and Where Is There

The final session of the symposium was devoted to presentations and discussions on visions for the future and the path forward. Preuss began the session by providing a perspective on the changing landscape of risk assessment and the need for modernization. He stated that advances in understanding of the gene environment and the pending test data from the European REACH program are driving the need for change. He described two paradigms for understanding the effects of toxic chemicals. One is the human-disease model in which genetic profiles of people with and without a disease are compared to yield fingerprints of disease and susceptibility, and the other is the current animal-testing model in which chemically induced events are matched to rodent test results and rodent modes of action. Preuss noted that the new science and technologies should allow movement between paradigms, although the two approaches will probably progress in tandem for many years. However, the question now is how to move from assessing a few chemicals each year to assessing thousands of chemicals each year as the REACH program anticipates. Dossiers on 40,000 chemicals are expected by 2012, and the U.S. government is ill prepared to use the volume and complexity of information resulting from that or a similar program. Anticipating the need for change, EPA sponsored several NRC reports over the last few years that focused on toxicity testing and risk assessment (for example, NRC 2007a, 2008, 2009). Overall, those reports concluded that risk-assessment data needs cannot be met with the current testing methods, that scientists need to determine how to use the new data being generated for risk assessment, and that the transformation of risk assessment has to occur with stakeholder input.

Preuss described EPA's dual approach to developing the next generation of risk assessments. First, EPA is considering creating a high-priority list of chemicals and streamlining the process for assessment by narrowing the scope, using off-the-shelf risk approaches, and focusing and coordinating stakeholder reviews. Second, EPA is considering broadening the scope of some assessments to synthesize more information into each assessment, such as assessments on cumulative effects of agents that cause the same effect or on families of chemicals that are physically similar. EPA intends to explore the new science, methods, and policies that could be incorporated into emerging and future risk assessments with the primary goal of mapping a path forward. Preuss listed many questions that will need to be addressed, including, How can the new information best be incorporated into risk assessment and used to inform risk managers?

What new policies and procedures will be needed? How can EPA ensure that decision-makers, the courts, and Congress see the new approach as an acceptable way to proceed? EPA's strategy for the next generation of risk assessment is to implement the framework presented in the NRC report *Science and Decisions* (NRC 2009); to develop an operational knowledge of bioinformatics, data mining, and gene-environment databases to support risk-assessment work; and to develop prototype examples of increasingly complex assessments that are responsive to risk context and refined through discussions with scientists, risk managers, and stakeholders. Preuss concluded that EPA estimates that it may take a decade before risk assessment can rely primarily on the new advances in science, but it is necessary to begin now to address the needed changes.

Bucher continued the discussion by providing a perspective from the National Institute of Environmental Health Sciences (NIEHS) and noted that current NTP research includes many projects that are amenable to high-throughput screening and high-content data analysis. Furthermore, NTP has made a commitment to the development and use of high-throughput screening that could be used to set priorities among chemicals for further in-depth toxicologic evaluation, to identify mechanisms of toxicity, and to develop predictive models of in vivo biologic responses in humans. NTP's commitment is consistent with its vision, articulated 5 years ago, of supporting the evolution of toxicology from an observational science to a predictive one that is based on a broad array of target-specific, mechanism-based biologic observations.

Bucher noted that several people had commented during the symposium that tools need to be developed to handle the enormous volume of data being generated. He described a program at NIEHS led by Christopher Portier that is attempting to create such a tool. The approach is to use knowledge about genes associated with diseases to determine pathways linked to the genes and thus link the pathways to the diseases (that is, to elucidate "disease" pathways). The next step is to use toxicogenomic and proteomic databases on well-studied chemicals to link chemicals to diseases through pathways and then to analyze the toxicity pathways to find the best points for screening, such as critical nodes or connection points. –Omics and other molecular tools can then be used to validate the choices. What is ultimately created is an interaction network (see Figure 14). Bucher concluded that NTP expectations for the 21st century are to continue to refine traditional methods and to develop new methods to generate information on mechanisms, exposure-response relationships, and life-stage and genetic susceptibility that will allow better prediction of toxicity to humans and ultimately better protection of public health; to reconcile results from new data-rich techniques—such as genomics, proteomics, and high-throughput screens—with existing testing information for conceptual validation; and to develop approaches to accomplish formal validation of new methods for human hazard and risk estimations.

Tina Bahadori, of the American Chemistry Council Long-Range Research Initiative, continued the discussion of the future vision and path forward by pro-

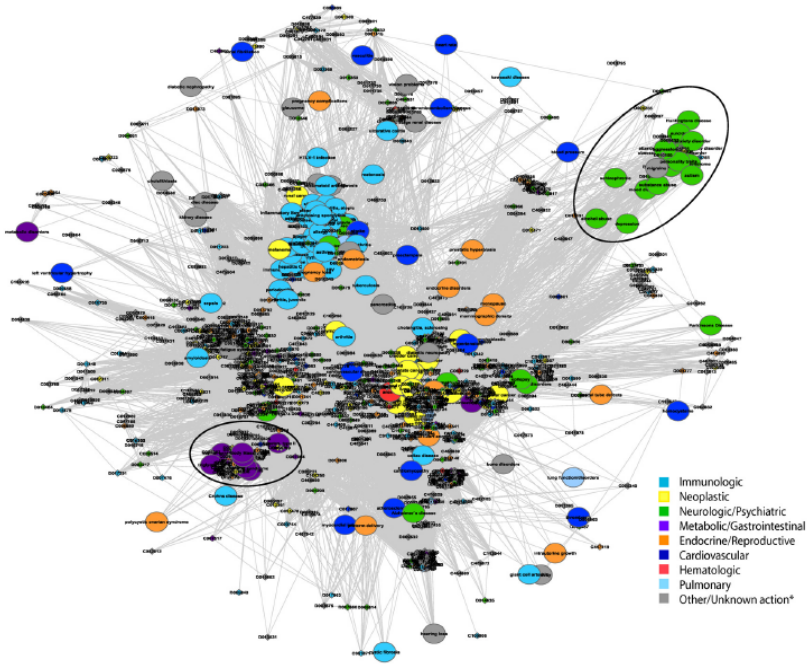

FIGURE 14 Interaction network that can be used to associate environmental factors with toxicity pathways and associated human diseases. Source: Gohlke et al. 2009. Reprinted with permission; copyright 2009, *BMC Systems Biology*. J. Bucher, National Institute of Environmental Health Sciences, presented at the symposium.

viding an industry perspective. She stated that an unprecedented opportunity exists to improve the scientific understanding of the potential effects of chemicals on human health and the environment. New technologies to test the effects of chemicals have the potential to revolutionize risk assessment, and if it is done in a scientifically robust way, risks could be understood better, faster, and less expensively. The concern, however, is that the technology is advancing faster than the means to interpret the data accurately. Although investments are made to generate volumes of data, comparable investments to interpret the data are lacking; without investment in the "science of interpretation," the tendency will be to rely on high-throughput hazard data as a surrogate for risk assessment.

Bahadori stated that scientists need to determine how information is going to be translated into a real-world context to protect public health and the environment. Information on host susceptibility and background exposures will be needed for interpretation and extrapolation of in vitro test results. Furthermore, information on actual human exposure will be needed for selection of doses for toxicity testing so that hazard information can be developed on environmentally relevant effects and for determination of whether concentrations that perturb

toxicity pathways are biologically relevant. Scientists also will need to understand the progression from exposure to effect. Exposure science will need to predict and link exposure among all levels of biologic organization and to use the new technologies to characterize exposure. Bahadori emphasized the need to invest in the new tools and technologies and to stay committed to the long-range vision, recognizing that it may be years before the new science can be used to advance risk assessment. She concluded by saying that her organization has developed a research strategy to support the vision and is investing in research on interpreting the new data being generated, on developing innovative approaches to characterize biologically relevant exposures and their relation to health risk, and on determining the genetic influences and gene-environment interactions of susceptible populations.

Roger Ulrich, of Calistoga Pharmaceuticals, provided a perspective from the pharmaceutical industry on what the future holds. The key difficulties that the pharmaceutical industry faces are late-stage failures and product withdrawals, which are extremely expensive and reduce the ability to reinvest in research; the erosion of consumer confidence in and the increased consumer expectations for product safety; the paucity of new products; and the shift of risk and product development from large pharmaceutical companies to small ones. To overcome the difficulties, Ulrich stated, the industry must focus on the pipeline and do a better job of assessing products, and this requires more thorough preclinical assessment of toxicity and more research on mechanisms and affected biologic processes or pathways. The goal is to identify prognostic biomarkers—markers that tell what might happen rather than markers that tell what *has* happened (diagnostic biomarkers). He also stated that the industry must focus on patients and identify at-risk people or populations in parallel with clinical development.

Ulrich stated that the new technologies can help to improve the processes of drug discovery and development. They can help to identify molecular pathways involved in pharmacologic and toxicologic mechanisms; this will help in making decisions as to whether to pursue specific compounds earlier in the process. The new technologies can also help to identify potential biomarkers and to detect adverse drug effects in animals that do not necessarily result in the expression of an adverse outcome in an animal model. Regarding the patient, the new technologies can be used to understand the idiosyncrasies that may increase the risk or decrease the benefit for individual patients. For example, they can be used to identify genetic defects or susceptibilities that can lead to adverse events in response to specific drugs. Thus, the vision for the drug industry is to use contemporary tools to understand the full spectrum of drug effects on biologic pathways in the preclinical phase before formal development, to overlay drug-response networks on patient phenotype and genetic networks to understand individual patient risk, and to identify benefits, as well as risks, and apply the knowledge in prescription practices. In the next 5 years, Ulrich stated, scientists will continue to develop and explore the potential of the new tools and technologies, but the excitement will be in the development of in silico models and

application of new discoveries to the clinical setting. He concluded by listing several resources needed, including an open-source genetic and functional genomic data platform, most likely funded by government, and training for the next generation of scientists.

Helmut Zarbl, of the University of Medicine and Dentistry of New Jersey, continued the discussion with an academic perspective on the future vision and discussed the NRC report *Applications of Toxicogenomic Technologies to Predictive Toxicology and Risk Assessment* (NRC 2007c), which was released shortly after *Toxicity Testing in the 21st Century*. The committee that produced the report was asked to evaluate the status of toxicogenomics and to discuss potential applications, including applications to risk assessment. It concluded that toxicogenomic technologies have strong potential to affect decision-making but are not ready to replace existing testing regimens in risk assessment and regulatory toxicology; that toxicogenomics can provide information to be added to the weight of evidence for refining risk judgments; and that ultimately the technologies are envisioned as more sensitive and informative than existing technologies and have the potential to replace some current approaches or tests. That committee recommended a human toxicogenomics initiative to accomplish the following tasks: create and manage a large public database for storing and integrating the results of toxicogenomic analysis with conventional toxicity-testing data; assemble toxicogenomic and conventional toxicologic data on hundreds of compounds into a single database; create a centralized national biorepository for human clinical and epidemiologic samples; develop bioinformatic tools—such as software, analysis, and statistical tools—further; consider ethical, legal, and social implications of collecting and using toxicogenomic data and samples; and coordinate subinitiatives to evaluate the application of toxicogenomic technologies to the assessment of risks associated with chemical exposures.

Zarbl concluded by discussing the path forward and stated that improvements in technology and science often build on previous knowledge and that scientists should not abandon the tools and knowledge of classical toxicology and risk assessment. He continued, saying that the paradigm shift will require a reduction in reliance on apical end points, and the challenge will be to validate toxicogenomic data to ensure that they are predictive of outcomes that occur much further downstream. Thus, the path for the next several years will be to develop in vitro assays, tools, and strategies; to continue to populate public databases of curated data; to invest in systems toxicology and computational tools for pathway-based risk assessment; to incorporate toxicogenomic data into the weight of evidence for risk assessment; and to continue to explore and validate the utility of the pathway-based approach for risk assessment. Further in the future, pathway-based approaches may be used for routine hazard screening of both new and legacy compounds. Zarbl again highlighted, however, the need for validation of the new approaches before they become stand-alone processes, and he cautioned that if the assays yield negative results, we need to proceed with care to ensure that nothing was missed.

Gina Solomon, of the Natural Resources Defense Council, closed with a perspective from an environmental-advocacy group and stated that she is encouraged by the research presented at the symposium. However, she noted that many people have concerns about the new direction, and some effort will be needed to persuade the broader community that the new approach has merit. The NRC report *Toxicity Testing in the 21st Century* offered the hope of rapid, cost-effective, animal-sparing testing of thousands of chemicals and a chance to intervene to prevent pathway perturbations before the occurrence of disease. Many data have been generated since publication of that report, but few of them have been incorporated or used in risk assessment, and the backlog of 80,000 chemicals remains. Solomon noted that the situation is reminiscent of EPA's Endocrine Disruptor Screening Program (EDSP), which was originally designed to screen thousands of chemicals but was delayed for more than a decade by an overcumbersome validation process. She stated that the EDSP has not lived up to its promise, and the scientific community needs to work hard not to repeat the history of that program. She acknowledged that some effort will be required to bring the new science into the process of regulatory decision-making in a timely fashion. However, the revision and reauthorization of the Toxic Substances Control Act now under way may help to facilitate the adoption of new scientific tools. Solomon concluded that at the end of the day, if the pathway-based assays are concerned only with generating more in-depth, detailed mode-of-action data on the same subset of old chemicals, the new paradigm will fail. However, if it can deal with the backlog of chemicals and foster faster, health-protective hazard identification and risk assessment, it will be heralded as an important and valuable advance.

Panel Discussion

The symposium closed with a panel discussion focused on questions about and hopes for advancing the new paradigm. Preuss expressed concern that the field is quickly becoming, if it is not already, extremely complex and that distinguishing important signals from ones that are not important is going to be challenging. Furthermore, validating the sophisticated, complex models and approaches is going to present another challenge. The new paradigm will not be successful if scientists create models that are intelligible only to a select few. The fear is that the research community is far outpacing the ability of the regulatory community to use the information that is generated and to make intelligent decisions about it. If that happens, much will fall by the wayside. The problem is that the EPA's research budgets have decreased each year to the point where EPA now has 50% of the purchasing power that it had 10 years ago. The available resources are not commensurate with the kind of effort needed.

Zarbl noted that pathway predictions at this point may be premature given that a majority of gene expression is still not understood and that huge gaps must be addressed before the data should be used in risk assessment. Ulrich agreed

that huge gaps exist but stated that the gaps are being systematically filled. His concern was that realistic expectations be set. For example, it is unrealistic to expect that in 10 years scientists will be able to screen 200,000 chemicals in cell cultures and know the human risks on the basis of the resulting data. Bahadori added that the paradigm will fail if perfection of the science is required. Information that can inform and improve risk assessment will be available soon, if it is not already available. The scientific community needs to determine the level of knowledge needed for different purposes, that is, what is good enough for a given task. Bahadori expressed concern, however, about funding and emphasized the need to articulate the value of the current effort to ensure that resources are available. The scientific community needs to create a compelling case for requesting resources.

Bucher remarked on the need expressed throughout the symposium for creating a large, publicly accessible data platform and the need for a concerted effort to overcome the inertia that might exist in creating such a platform. He stated that expectations for the new science and approaches will not be fulfilled if the necessary computational and analytic tools are not made available. Zarbl clarified that what is needed is a platform where data are curated; standards would have to be met to enter data so that they are standardized or uniform. What is being proposed is not simply a data repository.

The question arose about what must be accomplished in the near term to illustrate the value of the new science. Bucher commented that the results of high-throughput screening should be used to design and guide current toxicity testing. The resulting data can help to move standard toxicology models forward. Zarbl stated that studies that demonstrate that pathway-based risk assessment can produce results at least as good as standard approaches (such as the studies described by Thomas earlier in the symposium) are the milestones needed. Ulrich noted that high priority should be given to a concerted effort to pull together the existing knowledge to create a cohesive and comprehensive output. Kenny Crump, of Environ, concurred: the key is to start applying and using the data and comparing the results with those of the current path. That would illuminate the gaps and help to differentiate between data that EPA might need and data that some other application would need.

Hal Zenick, of EPA, commented that application of the new approaches may depend on what information is needed. One size does not fit all risk assessments. For example, the information needed to make a clean-up decision will be quite different from that needed to set exposure guidelines on a nationally pervasive bioaccumulative chemical. The nature of the question that needs to be answered may determine how soon the new approaches can be applied to risk assessment and risk management. Whenever the new science is finally used to make risk-assessment and risk-management decisions, several noted, a major challenge will be risk communication, that is, explaining the new approaches to policy-makers and the public.

REFERENCES

Amundson, S.A., K.T. Do, L.C. Vinikoor, R.A. Lee, C.A. Koch-Paiz, J. Ahn, M. Reimers, Y. Chen, D.A. Scudiero, J.N. Weinstein, J.M. Trent, M.L. Bittner, P.S. Meltzer, and A.J. Fornace, Jr. 2008. Integrating global gene expression and radiation survival parameters across the 60 cell lines of the National Cancer Institutes anticancer drug screen. Cancer Res. 68(2): 415-424.

Ashburner, M., C.A. Ball, J.A. Blake, D. Botstein, H. Butler, J.M. Cherry, A.P. Davis, K. Dolinski, S.S. Dwight, J.T. Eppig, M.A. Harris, D.P. Hill, L. Issel-Tarver, A. Kasarskis, S. Lewis, J.C. Matese, J.E. Richardson, M. Ringwald, G.M. Rubin, and G. Sherlock. 2000. Gene ontology: Tool for the unification of biology. Nat Genet. 25(1):25-29.

Beck, M.B. 2002. Model evaluation and performance. Pp. 1275-1279 in Encyclopedia of Environmetrics, A.H. El-Shaarawi, and W.W. Piegorsch, eds. Chichester, UK: Wiley.

Begley, T.J., A.S. Rosenback, T. Ideker, and L.D. Samson. 2002. Damage recovery pathways in Saccharomyces cerevisiae revealed by genomic phenotyping and interactome mapping. Mol. Cancer Res. 1(2):103-112.

Bucher, J.R., and C. Portier. 2004. Human carcinogenic risk evaluation, Part V: The national toxicology program vision for assessing the human carcinogenic hazard of chemicals. Toxicol. Sci. 82(2): 363-366.

Collins, F.S., G.M. Gray, and J.R. Bucher. 2008. Toxicology. Transforming environmental health protection. Science 319(5865):906-907.

EC (European Commission). 2007. REACH in Brief. European Commission [online]. Available: http://ec.europa.eu/environment/chemicals/reach/pdf/2007_02_reach_in_brief.pdf [accessed Apr. 30, 2010].

EPA (U.S. Environmental Protection Agency). 2009. The U.S Environmental Protection Agency's Strategic Plan for Evaluating the Toxicity of Chemicals. EPA/100/K-09/001. Office of the Science Advisor, Science Policy Council, U.S. Environmental Protection Agency, Washington, DC. March 2009 [online]. Available: http://www.epa.gov/spc/toxicitytesting/docs/toxtest_strategy_032309.pdf [accessed Apr. 30, 2010].

Fry, R.C., T.J. Begley, and L.D. Samson. 2005. Genome-wide responses to DNA-damaging agents. Annu. Rev. Microbiol. 59:357-377.

Gohlke, J.M., R. Thomas, Y. Zhang, M. Rosenstein, A.P. Davis, C. Murphy, K.G. Becker, C.J. Mattingly, and C.J. Portier. 2009. Genetic and environmental pathways to complex diseases. BMC Systems Biology 3:46.

Hartung, T., and M. Leist. 2008. Food for thought…on the evolution of toxicology and the phasing out of animal testing. ALTEX 25(2):91-96.

Johnson, C.D., Y. Balagurunathan, K.P. Lu, M. Tadesse, M.H. Falahatpisheh, R.J. Carroll, E.R. Dougherty, C.A. Afshari, and K.S. Ramos. 2003. Genomic profiles and predictive biological networks in oxidant-induced atherogenesis. Physiol. Genomics 13(3):263-275.

Johnson, C.D., Y. Balagurunathan, M.G. Tadesse, M.H. Falahatpisheh, M. Brun, M.K. Walker, E.R. Dougherty, and K.S. Ramos. 2004. Unraveling gene-gene interactions regulated by ligands of the aryl hydrocarbon receptor. Environ. Health Perspect. 112(4):403-412.

Judson, R., A. Richard, D.J. Dix, K. Houck, M. Martin, R. Kavlock, V. Dellarco, T. Henry, T. Holderman, P. Sayre, S. Tan, T. Carpenter, and E. Smith. 2009. The tox-

icity data landscape for environmental chemicals. Environ. Health Perspect. 117(5):685-695.

Lu, K.P., L.M. Hallberg, J. Tomlinson, and K.S. Ramos. 2000. Benzo(a)pyrene activates L1Md retrotransposon and inhibits DNA repair in vascular smooth muscle cells. Mutat. Res. 454(1-2): 35-44.

NRC (National Research Council). 2000. Scientific Frontiers in Developmental Toxicology and Risk Assessment. Washington, DC: National Academy Press.

NRC (National Research Council). 2007a. Toxicity Testing in the 21st Century: A Vision and a Strategy. Washington, DC: National Academies Press.

NRC (National Research Council). 2007b. Models in Environmental Regulatory Decision Making. Washington, DC: National Academies Press.

NRC (National Research Council). 2007c. Applications of Toxicogenomic Technologies to Predictive Toxicology and Risk Assessment. Washington, DC: National Academies Press.

NRC (National Research Council). 2008. Phthalates and Cumulative Risk Assessment: The Tasks Ahead. Washington, DC: National Academies Press.

NRC (National Research Council). 2009. Science and Decisions: Advancing Risk Assessment. Washington, DC: National Academies Press.

Oberdörster, G., E. Oberdörster, and J. Oberdörster. 2005. Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles. Environ. Health Perspect. 113(7):823-839.

Robinson, J.F., X. Yu, S. Hong, C. Zhou, N. Kim, D. DeMasi, and E.M. Faustman. 2010. Embryonic toxicokinetic and dynamic differences underlying strain sensitivity to cadmium during neurulation. Reprod. Toxicol. 29(3):279-285.

Rudén, C. 2001. The use and evaluation of primary data in 29 trichloroethylene carcinogen risk assessments. Regul. Toxicol. Pharmacol. 34(1):3-16.

Stratton, M.R., P.J. Campbell, and P.A. Futreal. 2009. The cancer genome. Nature 458(7239):719-724.

Teeguarden, J.G., P.M. Hinderliter, G. Orr, B.D. Thrall, and J.G. Pounds. 2007. Particokinetics in vitro: Dosimetry considerations for in vitro nanoparticle toxicity assessments. Toxicol. Sci. 95(2):300-312.

Waters, K.M., L.M. Masiello, R.C. Zangar, B.J. Tarasevich, N.J. Karin, R.D. Quesenberry, S. Bandyopadhyay, J.G. Teeguarden, J.G. Pounds, and B.D. Thrall. 2009. Macrophage responses to silica nanoparticles are highly conserved across particle sizes. Toxicol. Sci. 107(2):553-569.

Yu, X., W.C. Griffith, K. Hanspers, J.F. Dillman III, H. Ong, M.A. Vredevoogd, and E.M. Faustman. 2006. A system based approach to interpret dose and time-dependent microarray data: Quantitative integration of gene ontology analysis for risk assessment. Toxicol. Sci. 92(2):560-577.

Yu, X., S. Hong, E.G. Moreira, and E.M. Faustman. 2009. Improving in vitro Sertoli cell/gonocyte co-culture model for assessing male reproductive toxicity: Lessons learned from comparisons of cytotoxicity versus genomic responses to phthalates. Toxicol. Appl. Pharmacol. 239(3):325-336.

Yu, X., J.F. Robinson, J.S. Sidhu, S. Hong, and E.M. Faustman. 2010. A system-based comparison of gene expression reveals alterations in oxidative stress, disruption of ubiquitin-proteasome system and altered cell cycle regulation after exposure to cadmium and methylmercury in Mouse Embryonic Fibroblast (MEF). Toxicol. Sci. 114(2):356-377.

Appendixes

Appendix A

Biographic Information on the Standing Committee on Risk Analysis Issues and Reviews

Bernard D. Goldstein (*Chair*) is dean of the University of Pittsburgh Graduate School of Public Health. Previously, he served as the director of the Environmental and Occupational Health Sciences Institute, a joint program of Rutgers, the State University of New Jersey, and the University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School. He was also principal investigator for the Consortium of Risk Evaluation with Stakeholder Participation. Dr. Goldstein was assistant administrator for research and development in the U.S. Environmental Protection Agency (EPA) in 1983-1985. His past activities include serving as a member and chair of the National Institutes of Health Toxicology Study Section and EPA's Clear Air Scientific Advisory Committee. He has also served on numerous National Research Council and Institute of Medicine (IOM) committees, including being chair of the Committee on the Role of the Physician in Occupational and Environmental Medicine, the Committee on Biomarkers in Environmental Health Research, and the Committee on Risk Assessment Methodology. He chairs the Committee to Review the NIOSH Hearing Loss Research Program. Dr. Goldstein is a member of IOM and chaired its Section on Public Health, Biostatistics, and Epidemiology. He is a member and past president of the Society for Risk Analysis. He is a member and fellow of the American College of Environmental and Occupational Medicine (whose Robert A. Kehoe Award of Merit he has received) and a member of the Collegium Ramazzini, the Society for Occupational and Environmental Health, the Society of Toxicology, and the American Public Health Association. Dr. Goldstein earned an MD from New York University.

Frederic Bois is scientific officer for the Chronic Risks Division of the Institut National de l'Environnement Industriel et des Risques (INERIS) in France. Dr. Bois's expertise is in the development, statistical analysis, and application of physiologically based pharmacokinetic models; Bayesian analysis of toxicologic

or epidemiologic data; dose-response modeling; decision analysis; and risk assessment. Dr. Bois has been head of the toxicology unit at INERIS and staff scientist at the Lawrence Berkeley National Laboratory in California. He is a member of the French Committee for Precaution and Prevention, the Scientific Committee of the French Agency for Environmental and Occupational Health, the Society for Mathematical Biology, the European Science Foundation-EERO Association, the American Chemical Society, the French Statistical Society, and the French National Association for Technological Research. He has served as a reviewer for a number of scientific journals—including *Environmental Health Perspectives*, *Risk Analysis*, the *Journal of Pharmacokinetics and Biopharmaceutics*, the *Human and Ecological Risk Assessment Journal*, and *Toxicology and Industrial Health*—and for the National Science Foundation. Dr. Bois earned a PhD in pharmacy from the University of Nancy and a PhD in toxicology from the University of Metz, both in France.

Michael Brauer is a professor and director of the School of Occupational and Environmental Hygiene of the University of British Columbia. His research interests include assessment of exposure to air pollutants, application of advanced exposure techniques to assess the health effects of air pollution, air pollution from mobile sources and vegetation fires, and air quality and health in developing countries. He has been a member of National Research Council and Institute of Medicine (IOM) committees, including the IOM Committee on Gulf War and Health: Literature Review of Selected Environmental Particulates, Pollutants, and Synthetic Chemical Compounds and the National Research Council Committee to Review NARSTO's Scientific Assessment of Airborne Particulate Matter. Dr. Brauer received his ScD from the Harvard School of Public Health.

Richard Corley is laboratory fellow in the biologic monitoring and biologic modeling group at the Pacific Northwest Laboratory operated by Battelle for the U.S. Department of Energy in Richland, WA. Dr. Corley specializes in the development of physiologically based pharmacokinetic models, real-time breath analysis, dermal and inhalation bioavailability, and the development of three-dimensional computational fluid-dynamic models of the respiratory system. He has published numerous peer-reviewed papers on oral, dermal, and inhalation toxicology; modes of action of a variety of industrial and consumer chemicals; and pharmacokinetic modeling and its applications in human health risk assessments. Dr. Corley served on the National Research Council Committee to Assess the Health Implications of Perchlorate Ingestion. He received a PhD in environmental toxicology from the University of Illinois at Urbana-Champaign.

Linda Cowan is George Lynn Cross Professor in the Department of Biostatistics and Epidemiology at the University of the Oklahoma Health Sciences Center. Her research interests include cardiovascular disease and the relative importance of risk factors in American Indian men and women, neurologic disorders, and perinatal epidemiology. Her recent research includes analysis of risk-factor

profiles for early-onset and late-onset coronary heart disease in American Indians, investigation of the role of environmental toxicants and congenital hearing loss, and studies in west Africa of the prevalence of and risk factors for epilepsy associated with neurocysticercosis. Dr. Cowan has served on several National Research Council and Institute of Medicine (IOM) committees, including the Committee to Assess the Health Implications of Perchlorate Ingestion and the Committee to Assess the Safety and Efficacy of the Anthrax Vaccine, and she is a member of the IOM Board on Military and Veterans Health. She earned a PhD in epidemiology from Johns Hopkins University.

Kenny S. Crump is a principal with ENVIRON. He has over 25 years of experience in assessing risk related to exposure to toxic materials. Statistical models for assessing risk developed by Dr. Crump have been widely used by regulatory agencies and private groups. He has served on science advisory boards of the Environmental Protection Agency (EPA), the National Center for Toxicological Research, the Mickey Leland National Urban Air Toxics Research Center, and the National Institute of Environmental Health Sciences and on several committees of the National Research Council, including the Committee on Risk Assessment Methodology and the Committee on Institutional Means for Assessment of Risks to Public Health. He has experience in assessing risk posed by exposure to many toxic substances, including asbestos and dioxin. Dr. Crump was a member of the EPA Science Advisory Board Dioxin Reassessment Review Committee on two occasions and was an adviser to the Food and Agriculture Organization and World Health Organization Committee on Food Additives that assessed risk posed by dioxin in food. He earned a PhD in mathematics from Montana State University.

Lynn R. Goldman, a pediatrician and an epidemiologist, is a professor at the Johns Hopkins University Bloomberg School of Public Health, where she focuses on environmental health policy, public-health practice, and children's environmental health. Dr. Goldman previously served as assistant administrator for the Environmental Protection Agency (EPA) Office of Prevention, Pesticides and Toxic Substances. During her tenure at EPA, Dr. Goldman was responsible for the nation's pesticide, toxic-substances, and pollution-prevention laws and was successful in promoting children's health issues and furthering the international agenda for global chemical safety. Before joining EPA, Dr. Goldman served in several positions in the California Department of Health Services, most recently as head of the Division of Environmental and Occupational Disease Control. She has served on numerous boards and expert committees, including the Committee on Environmental Health of the American Academy of Pediatrics and the Centers for Disease Control and Prevention Advisory Committee on Childhood Lead Poisoning Prevention. She has served on numerous National Research Council committees, including the Committee on Identifying and Assessing Unintended Effects of Genetically Engineered Foods on Human Health, the Committee on Clinical Trial Registries, and the Committee to Evalu-

ate the Hazardous Materials Management Program of the Bureau of Land Management. She is vice chair of the Institute of Medicine (IOM) Roundtable on Environmental Health Sciences and chair of the IOM Gulf War and Health study. Dr. Goldman received her MD from the University of California, San Francisco.

Philip J. Landrigan is director of the Center for Children's Health and the Environment, Ethel H. Wise Professor and chair of the Department of Community and Preventive Medicine, and director of environmental and occupational medicine at Mount Sinai School of Medicine. He also holds a professorship in pediatrics at Mount Sinai. From 1970 to 1985, Dr. Landrigan served as a commissioned officer in the U.S. Public Health Service, where he served as an Epidemic Intelligence Service officer and then as a medical epidemiologist with the Centers for Disease Control and Prevention (CDC). At CDC, Dr. Landrigan participated in epidemiologic studies of measles and rubella, directed research and developed activities for the Global Smallpox Eradication Program, and established and directed the Environmental Hazards Branch of the Bureau of Epidemiology. From 1979 to 1985, as director of the Division of Surveillance, Hazard Evaluations, and Field Studies of the National Institute for Occupational Safety and Health, he directed the U.S. national program in occupational epidemiology. He is editor-in-chief of the *American Journal of Industrial Medicine* and previously was editor of *Environmental Research*. From 1995 to 1997, Dr. Landrigan served on the Presidential Advisory Committee on Gulf War Veterans' Illnesses. From 1997 to 1998, he served as senior adviser to the administrator of the Environmental Protection Agency (EPA) on children's health. He is a member of the Institute of Medicine (IOM) and has served on numerous National Research Council and IOM committees, including the Committee on Pesticides in the Diets of Infants and Children, the committee for the Conference on Human Health and Global Climate Change, the Committee on Biologic Markers, and the Committee on Neurotoxicology and Models for Assessing Risk. Dr. Landrigan earned an MD from Harvard Medical School and a DIH from the London School of Hygiene & Tropical Medicine.

Thomas A. Louis is professor of biostatistics at the Johns Hopkins Bloomberg School of Public Health. His research interests include risk assessment, environmental health and public policy, and development of related statistical approaches. He is a fellow of the American Statistical Association and of the American Association for the Advancement of Science. Dr. Louis serves on the Health Review Committee of the Health Effects Institute and on the Environmental Protection Agency's Science Advisory Board Drinking Water Committee. Previous and current National Research Council and Institute of Medicine (IOM) service includes membership on the Committee on the Effects of Changes in New Source Review Programs for Stationary Sources of Air Pollutants, the Committee on Applied and Theoretical Statistics, the Committee on National Statistics, the board of IOM's Medical Follow-up Agency, the Panel to Assess the Health Consequences of Service in the Persian Gulf War, the Panel

on Estimates of Poverty for Small Geographic Areas, and the Committee on the Use of Third-Party Toxicity Research with Human Research Participants. Dr. Louis earned a PhD in mathematical statistics from Columbia University.

Nu-May Ruby Reed is a staff toxicologist with the California Environmental Protection Agency (Cal/EPA) Department of Pesticide Regulation, where she is the lead person on risk-assessment issues in the Health Assessment Section. Her research interests are in evaluating health risks and developing dietary-assessment guidelines for pesticides. She has been on several Cal/EPA working groups that initiate, research, and revise risk-assessment guidelines and policies. Dr. Reed represented her department in task forces on community concerns and emergency response, risk-management guidance, and public education. She is also a lecturer on health risk assessment at the University of California, Davis. Dr. Reed served on the National Research Council Subcommittee on Fluoride in Drinking Water. She received her PhD from the University of California, Davis and is a diplomate of the American Board of Toxicology.

Lorenz Rhomberg is a principal with Gradient Corporation. He is an expert in quantitative risk assessment, including dose-response analysis, pharmacokinetic modeling, and probabilistic methods, with special expertise in chlorinated solvents and endocrine-active agents. Before joining Gradient, Dr. Rhomberg was on the faculty of the Harvard School of Public Health and at the Environmental Protection Agency. Dr. Rhomberg is active in professional groups and environmental policy development, focusing on the interpretation of toxicologic data in human health risk assessment through service on panels sponsored by government, industry, and such organizations as the International Life Sciences Institute. He has served on several National Research Council committees, including the Committee on Assuring the Safety of the Defense Department's Mail, the Committee on Testing and Evaluation of Standoff Chemical Agent Detectors, and the Subcommittee on Manufactured Vitreous Fibers. Dr. Rhomberg earned his PhD in population biology from the State University of New York at Stony Brook.

Joyce Tsuji is a principal with Exponent's Health Sciences Practice and is a board-certified toxicologist with 19 years of experience in toxicology and risk assessment on projects in the United States and internationally for corporations, trade associations, regulatory agencies, and state and local municipalities. Her research interests include exposure assessment and toxicology of a variety of chemicals, including those from industrial releases and in consumer products. She also has experience in design and direction of exposure studies involving health education, environmental sampling, and biomonitoring of populations potentially exposed to chemicals in soil, water, and the food chain. Dr. Tsuji has served on expert committees for the National Research Council, the Environmental Protection Agency, the U.S. Army, and the state of Washington. Her Research Council service has included membership on the Committees on Cop-

per in Drinking Water, Spacecraft Exposure Guidelines, Emergency and Continuous Exposure Guidance Levels for Selected Submarine Contaminants, and Submarine Escape Action Levels. She is currently serving on the Committee on Toxicology and the Standing Committee on Risk Analysis Issues and Reviews. She received her PhD with a focus in physiology and ecology from the University of Washington.

Appendix B

Biographic Information on the Planning Committee for a Symposium on Toxicity-Pathway-Based Risk Assessment

Lorenz R. Rhomberg (*Chair*) is a principal with Gradient Corporation, an environmental sciences consulting firm. He is an expert in quantitative risk assessment, including dose-response analysis, pharmacokinetic modeling, and probabilistic methods, with special expertise in chlorinated solvents. His focus includes science policy and methodology for human health risk assessment, including approaches to weight of toxicologic evidence for human hazard and cross-species extrapolation. Before joining Gradient, Dr. Rhomberg was on the faculty of the Harvard School of Public Health and also worked at the U.S. Environmental Protection Agency. Dr. Rhomberg is active in professional groups and environmental policy development, focusing on current issues in the interpretation of toxicologic data in human health risk assessment through service on panels sponsored by government, industry, and such organizations as the International Life Sciences Institute. He has served on several National Research Council committees, including the Committee on Assuring the Safety of the Defense Department's Mail, the Committee on Testing and Evaluation of Standoff Chemical Agent Detectors, and the Subcommittee on Manufactured Vitreous Fibers, and he is a member of the Standing Committee on Risk Analysis Issues and Reviews. Dr. Rhomberg earned his PhD in population biology from the State University of New York at Stony Brook.

Elaine M. Faustman is a professor of environmental and occupational health sciences at the University of Washington School of Public Health and Community Medicine and directs the Institute for Risk Analysis and Risk Communication. Her research interests include understanding mechanisms of developmental and reproductive toxicants, characterizing in vitro techniques for developmental-toxicology assessment, and developing biologically based dose-response models for noncancer risk assessment. Her research expertise includes the development of tools for incorporating new scientific findings into risk-assessment decisions.

Dr. Faustman is an elected fellow of the American Association for the Advancement of Science and of the Society for Risk Analysis. She has also been involved in several National Research Council committees, including the Committee on Spacecraft Exposure Guidelines, the Committee on Developmental Toxicology, and the Committee on Toxicology. Dr. Faustman received a PhD in toxicology from Michigan State University.

Lynn R. Goldman, a pediatrician and epidemiologist, is a professor at the Johns Hopkins University Bloomberg School of Public Health, where she focuses on environmental health policy, public-health practice, and children's environmental health. Dr. Goldman previously served as the assistant administrator for the U.S. Environmental Protection Agency (EPA) Office of Prevention, Pesticides and Toxic Substances. During her tenure at EPA, Dr. Goldman was responsible for the nation's pesticide, toxic-substances, and pollution-prevention laws, and she was successful in promoting children's health issues and furthering the international agenda for global chemical safety. Before joining EPA, Dr. Goldman served in several positions in the California Department of Health Services, most recently as head of the Division of Environmental and Occupational Disease Control. She has served on numerous boards and expert committees, including the Committee on Environmental Health of the American Academy of Pediatrics and the Centers for Disease Control and Prevention Advisory Committee on Childhood Lead Poisoning Prevention. She has also served as a member of numerous National Research Council (NRC) committees, including the Committee on Identifying and Assessing Unintended Effects of Genetically Engineered Foods on Human Health, the Committee on Clinical Trial Registries, and the Committee to Evaluate the Hazardous Materials Management Program of the Bureau of Land Management. She is vice chair of the Institute of Medicine (IOM) Roundtable on Environmental Health Sciences, chair of the IOM Committee on Secondhand Smoke Exposure and Acute Coronary Events, and a member of the NRC Standing Committee on Risk Analysis Issues and Reviews. Dr. Goldman received her MD from the University of California, San Francisco.

Michael Lawton is an associate research fellow and head of the Molecular Toxicology Group at Pfizer in Groton, CT. His molecular-toxicology laboratory applies molecular approaches in support of predictive toxicology, identifies molecular biomarkers, and investigates mechanistic toxicology for early- and late-stage safety assessment. Dr. Lawton participates in the Vascular Injury Working Group of the external Predicative Safety Testing Consortium and supports the Internal Hepatic and Vascular Injury Project teams. He is also the principal investigator for several external technology collaborations. He is a member of the Society of Toxicology and councilor of its Drug Discovery Toxicology Specialty Section. Dr. Lawton received a PhD in toxicology from North Carolina State University.

George D. Leikauf is a professor in the Department of Environmental and Occupational Health of the University of Pittsburgh. His research investigates the functional genomics of acute lung injury, asthma, and chronic obstructive pulmonary disease. He is interested in uncovering the genetic basis of increased susceptibility to pulmonary epithelial injury and repair and in examining the transcriptional regulation of molecular targets. Dr. Leikauf has served on numerous national and international committees, including the National Institutes of Health's National Advisory Environmental Health Sciences Council and Center for Scientific Review Advisory Committee, the American Physiological Society's Perkins Memorial Fund Committee, the American Thoracic Society's Program Committee, and the International Advisory Committee for the 8th and 9th Inhalation Symposiums. He has also served as a member of the National Research Council Committee on Applications of Toxicogenomics Technologies to Predictive Toxicology. Dr. Leikauf received a PhD in environmental health sciences from the New York University Medical Center.

Joel G. Pounds is a senior staff scientist in cell biology and biochemistry in the Biological Sciences Division and science adviser to the Environmental Biomarkers Initiative of the Pacific Northwest National Laboratory (PNNL) and director of the Center for Novel Biomarkers of Response Genes (Genes and Environment Initiative, Exposure Biology Program) funded by the National Institutes of Health. Dr. Pounds has directed research programs in government (at the National Center for Toxicological Research, 1977-1985), national laboratories (at Brookhaven National Laboratory, 1985-1990), and academe (at Wayne State University, 1990-1999). His current research includes the use of mass-spectrometry-based proteomic and nuclear-magnetic-resonance-based metabonomic instrumentation for characterization of biologic responses of pulmonary disease in humans and in experimental animals, including response to engineered nanomaterials. Dr. Pounds received a PhD in environmental toxicology from the University of Wisconsin.

Joyce S. Tsuji is a principal with Exponent's Health Sciences Practice and is a board-certified toxicologist and fellow of the Academy of Toxicological Sciences. She has over 20 years of experience in toxicology and risk-assessment projects in the United States and internationally for corporations, trade associations, regulatory agencies, and state and local municipalities. Particular interests include exposure assessment and the toxicology of a variety of chemicals, including those from industrial releases and in consumer products. She has specific experience in design and direction of exposure studies involving health education, environmental sampling, and biomonitoring of populations potentially exposed to chemicals in soil, water, and the food chain. Dr. Tsuji has served on expert committees for the National Research Council, the Environmental Protection Agency, the U.S. Army, and the state of Washington. Her Research Council service has included membership on the Committees on Copper in Drinking Water, Spacecraft Exposure Guidelines, Emergency and Continuous Exposure Guidance Levels for Selected

Submarine Contaminants, and Submarine Escape Action Levels. She is currently serving on the Committee on Toxicology and the Standing Committee on Risk Analysis Issues and Reviews. Dr. Tsuji received her PhD with a focus in physiology and ecology from the University of Washington.

Lauren Zeise is chief of the Reproductive and Cancer Hazard Assessment Branch of the California Environmental Protection Agency. She oversees a variety of risk-assessment activities, including development of approaches for assessing cumulative impacts, nanotechnology, green chemistry, safer alternatives, susceptible populations; cancer and reproductive-toxicant assessments; health risk characterizations for environmental media, food, and consumer products; and her department's biomonitoring activities. Dr. Zeise has served on advisory boards of the U.S. Environmental Protection Agency (EPA), the World Health Organization, the Office of Technology Assessment, and the National Institute of Environmental Health Sciences. She has also served on the National Research Council (NRC) Board on Environmental Studies and Toxicology, the Institute of Medicine Board of Health Promotion and Disease Prevention and Committee on Evaluation of Presumptive Disability Decision-Making Process for Veterans, and several NRC committees, including the Committee on Toxicity Testing and Assessment of Environmental Agents, the Committee on Improving Risk Analysis Approaches Used by U.S. EPA, the Committee on Risk Characterization, and the Committee on Comparative Toxicology of Naturally Occurring Carcinogens. She is a member, fellow, and councilor of the Society of Risk Analysis and received the society's Outstanding Risk Practitioner Award in 2008. Dr. Zeise received her PhD from Harvard University.

Appendix C

Symposium Agenda

**TOXICITY-PATHWAY-BASED RISK ASSESSMENT:
PREPARING FOR PARADIGM CHANGE**

Public Meeting: May 11-13, 2009
National Academy of Sciences
2101 Constitution Avenue, NW
Washington, DC 20418

MONDAY, MAY 11, 2009

A PARADIGM CHANGE ON THE HORIZON

8:30 Welcome and Introduction

Warren Muir
Executive Director, Division on Earth and Life Studies
National Academies

8:35 Background on Standing Committee on Risk Analysis Issues and Reviews

Bernard Goldstein
Chair, Standing Committee on Risk Analysis Issues and Reviews
Professor, Department of Environmental and Occupational Health
University of Pittsburgh

8:40 EPA Expectations on Symposium

Peter Preuss
Director, National Center for Environmental Assessment
U.S. Environmental Protection Agency

8:45 Making Risk Assessment More Useful to Public and Private Decision-Makers in an Era of Paradigm Change

E. Donald Elliott
Member, Board on Environmental Studies and Toxicology
National Academies
Former EPA General Counsel

9:15 Emerging Science and Public Health

Lynn Goldman
Member, Symposium Planning Committee
Member, Standing Committee on Risk Analysis Issues and Reviews
Professor and Chair, Environmental Health Sciences
Johns Hopkins Bloomberg School of Public Health

9:45 Toxicity Testing in the 21st Century

Kim Boekelheide
Professor of Medical Sciences
Brown University

10:15 Symposium Roadmap

Lorenz Rhomberg
Chair, Symposium Planning Committee
Member, Standing Committee on Risk Analysis Issues and Reviews
Principal, Gradient Corporation

10:45 Break

THE NEW SCIENCE

Moderator: Lorenz Rhomberg, Chair, Symposium Planning Committee; Member, Standing Committee on Risk Analysis Issues and Reviews; Principal, Gradient Corporation

11:00 Overview of New Science

John Groopman
Anna M. Baetjer Professor of Environmental Health and Chair
Department of Environmental Health Sciences
Johns Hopkins Bloomberg School of Public Health

11:30 Gene-Environment Interactions

George Leikauf
Member, Symposium Planning Committee
Professor, University of Pittsburgh

12:00 Tools and Technologies for Pathway-Based Research

Ivan Rusyn
Associate Professor, Department of Environmental Science and Engineering
University of North Carolina at Chapel Hill

12:30 Lunch

PATHWAY-BASED APPROACHES FOR HAZARD ID

Moderator: Michael Lawton, Member, Symposium Planning Committee; Associate Research Fellow, Head, Molecular Toxicology Group, Pfizer, Inc.

1:30 ToxCast: Redefining Hazard Identification

Robert Kavlock
Director, National Center for Computational Toxicology
U.S. Environmental Protection Agency

2:00 Practical Applications: Pharmaceuticals

William Pennie
Executive Director, Compound Safety Prediction
Worldwide Medicinal Chemistry
Pfizer Global Research and Development

2:30 Practical Applications: Consumer Products

George Daston
Research Fellow
Procter & Gamble Company

3:00 Break

3:15 Practical Applications: Mixtures

John Groten
Vice President, Drug Safety Europe
Schering-Plough

3:45 Pathway-Based Approaches: A European/REACH Perspective

Thomas Hartung
Director, Center for Alternatives to Animal Testing
Johns Hopkins University

4:15 Panel Discussion

Panelists: Speakers and invited panelists (Charles Auer, former Director, Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency (retired); David Jacobson-Kram, Associate Director for Pharmacology and Toxicology, U.S. Food and Drug Administration)

5:00 Break

5:30 Poster Session

7:00 Adjourn

TUESDAY, MAY 12, 2009

APPLICATION TO MODE-OF-ACTION ANALYSIS

Moderator: Lauren Zeise, Member, Symposium Planning Committee; Chief, Reproductive and Cancer Hazard Assessment Section, California Environmental Protection Agency

8:30 What is Required for Acceptance

John Bucher
Associate Director, National Toxicology Program
National Institute of Environmental Health Sciences

9:00 Environmental Disease: Evaluation at the Molecular Level

Kenneth Ramos
Distinguished Professor, Department of Biochemistry and Molecular Biology
University of Louisville Health Science Center

9:30 Dioxin: Evaluation of Pathways at the Molecular Level

Alvaro Puga
Professor
University of Cincinnati

10:00 Systems Level Approaches for Understanding Principals of Nanomaterial Biocompatibility

Brian Thrall
Technical Group Leader, Cell Biology Group
Pacific Northwest National Laboratory

10:30 Break

11:00 Panel Discussion

Panelists: Speakers and invited panelists (William Kaufmann, Professor of Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill; Jonathan Wiener, Perkins Professor of Law and Professor of Environmental Policy & Public Policy Studies, Duke University; Past President, Society for Risk Analysis)

12:00 Lunch

CHALLENGES AND OPPORTUNITIES FOR RISK ASSESSMENT IN THE CHANGING PARADIGM

Moderator: Joel Pounds, Member, Symposium Planning Committee; Senior Staff Scientist, Advisor to the Environmental Biomarkers Initiative, Pacific Northwest National Laboratory

1:00 Dose and Temporal Response

Elaine Faustman
Member, Symposium Planning Committee
Professor, Environmental and Occupational Health
University of Washington

1:30 Application of Genomic Dose-Response Data to Define Mode-of-Action and Low-Dose Behavior of Chemical Toxicants

Rusty Thomas
Director, Functional Genomics
The Hamner Institutes for Health Sciences

2:00 Using PBPK Models to Interpret Dose-Response of –Omics Data

Gregory L. Kedderis
Independent Researcher and Consultant
Chapel Hill, North Carolina

2:30 Modular Network Modeling of Toxicity Pathways for Extrapolation

Katrina Waters
Senior Research Scientist, Computational Biology & Bioinformatics
Pacific Northwest National Laboratory

3:00 Break

3:15 Integrating Global Gene Expression and Survival Across 60 Cell Lines: Applications for Risk Assessment

Albert J. Fornace, Jr.
Professor, Department of Biochemistry and Molecular and Cellular Biology, Biomedical Graduate Research Organization; Department of Oncology, Lombardi Comprehensive Cancer Center
Georgetown University

3:45 The FDA Experience in Analyzing Genomic Data

Federico Goodsaid
Associate Director for Operations in Genomics
U.S. Food and Drug Administration

4:15 Panel Discussion

5:30 Adjourn

WEDNESDAY, MAY 13, 2009

WHAT THE FUTURE HOLDS

Moderator: Lorenz Rhomberg, Chair, Symposium Planning Committee; Member, Standing Committee on Risk Analysis Issues and Reviews; Principal, Gradient Corporation

8:30 Are we "there" yet and Where is "there"?
The Path Forward (1, 5, and 10 years in the future)

A Perspective from EPA
Peter Preuss
Director, National Center for Environmental Assessment
U.S. Environmental Protection Agency

A Perspective from NIEHS
John Bucher
Associate Director, National Toxicology Program
National Institute of Environmental Health Sciences

A Perspective from the Chemical Industry
Tina Bahadori
Managing Director Long Range Research Initiative
American Chemistry Council

A Perspective from the Pharmaceutical Industry
Roger Ulrich
Chief Development Officer
Calistoga Pharmaceuticals, Inc.

A Perspective from Academia
Helmut Zarbl
Professor of Environmental and Occupational Medicine
University of Medicine and Dentistry of New Jersey

A Perspective from an Environmental Advocacy Group
Gina Solomon
Senior Scientist
Natural Resources Defense Council

10:30 Break

10:45 Panel Discussion

11:45 Symposium Close

Lorenz Rhomberg
Chair, Symposium Planning Committee
Member, Standing Committee on Risk Analysis Issues and Reviews
Principal, Gradient Corporation

12:00 Adjourn

Appendix D

Biographic Information on the Speakers and Panelists for a Symposium on Toxicity-Pathway-Based Risk Assessment

SPEAKER BIOGRAPHIES

Tina Bahadori is the managing director for the Long-Range Research Initiative (LRI) program of the American Chemistry Council. She is responsible for the direction of the LRI, which sponsors a multimillion-dollar independent research program that advances the science of risk assessment of the health and ecologic effects of chemicals to support decision-making by government, industry, and the public. Dr. Bahadori was instrumental in developing the LRI's strategic plan and shaping its global initiatives through the International Council of Chemical Associations. Dr. Bahadori is the president (2009-2010) of the International Society of Exposure Science. She is an associate editor of the *Journal of Exposure Science and Environmental Epidemiology*. She has served as a member of several committees of the National Academies; as a peer reviewer for the U.S. Environmental Protection Agency's STAR grants; as a member of the Advisory Panel for the Aerosol Research Inhalation Epidemiology Study; and as a member of the internal steering committee and one of the principal investigators for the St. Louis-Midwest Particulate Matter Supersite. She was also a member of the Chemical Exposure Working Group on the National Children's Study. She received her doctorate in environmental science and engineering from the Harvard School of Public Health.

Kim Boekelheide is professor of medical sciences in the Department of Pathology and Laboratory Medicine of Brown University and director of the university's Superfund Basic Research Program. His research examines fundamental molecular mechanisms by which environmental and occupational toxicants induce tes-

ticular injury. Current projects include the study of co-exposure synergy using model testicular toxicants and of the effects of in utero endocrine-disruptor exposure on steroidogenesis and a predisposition to cancer. Dr. Boekelheide serves on the National Research Council Standing Committee on Use of Emerging Science for Environmental Health Decisions and previously served on the Committee on Toxicity Testing and Assessment of Environmental Agents (which produced *Toxicity Testing in the 21st Century*), the Subcommittee on Fluoride in Drinking Water, and the Committee on Gender Differences in Susceptibility to Environmental Factors: A Priority Assessment. He is a councilor of the Society of Toxicology and a past member of the Board of Scientific Counselors of the National Toxicology Program (NTP) and has been a member of various expert panels (such as those on bisphenol A, phthalates, and bromopropanes) of the NTP Center for the Evaluation of Risks to Human Reproduction. Dr. Boekelheide received his MD and his PhD in pathology from Duke University and is board-certified in anatomic and clinical pathology.

John Bucher is associate director of the National Toxicology Program (NTP) of the National Institutes of Health's National Institute of Environmental Health Sciences. His research interests include characterization of the toxic and carcinogenic potential of a variety of chemicals, mixtures, and physical agents of interest to the NTP, and issues related to the improvement of research tools and assays. As associate director of the NTP, Dr. Bucher is responsible for oversight of the NTP toxicology and carcinogenesis studies, of the NTP *Report on Carcinogens*, and of the NTP Center for the Evaluation of Risks to Human Reproduction and for administrative support for the Alternative Animal Assay Validation Program of the Interagency Coordinating Committee on the Validation of Alternative Methods. Newer efforts include the development of initiatives that examine the genetic basis of variations in response to environmental agents and implementation of new tools for toxicity testing as outlined in the NTP vision and roadmap for the future. Dr. Bucher earned a PhD in pharmacology from the University of Iowa and is a diplomate of the American Board of Toxicology.

George Daston is a Victor Mills Society Research Fellow at Procter & Gamble Company. He is also an adjunct professor in the Department of Pediatrics and the Developmental Biology Program of the University of Cincinnati. Dr. Daston's current research efforts are in toxicogenomics and mechanistic toxicology, particularly in addressing how findings in these fields can improve risk assessment of chemicals. He has published over 100 peer-reviewed articles, reviews, and book chapters and has edited three books. His professional activities include serving as president of the Teratology Society, as a councilor of the Society of Toxicology, as a member of the U.S. Environmental Protection Agency (EPA) Board of Scientific Counselors, and as a member of the National Toxicology Program Board of Scientific Councilors. He is a member of the National Research Council (NRC) Standing Committee on Use of Emerging Science for Environmental Health Decisions and has served as a member of the Committee on Developmental Toxicology, the

Subcommittee on Arsenic in Drinking Water, the Committee on Research Opportunities and Priorities for EPA, and the Board on Environmental Studies and Toxicology. Dr. Daston is editor of *Birth Defects Research: Developmental and Reproductive Toxicology*. He has been awarded the Josef Warkany Lectureship by the Teratology Society and the George H. Scott Award by the Toxicology Forum and was elected a fellow of the American Association for the Advancement of Science. Dr. Daston received his PhD from the University of Miami and postdoctoral training at EPA's laboratories in Research Triangle Park, NC.

E. Donald Elliott is an adjunct professor of law at Yale Law School and Georgetown Law Center, where he teaches a course comparing chemical regulation in the United States and the European Union. He has taught environmental law, administrative law, and law and science, and he is the author of over 60 articles. As a practicing lawyer, Mr. Elliott heads the Environment, Health and Safety Department of Willkie Farr & Gallagher LLP and is a partner in its Washington, DC, office. Formerly, Mr. Elliott was assistant administrator and general counsel for the U.S. Environmental Protection Agency (EPA), and he has served as a consultant in improving the relationship of law and science for the Federal Courts Study Committee and the Carnegie Commission for Law, Science, and Government. He has cochaired the National Environmental Policy Institute's Committee on Improving Science at EPA, he is a member of the National Research Council's Board on Environmental Studies and Toxicology, and he serves on the boards of the Environmental Law Institute and the Center for Clean Air Policy. Mr. Elliott earned his JD from Yale University.

Elaine M. Faustman is a professor of environmental and occupational health sciences at the University of Washington School of Public Health and Community Medicine and directs the Institute for Risk Analysis and Risk Communication. Her research interests include understanding mechanisms of developmental and reproductive toxicants, characterizing in vitro techniques for developmental-toxicology assessment, and developing biologically based dose-response models for noncancer risk assessment. Her research expertise includes the development of tools for incorporating new scientific findings into risk-assessment decisions. Dr. Faustman is an elected fellow of the American Association for the Advancement of Science and of the Society for Risk Analysis. She has also been involved in several National Research Council committees, including the Committee on Spacecraft Exposure Guidelines, the Committee on Developmental Toxicology, and the Committee on Toxicology. Dr. Faustman received a PhD in toxicology from Michigan State University.

Albert J. Fornace, Jr, is a professor in the Department of Biochemistry and Molecular and Cellular Biology, a professor in the Department of Oncology, and chair of molecular cancer research at the Lombardi Comprehensive Cancer Center of Georgetown University. He is also an adjunct professor at the Harvard School of Public Health. His research investigates the complex pathways in-

volved in cellular responses to environmental stressors and the detection of markers in gene expression and metabolites that could be used to detect exposure easily. Previously, he held positions in laboratories at the National Cancer Institute, including stints as an expert consultant, a senior investigator, and an acting chief. Dr. Fornace earned his MD from Jefferson Medical College.

Lynn R. Goldman, a pediatrician and an epidemiologist, is a professor at the Johns Hopkins University Bloomberg School of Public Health, where her areas of focus are environmental health policy, public-health practice, and children's environmental health. Dr. Goldman previously served as the assistant administrator for the U.S. Environmental Protection Agency (EPA) Office of Prevention, Pesticides and Toxic Substances. During her tenure at EPA, Dr. Goldman was responsible for the nation's pesticide, toxic substances, and pollution prevention laws, and she was successful in promoting children's health issues and furthering the international agenda for global chemical safety. Prior to joining EPA, Dr. Goldman served in several positions in the California Department of Health Services, most recently as head of the Division of Environmental and Occupational Disease Control. She has served on numerous boards and expert committees, including the Committee on Environmental Health of the American Academy of Pediatrics and the Centers for Disease Control and Prevention Advisory Committee on Childhood Lead Poisoning Prevention. She has served as a member of numerous National Research Council (NRC) committees, including the Committee on Identifying and Assessing Unintended Effects of Genetically Engineered Foods on Human Health, the Committee on Clinical Trial Registries, and the Committee to Evaluate the Hazardous Materials Management Program of the Bureau of Land Management. She currently is vice chair of the Institute of Medicine (IOM) Roundtable on Environmental Health Sciences, chair of the IOM Committee on Secondhand Smoke Exposure and Acute Coronary Events, and a member of the NRC Standing Committee on Risk Analysis Issues and Reviews. Dr. Goldman received her MD from the University of California, San Francisco.

Bernard D. Goldstein is dean of the University of Pittsburgh Graduate School of Public Health. Previously, he served as the director of the Environmental and Occupational Health Sciences Institute, a joint program of Rutgers, the State University of New Jersey, and the University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School. He was also principal investigator for the Consortium of Risk Evaluation with Stakeholder Participation. Dr. Goldstein was assistant administrator for research and development in the U.S. Environmental Protection Agency (EPA) in 1983-1985. His past activities include serving as a member and chair of the National Institutes of Health Toxicology Study Section and EPA's Clear Air Scientific Advisory Committee. He has also served on numerous National Research Council and Institute of Medicine (IOM) committees, including being chair of the Committee on the Role of the Physician in Occupational and Environmental Medicine, the Committee on Biomarkers in Environ-

mental Health Research, and the Committee on Risk Assessment Methodology. He chairs the Committee to Review the NIOSH Hearing Loss Research Program. Dr. Goldstein is a member of IOM and chaired its Section on Public Health, Biostatistics, and Epidemiology. He is a member and past president of the Society for Risk Analysis. He is a member and fellow of the American College of Environmental and Occupational Medicine (whose Robert A. Kehoe Award of Merit he has received) and a member of the Collegium Ramazzini, the Society for Occupational and Environmental Health, the Society of Toxicology, and the American Public Health Association. Dr. Goldstein earned an MD from New York University.

Federico Goodsaid is associate director for operations in genomics at the Center for Drug Evaluation and Research of the U.S. Food and Drug Administration (FDA). In this role, he works on the regulatory application and development of genomics and biomarkers. Previously, he was a senior staff scientist of applied biosystems and lead for the Molecular Toxicology Group at Schering-Plough Research Institute, where his collaboration with FDA led to one of the first genomic-data submissions to FDA. He was also director of assay and development at the Fluidigm Corporation, a microfluidics company based in San Francisco, CA. Dr. Goodsaid earned a PhD in molecular biophysics and biochemistry from Yale University and was a postdoctoral fellow at Cornell University and Washington University in St. Louis.

John Groopman is a professor of oncology, Anna M. Baetjer Professor of Environmental Health, and director of the National Institute of Environmental Health Sciences Center in Urban Environmental Health at Johns Hopkins University. His research involves the development and application of molecular biomarkers of exposure to, dose of, and effect of environmental carcinogens and focuses on the translation of mechanistic research to public health on the basis of prevention strategies. Dr. Groopman has earned several honors, including the National Cancer Institute Research Career Development Award and membership in the Delta Omega Honor Society in Public Health and Phi Beta Kappa. He has served as a member of the National Research Council Committee on How Toxicogenomics Could Inform Critical Issues in Carcinogenic Risk Assessment of Environmental Chemicals, the Standing Committee on Emerging Issues and Data on Environmental Contaminants, and the Panel on Life Sciences. Dr. Groopman received a PhD in toxicology from the Massachusetts Institute of Technology.

John Groten is vice president of the Drug Safety Organization in Europe for Schering Plough, where he is responsible for providing toxicology support for pharmaceuticals during the discovery, preclinical, and full-development stages. He is also a part-time professor and chair of combination toxicology at Wageningen University, the Netherlands. Earlier, Dr. Groten was executive director of the Business Unit of BioSciences at TNO Quality of Life, an independent contract research and technology organization founded in the Netherlands. Throughout his

career, Dr. Groten has headed several toxicology and life-science departments and has completed business training to handle financial and general managerial work related to research and development efficiently. He has participated in many international scientific and regulatory committees on the safety assessment of chemical mixtures, and he has published over 100 papers in scientific journals and books on combination toxicology. Dr. Groten earned a PhD in toxicology from Wageningen University and is a certified toxicologist (the Netherlands and Eurotox).

Thomas Hartung is director of the Center for Alternatives to Animal Testing (CAAT) and the Doerenkamp-Zbinden Chair for Evidence-Based Toxicology in the Department of Environmental Health Sciences of the Johns Hopkins School of Public Health. His career began on the faculty of the University of Konstanz, Germany, where he was an assistant professor of biochemical pharmacology and an associate and later full professor of pharmacology and toxicology. He also served as the CEO of the Steinbeis Technology Transfer Center for In Vitro Pharmacology and Toxicology. Before his position at CAAT, Dr. Hartung was the head of the European Centre for the Validation of Alternative Methods (ECVAM) at the European Commission Joint Research Centre in Italy. As head of ECVAM, he was integral in accelerating the alternative-methods validation process and in enabling a network of more than 400 experts in all stakeholder groups to facilitate global regulatory harmonization in toxicity testing. Dr. Hartung received a PhD in biochemical pharmacology from the University of Konstanz and an MD in toxicology from the University of Tubingen, Germany.

Robert Kavlock is director of the National Center for Computational Toxicology in the Office of Research and Development (ORD) of the U.S. Environmental Protection Agency (EPA). During his 24 years at EPA, he has served as special assistant (computational toxicology) to the director of the National Health and Environmental Effects Research Laboratory (NHEERL); as acting associate director for health, NHEERL; as director of the Reproductive Toxicology Division, NHEERL; as chief of the Perinatal Toxicology Branch; and as research biologist of the Perinatal Toxicology Branch. Dr. Kavlock is a member of the Society of Toxicology and the Teratology Society. He has served on the editorial boards of *Toxicological Sciences* and *Teratogenesis, Carcinogenesis and Mutagenesis*, and he now serves on the editorial boards of the *Journal of Toxicology and Environmental Health, Part B*; the *Journal of Children's Health*; *Birth Defects Research, Part B*; and *Neurotoxicity and Teratology*. Dr. Kavlock has been the recipient of numerous honors and awards, most recently being named the EPA/ORD Statesman of the Year and receiving the EPA Bronze Medal. He is an active participant in expert committees, advisory panels, and organizing committees, such as the National Institute of Environmental Health Sciences Superfund Basic Research Program Peer Review Panel, the EPA Science Forum on Computational Toxicology, and the OECD Molecular Screening Initiative Working Group. Dr. Kavlock earned a PhD in embryology from the University of Miami.

Gregory Kedderis is an independent researcher and consultant in biochemistry, pharmacology, and toxicology in Chapel Hill, NC. He is also an adjunct associate professor in the Nicholas School of the Environment and Earth Sciences and in the Integrated Toxicology and Environmental Health Program of Duke University. His research interests include the relationship between chemical dosimetry and biologic effects, mechanisms of toxicity of drugs and xenobiotics, and mechanisms of genotoxicity and chemical carcinogenesis. Before his independent consultancy, he held positions as director of the Chemical Carcinogenesis Research Program and director of the Division of Biochemistry and Molecular Genetics at the Chemical Industry Institute of Toxicology (now Hamner Institutes for Health Sciences). Dr. Kedderis is author or coauthor of over 70 publications. He has served on a number of national and international committees and workshops sponsored by the International Life Sciences Institute, the European Centre for Validation of Alternative Methods, and other organizations. He has served on the editorial boards of *Drug Metabolism and Disposition* and the *Journal of Pharmacology and Experimental Therapeutics*, and he was reviews editor for *Chemico-Biological Interactions*. He is a member of the Society of Toxicology and of the Chemical Substances Threshold Limit Values Committee of the American Conference of Governmental Industrial Hygienists. Dr. Kedderis earned a PhD in biochemistry from Northwestern University Medical and Dental School.

George D. Leikauf is a professor in the Department of Environmental and Occupational Health of the University of Pittsburgh. His research investigates the functional genomics of acute lung injury, asthma, and chronic obstructive pulmonary disease. He is interested in uncovering the genetic basis of increased susceptibility to pulmonary epithelial injury and repair and in examining the transcriptional regulation of molecular targets. Dr. Leikauf has served on numerous national and international committees, including the National Institutes of Health's National Advisory Environmental Health Sciences Council and Center for Scientific Review Advisory Committee, the American Physiological Society's Perkins Memorial Fund Committee, the American Thoracic Society's Program Committee, and the International Advisory Committee for the 8th and 9th Inhalation Symposiums. He has also served as a member of the National Research Council Committee on Applications of Toxicogenomics Technologies to Predictive Toxicology. Dr. Leikauf received a PhD in environmental health sciences from the New York University Medical Center.

Warren Muir is executive director of the Division on Earth and Life Studies of the National Academies. He was founder and president of the Hampshire Research Institute, director of the Office of Toxic Substances of the U.S. Environmental Protection Agency, and senior staff member of the Council on Environmental Quality in the Executive Office of the President. Dr. Muir holds master's and doctoral degrees in chemistry from Northwestern University and did postdoctoral study in epidemiology at the Johns Hopkins University.

William Pennie is the executive director of compound safety prediction at Pfizer Global Research and Development, Groton, CN. After fellowships at the National Institutes of Health, Dr. Pennie started his industrial career at the Central Toxicology Laboratories of Zeneca in Macclesfield, England, working primarily on estrogen-receptor selectivity, receptor-mediated transcription, microarray technologies, mechanistic toxicology, and novel predictive toxicology approaches to help in compound selection. Since joining Pfizer in 2002, Dr. Pennie has led the Molecular and Investigative Toxicology Group and was the research site lead for drug safety research and development in Groton. Most recently, he has built, and leads, a new group in Pfizer Global Research that aims to develop mechanistic understanding of toxicity further, to build predictive models for toxicity mechanisms, and to integrate them into early design cycles of medicinal chemistry. Dr. Pennie received his PhD from the Beatson Institute for Cancer Research (Glasgow University).

Peter Preuss is director of the National Center for Environmental Assessment (NCEA) at the U.S. Environmental Protection Agency (EPA), where his role is to oversee all major activities in NCEA and to pursue innovative approaches to risk assessment. His expertise is in risk assessment and its applications, science policy and law, and the use of comparative risk assessment as a tool in setting environmental priorities domestically and internationally. Dr. Preuss has been a director at EPA since 1988; before then, he was the associate executive director for health sciences at the U.S. Consumer Product Safety Commission and director of the New Jersey Department of Environmental Protection. Dr. Preuss earned a PhD in biology from Columbia University.

Alvaro Puga is a professor of molecular biology and environmental health at the University of Cincinnati, deputy director of the National Institute of Environmental Health Sciences Center for Environmental Genetics, and associate director of the Superfund Basic Research Program at the University of Cincinnati Medical Center. His laboratory investigates the response of individuals and populations to toxic or carcinogenic environmental agents. The long-term objective of that work is to elucidate the molecular mechanisms that underlie the response. Before his work at the University of Cincinnati, Dr. Puga served for 15 years in several capacities at the National Institutes of Health in the Institute of Dental Research and the Institute of Child Health and Human Development. Dr. Puga is the recipient of the Society of Toxicology Lectureship Award and the University of Cincinnati College of Medicine Richard Akeson Award for Excellence in Teaching. He has participated in numerous national and international committees, including serving as a member of the National Research Council Committee on EPA's Exposure and Human Health Reassessment of TCDD and Related Compounds and the Institute of Medicine Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides. Dr. Puga earned a PhD in molecular biology and biophysics from Purdue University.

Kenneth Ramos is a professor of biochemistry and molecular biology in the University of Louisville Health Sciences Center, director of the Center for Genetics and Molecular Medicine, and director of the National Institute of Environmental Health Sciences Center for Environmental Genomics and Integrative Biology. His research on molecular mechanisms of environmental pathogenesis focuses on the molecular biology of L1 retroelements, inference of genetic regulatory networks, and developmental basis of environmental disease. Dr. Ramos has served on numerous National Research Council committees, including being a member of the Committee for a Review of Evidence Regarding the Link Between Exposure to Agent Orange and Diabetes, a member of the Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides for the first and second biennial updates, and chair of the Standing Committee on Emerging Issues and Data on Environmental Contaminants. Dr. Ramos earned a PhD in biochemical pharmacology at the University of Texas at Austin.

Lorenz Rhomberg is a principal with Gradient Corporation, an environmental-sciences consulting firm. He is an expert in quantitative risk assessment, including dose-response analysis, pharmacokinetic modeling, and probabilistic methods, with special expertise in chlorinated solvents. His focus includes science policy and methodology for human health risk assessment, including approaches to weight of toxicologic evidence for human hazard and cross-species extrapolation. Before joining Gradient, Dr. Rhomberg was on the faculty of the Harvard School of Public Health and worked at the U.S. Environmental Protection Agency. Dr. Rhomberg is active in professional groups and environmental policy development, focusing on current issues in the interpretation of toxicologic data in human health risk assessment through service on panels sponsored by government, industry, and such organizations as the International Life Sciences Institute. He has served on several National Research Council committees, including the Committee on Assuring the Safety of the Defense Department's Mail, the Committee on Testing and Evaluation of Standoff Chemical Agent Detectors, and the Subcommittee on Manufactured Vitreous Fibers, and he is currently a member of the Standing Committee on Risk Analysis Issues and Reviews. Dr. Rhomberg earned his PhD in population biology from the State University of New York at Stony Brook.

Ivan Rusyn is an associate professor with tenure in the Department of Environmental Sciences and Engineering of the School of Public Health of the University of North Carolina (UNC) at Chapel Hill. He directs the Laboratory of Environmental Genomics and the Carolina Center for Computational Toxicology in the Gillings School of Global Public Health of UNC. He also serves as associate director of the Curriculum in Toxicology and is a member of the Lineberger Comprehensive Cancer Center, the Center for Environmental Health and Susceptibility, the Bowles Center for Alcohol Studies, and the Carolina Center for Genome Sciences. Dr. Rusyn served on several working groups convened by the National Research Council and the World Health Organization-International Agency for Research on Cancer. Dr. Rusyn's laboratory has an active research portfolio funded

by the National Institutes of Health and the U.S. Environmental Protection Agency with a focus on the mechanisms of action of environmental toxicants and the genetic determinants of susceptibility to toxicant-induced injury. His laboratory applies molecular, biochemical, genetic, and genomics approaches to understanding the mechanisms of environmental-agent-related disease, and his studies on health effects of environmental agents have resulted in 80 peer-reviewed publications. Dr. Rusyn received his MD from the Ukrainian State Medical University in Kiev and his PhD in Toxicology from UNC-Chapel Hill. He also trained at the University of Düsseldorf in Germany and at the Massachusetts Institute of Technology.

Gina Solomon is a senior scientist at the Natural Resources Defense Council. She is also an associate clinical professor of medicine, director of the Occupational and Environmental Medicine Residency Program, and associate director of the Pediatric Environmental Health Specialty Unit of the University of California, San Francisco. Her work has included over 40 scientific papers, book chapters, and reports on air pollution, pesticides, global warming, and other environmental and occupational threats to health. Dr. Solomon serves on the U.S. Environmental Protection Agency's Science Advisory Board Drinking Water Committee, the National Toxicology Program Board of Scientific Counselors, and the California Scientific Guidance Panel for biomonitoring. Dr. Solomon earned her MD from Yale University and did her postgraduate training in internal medicine, public health, and occupational and environmental medicine at Harvard University.

Rusty Thomas is a senior investigator at the Hamner Institutes for Health Sciences and the director of the Center for Genomic Biology and Bioinformatics. His current research focuses on the development and application of genomic and high-throughput screening technologies and bioinformatics tools to address problems in environmental toxicology, drug safety, and chemical risk assessment. Dr. Thomas received his MS in radiation ecology and his PhD in toxicology from Colorado State University and performed his postdoctoral research in molecular biology and genomics at the McArdle Cancer Research Laboratory of the University of Wisconsin.

Brian Thrall is the technical group leader of cell biology and biochemistry in the Biological Sciences Division of the Pacific Northwest National Laboratory. He holds adjunct positions in the graduate research program of Washington State University and regularly serves on special-emphasis review panels for the National Institutes of Health in proteomic and genomic analysis of cancer and as a proposal reviewer for the Department of Energy, the Air Force Office of Scientific Research, and the Department of State. Dr. Thrall's research focuses on the mechanisms by which environmental agents modulate cell-signaling pathways to influence the balance between cell proliferation and cell death. His research uses genomic and proteomic strategies to investigate the molecular mechanisms of cross-regulation between growth-factor, inflammatory, and stress-signaling pathways with macrophages and various epithelial cells as model systems. Dr. Thrall is

the author or coauthor of over 50 peer-reviewed publications. He is a member of the American Society for Cell Biology, the Society of Toxicology Molecular Biology Specialty Section, and the Pacific Northwest Association of Toxicologists. He earned his PhD in pharmacology from Washington State University.

Roger Ulrich is founder and chief development officer of Calistoga Pharmaceuticals, Inc., a company focused on the development of PI3 kinase inhibitors for cancer and inflammatory diseases. He is responsible for all aspects of drug development, and he continues to pursue his interests in the phenotypic and genotypic traits that contribute to adverse drug reactions. Dr. Ulrich's industrial career began at the Upjohn Company, and he has held various scientific and leadership positions in the pharmaceutical industry, including those of senior scientist with the Upjohn Company and Pharmacia & Upjohn Inc., director of regulatory toxicology and safety pharmacology with Abbott Laboratories, and senior scientific director with Merck Research Laboratories Rosetta Inpharmatics. He is a member of the Academy of Toxicological Sciences and the author of more than 125 publications. He is an internationally recognized lecturer on toxicology, genomics, and drug discovery; he continues to maintain adjunct academic appointments; and he serves on various editorial boards, government and academic advisory boards, and working committees. Dr. Ulrich earned a PhD in cellular and molecular biology from West Virginia University.

Katrina Waters is a senior research scientist and bioinformatics team leader in the Computational Biology and Informatics Group of the Pacific Northwest National Laboratory (PNNL). Her research interests are focused on the reconstruction of cell-response networks from integrated gene and protein expression data to enable predictive mechanistic modeling of disease and toxicity pathways. Her current projects include pathway-based biomarker discovery for environmental exposure to lung toxicants, including disease-susceptibility factors, and network modeling of lung inflammation by examining macrophage and epithelial cell paracrine communication. She also has two collaborative projects with the University of Washington and Oregon Health Sciences University to perform data modeling of host-pathogen interactions. Before her position at PNNL, Dr. Waters was a senior research biochemist in molecular and investigative toxicology for drug safety risk assessment at Merck Research Laboratories. At Merck, she used microarray technologies to determine mechanisms of toxicity of drugs about which there were safety concerns and to identify biomarkers of tissue-specific toxicity. Dr. Waters earned a PhD in biochemistry from the University of Wisconsin, Madison.

Helmut Zarbl is a professor of environmental and occupational medicine at the Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey (UMDNJ). He is a member of the Environmental and Occupational Health Sciences Institute (EOHSI), a joint institute of UMDNJ and Rutgers University. He is also director of the National Institute of Environmental Health

Sciences Center for Environmental Exposures and Disease at EOHSI and the associate director for public-health science at the Cancer Institute of New Jersey. Dr. Zarbl's research has focused largely on toxicogenomics and functional genomics, carcinogenesis, molecular and cellular biology, and toxicology. Specifically, his work seeks to understand molecular mechanisms of chemical carcinogenesis and chemoprevention and the genetic basis of differential susceptibility to mammary carcinogenesis in animal and in vitro model systems. He is also involved in technology development, including his patented work on RNAi and its application to the development of novel platforms for function genomics. Dr. Zarble is a fellow of the Academy of Toxicological Sciences and is a member of the National Research Council (NRC) Standing Committee on Use of Emerging Science for Environmental Health Decisions. He previously served as a member of the NRC Committee on Applications of Toxicogenomic Technologies to Predictive Toxicology. Dr. Zarbl earned a PhD in biochemistry from McGill University.

PANELIST BIOGRAPHIES

Charles Auer was the director of the U.S. Environmental Protection Agency (EPA) Office of Pollution Prevention and Toxics (OPPT) and retired from EPA in January 2008 after a 32-year career there. As the director of OPPT, he was responsible for oversight of the Toxic Substances Control Act and had a lead role in promoting pollution prevention, both in the agency and with external stakeholders. During his time at EPA, OPPT was responsible for numerous collaborative efforts, such as the High Production Volume Challenge Program, the Presidential Green Chemistry Challenge, Design for the Environment, the Nanoscale Materials Stewardship Program, the Chemical Assessment and Management Program, the PFOA 2010/2015 Stewardship Program, and other voluntary regulatory efforts. A chemist by training, Mr. Auer has broad experience in assessment and management of new and existing chemicals, genetically engineered microorganisms, and nanoscale materials. He has also worked with the Organisation for Economic Co-operation and Development and has had extensive bilateral and multilateral interactions with the European Union, Canada, Japan, China, and other major U.S. trading partners.

David Jacobson-Kram is associate director for pharmacology and toxicology in the U.S. Food and Drug Administration's Office of New Drugs. Over the last 25 years, he has served as principal and co-principal investigator on several National Institutes of Health (NIH) grants and government contracts. Dr. Jacobson-Kram has published over 80 abstracts, 57 original articles in peer-reviewed journals, and 30 review articles and book chapters. He has served as a council member, treasurer, and chairman of the Genetic Toxicology Association; an executive council member of the Environmental Mutagen Society; the editor of *Cell Biology and Toxicology;* the president of the National Capital

Area Chapter of the Society of Toxicology; and member of NIH special study sections. He has also been elected as a diplomate of the American Board of Toxicology. Dr. Jacobson-Kram received a PhD in embryology from the University of Connecticut.

William Kaufmann is a professor of pathology and laboratory medicine, director of the genetic susceptibility research core at the Center for Environmental Health and Susceptibility, and director of the Program in Toxicogenomics of the University of North Carolina (UNC) at Chapel Hill. His laboratory is concerned with the mechanisms of human carcinogenesis with special interest in cell-cycle checkpoints that act to preserve chromosomal stability. Dr. Kaufmann is a member of the Environmental Mutagen Society and of the American Association for Cancer Research. He has served as associate editor of *Mutagenesis* and has served on the editorial board of *Carcinogenesis*. He has also served on numerous program, grant, and contract review groups; UNC committees; and national committees, including service as the chair of the Toxicogenomics Research Consortium Steering Committee, a member of the Environmental Mutagen Society Awards Committee, and a member of the American Society of Investigative Pathology Program Committee. Dr. Kaufmann earned a PhD in experimental pathology from UNC at Chapel Hill.

Jonathan Wiener is the William R. and Thomas L. Perkins Professor of Law at Duke Law School, professor of environmental policy at the Nicholas School of the Environment, and professor of public-policy studies at the Sanford Institute of Public Policy of Duke University. In 2008, he served as president of the Society for Risk Analysis (SRA) and was inducted as a fellow of SRA. In 2003, he received the Chauncey Starr Young Risk Analyst Award from SRA for exceptional contributions to the field of risk analysis by a scholar 40 years old or younger. Since 2002, he has been a university fellow of Resources for the Future. From 2000 to 2005, he was the founding faculty director of the Duke Center for Environmental Solutions. Prof. Wiener has been a visiting professor at Harvard Law School, the University of Chicago Law School, Sciences Po, and l'Ecole des Hautes Etudes en Sciences Sociales in Paris. He has written widely on U.S., European, and international environmental law and risk regulation, including several books and numerous articles in diverse journals in law, policy, risk, toxicology, and other fields. Before coming to Duke in 1994, Prof. Wiener served in the G.H.W. Bush and Clinton administrations in the Department of Justice, the Office of Science and Technology Policy, and the Council of Economic Advisers. He was a law clerk to federal judges Stephen Breyer and Jack Weinstein after earning his AB in economics and JD at Harvard University.

Appendix E

Symposium Presentations

All presentations are available on the CD on the inside of the back cover of this report.

Disclaimer: Some of the presentations on the CD differ slightly from the presentations given during the Symposium on Toxicity-Pathway-Based Risk Assessment: Preparing for Paradigm Change because those given during the symposium contained unpublished data that cannot be included in a published summary report. The presentations in question are by Elaine Faustman, Albert J. Fornace, Jr., Federico Goodsaid, and Ivan Rusyn.

Appendix F

Poster Abstracts

Use of early effect biomarker data to enhance dose-response models of lung tumors in rats exposed to titanium dioxide

Bruce Allen,[1] Andrew Maier,[2] Alison Willis,[2] and Lynne T. Haber[2]
[1]Bruce Allen Consulting, Chapel Hill, NC; [2]Toxicology Excellence for Risk Assessment, Cincinnati, OH

The use of precursor data (biomarkers of effect) directly in risk assessments is increasingly emphasized as the future of toxicology. Because of the limited validation that has been conducted for the biomarkers themselves and the lack of vetted approaches for incorporating biomarker data into dose-response assessments quantitatively, current applications for the growing pool of biomarker data are constrained to hazard characterization. This paper presents a dose-response modeling approach that incorporates biomarker data on the lung-tumor response in rats exposed to titanium dioxide (TiO_2). We used a series of linked "cause-effect" functions, fitted with a likelihood approach, to describe the relationships between successive key events and the ultimate tumor response. That approach was used to evaluate a hypothesized pathway for biomarker progression from a biomarker of exposure (lung burden), through several intermediate potential biomarkers of effect, to the clinical effect of interest (lung-tumor production). The model evaluated the contribution of several intermediate effect biomarkers to the dose-response behavior for lung tumors. These effect biomarkers included the polymorphonuclear-lymphocyte (PMN) count indicative of inflammation, proteins in bronchoalveolar-lavage fluid (BALF) indicative of alveolar damage, pulmonary-fibrosis incidence, and alveolar-cell proliferation. Interestingly, when the model allowed either fibrosis (and its precursors) or cell proliferation (and its precursors) or both to predict the tumor response, the cell-proliferation data provided no additional predictive power beyond that of the fibrosis response. Overall, the likelihood-maximization approach allowed the calculation of a lung-burden-based benchmark dose for lung tumors that directly incorporated data on biomarkers of exposure and effect. The biomarker-based modeling approach provided a more refined dose-response estimate than that

obtained by using the traditional approach of dose-response modeling based only on tumor data.

Physiological modeling of metabolic interactions

Frédéric Y. Bois
INERIS, Verneuil en Halatte, France

Purpose: Modeling metabolic interactions between chemicals can be a formidable task in model development. This presentation demonstrates a new approach and the capabilities of new tools to facilitate that development.

Methods: Individual models of metabolic pathways are automatically merged and coupled to a template physiologically based pharmacokinetic (PBPK) model by using the GNU MCSim software. The global model generated is very efficient and able to simulate the interactions between a theoretically unlimited number of substances. Development time increases only linearly with the number of substances considered while the number of possible interactions increases exponentially.

Results: An example of application of the approach to the prediction of the kinetics of a mixture of 30 arbitrary chemicals is shown. The qualitative and quantitative behavior of the corresponding pathway network is analyzed by using Monte Carlo simulations. In our example, the number of significant interactions, given the uncertainty and variability in the pharmacokinetics and metabolism of those substances, is much lower than the theoretically possible number of interactions.

Conclusion: The integrative approach to interaction modeling is efficient and can be extended beyond metabolic interactions. It relies on the availability of specific data on the rate constants of individual reactions. Such data could be obtained through unconventional experiments in enzyme kinetics or through ab initio chemical modeling of enzymatic reactions. We are currently exploring both approaches.

The role of oxysterols in a computational steroidogenesis model of human H295R cells to improve predictability of biochemical responses to endocrine disruptors

M. Breen,[1,2] M.S. Breen,[3] A.L. Lloyd,[1] and R.B. Conolly[2]
[1]Biomathematics Program, Department of Statistics, North Carolina State University, Raleigh, NC; [2]National Center for Computational Toxicology, U.S. Environmental Protection Agency, Research Triangle Park, NC; [3]National Exposure Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC

Steroids, which have an important role in a wide range of physiological

processes, are synthesized primarily in the gonads and adrenal glands through a series of enzyme-mediated reactions. The activity of steroidogenic enzymes can be altered by a variety of endocrine disruptors (EDs), some of which are environmental contaminants. We are developing a dynamic computational model of the metabolic network of adrenal steroidogenesis in a human H295R cell line to predict the synthesis and secretion of adrenocortical steroids (such as mineralocorticoids, glucocorticoids, androgens, and estrogens) and the biochemical response to EDs. We previously developed a deterministic model that describes the biosynthetic pathways for the conversion of cholesterol to adrenocortical steroids and the kinetics of enzyme inhibition by the ED metyrapone (MET). In this study, we extended the model by adding the pathway of oxysterol biosynthesis. Oxysterols are endogenous products of cholesterol unrelated to steroidogenesis. Experiments were performed to measure concentrations of cholesterol and 14 steroids in human H295R cells by using LC/MS/MS and ELISA methods. Model parameters were estimated with an iterative optimization algorithm. Results show that the model fit improved with the extended model. Model predictions closely correspond to time-course measurements of both cholesterol and steroid concentrations from control and dose-response experiments with MET. Our study demonstrates the feasibility of using the computational model of adrenal steroidogenesis to predict the in vitro adrenocortical steroid concentrations from human H295R cells. This capability could be useful to define mechanisms of action of poorly characterized chemicals and mixtures in support of the H295R steroidogenesis screening system and predictive risk assessments.

Disclaimer: This work was supported in part by the NCSU/EPA Cooperative Training Program in Environmental Sciences Research, Training Agreement CT833235-01-0 with North Carolina State University. Although this work was reviewed by the U.S. Environmental Protection Agency and approved for publication, it may not necessarily reflect official agency policy.

Toxicity pathway modeling of chemically induced oxidative stress: a case study with trichloroethylene

C.J. Brinkerhoff, S.S. Isukapalli, and P.G. Georgopoulos
Environmental and Occupational Health Sciences Institute, A Joint Institute of the University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School and Rutgers University, Piscataway, NJ

Oxidative stress is an important toxicity pathway that can lead to cellular injury and carcinogenesis. Mechanistic understanding and predictive modeling of this pathway is expected to improve risk assessment for environmental exposures significantly. Trichloroethylene (TCE) is a commonly encountered contaminant that is known to cause oxidative stress in liver tissue, where it is metabolized; it is a known carcinogen in rodents and a suspected carcinogen in humans. Human exposures to TCE are common because it is a ubiquitous con-

taminant in air and water owing to its widespread use as a degreaser and general-purpose solvent.

A new combined physiologically based pharmacokinetic (PBPK) and biologically based dose-response (BBDR) model is presented for studying the impact of TCE exposures. This model describes oxidative stress in a mechanistic manner. Many of the toxic effects of TCE are hypothesized to be due to the metabolites trichloroacetate and dichloroacetate rather than to TCE alone. Therefore, the mathematical formulation of the toxicity pathway for oxidative stress generation includes TCE and its metabolites and considers direct exposures to the metabolites. This new model improves on existing TCE models by including a toxicity response pathway and by considering the impact of direct exposures to metabolites. Model parameters were estimated from in vitro mouse liver slice measurement data from the literature; these data include amounts of free radicals and lipid peroxidation after TCE exposures. Specifically, model parameters were identified in a sequential manner, first using in vivo measurements of oxidative stress in mice after exposures to TCE metabolites and then using data from experiments involving exposures to TCE. The model can thus estimate relative contributions of each chemical to the total oxidative stress caused by a dose of TCE. Furthermore, the addition of the toxicity response pathway in the new model resulted in successful predictions of oxidative stress produced in the liver over periods ranging from hours to weeks; such consistent predictions on large time scales were not possible with existing models. The modeling approach used here can be generalized to other chemicals and toxicity pathways.

Disclaimer: Support for this work has been provided by U.S. Environmental Protection Agency grant GAD R 832721-010 and National Institute of Environmental Health Sciences grant P30ES005022. This work has not been reviewed by and does not represent the opinions of the funding agencies.

Use of renal biomarkers to characterize toxicity-based pathways of nephrotoxicity

Ronald Brown,[1] Peter Goering,[1] and Jun Zhang[2]
[1]U.S. Food and Drug Administration, Center for Devices and Radiological Health, Silver Spring, MD; [2]U.S. Food and Drug Administration, Center for Drug Evaluation and Research, Silver Spring, MD

The classic nephrotoxic agents gentamicin (Gen), mercury (Hg), and chromium (Cr) have been shown to produce acute kidney injury (AKI) at fairly well-defined sites in the nephron. This study was conducted to (1) determine the role of the nitric oxide (NO) toxicity-based pathway in renal pathogenesis of those agents and (2) determine the correlation between immunohistochemical expression of biomarkers of NO and expression of two AKI biomarkers, kidney injury molecule-1 (KIM-1) and renal papillary antigen-1(RPA-1), in the proximal tubules and collecting ducts, respectively. Sprague-Dawley rats were given

injections of Gen (100 mg/kg, sc, daily for 3 days), $HgCl_2$ (0.25 mg Hg/kg, iv), $K_2Cr_2O_7$ (5 mg Cr/kg, sc), or vehicle (control). At 24 or 72 h after the last dose, kidneys were collected, and formalin-fixed renal sections were used for histopathologic evaluation and immunostaining for inducible NO synthase (iNOS), endothelial NO synthase (eNOS), nitrotyrosine (NT), KIM-1, and RPA-1. Kidneys from rats treated with Gen, Hg, or Cr exhibited increased expression of iNOS, eNOS, and NT, which correlated with the severity of renal histopathology and the tissue expression of biomarkers of AKI, specifically KIM-1 and RPA-1. Those findings suggest that acute nephrotoxic effects of Gen, Hg, or Cr are associated with a toxicity-based pathway involving NO and nitrosative stress. Findings also show how renal biomarkers can be used to characterize toxicity-based pathways of kidney injury.

PBPK models, BBDR models, and virtual tissues: How will they contribute to the use of toxicity pathways in risk assessment?

Rory Conolly, Imran Shah, and Thomas Knudsen
U.S. Environmental Protection Agency, National Center for Computational Toxicology, Office of Research and Development, Research Triangle Park, NC

Accuracy in risk assessment, which is desirable to ensure protection of the public health while avoiding overregulation of economically important substances, requires quantitatively accurate descriptions of in vivo dose-response and time-course behaviors. This level of detailed characterization is desirable when substances are economically important or environmentally persistent. The report *Toxicity Testing in the 21st Century: A Vision and a Strategy* emphasizes in vitro studies, with bioinformatics and systems modeling approaches used to predict in vivo behavior. Physiologically based pharmacokinetic (PBPK) and biologically based dose-response (BBDR) models and virtual tissues (VTs) will all be important in these extrapolations. PBPK models describe the relationship between external exposure and target-site dose. BBDR models extend PBPK models to include the linkage between target-site dose, key events, and endpoint effect. These mathematical models typically have compartments that correspond to whole tissues (such as liver, kidney, and lung) and typically contain limited tissue-specific data. VT models, such as the U.S. Environmental Protection Agency v-Liver™ and v-Embryo™ projects, are computational models that will encode sufficient biological information to support significant predictive capabilities, with higher-level behaviors emerging from the structures encoded at more fundamental levels of organization. PBPK, BBDR, and VT models are thus complementary to one another, each having the ability to facilitate the interpretation of in vitro data with respect to in vivo significance and predicting dosimetry at finer levels of biological detail. This enhanced capability will help to identify (1) the doses or concentrations for in vitro studies that correspond to realistic levels of exposure in vivo and (2) relevant descriptions of the tissue-

dose-end-point response continuum. It will also contribute to pathway-based assessment of specific biological processes and toxicities.

Disclaimer: Although this work was reviewed by the U.S. Environmental Protection Agency and approved for publication, it may not necessarily reflect official agency policy.

Improving mode-of-action analysis using transcript profiling in nullizygous mouse models

Chris Corton,[1] Susan Hester,[1] Lauren Aleksunes,[2] Hongzu Ren,[1] Beena Vallanat,[1] Michael George,[1] Mitch Rosen,[1] Barbara Abbott,[1] Curt Klaassen,[2] Stephen Nesnow,[1] and Chris Lau[1]
[1]U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Research Triangle Park, NC; [2]Kansas University Medical Center, Kansas City, KS

A number of nuclear receptors (NRs) mediate transcriptional hepatocyte growth and carcinogenic effects in the rodent liver after chemical exposure. These receptors include the constitutive activated/androstane receptor (CAR), pregnane X receptor (PXR), and peroxisome proliferator-activated receptor alpha (PPAR-α). We hypothesized that transcriptional analysis in the livers of exposed wild-type and NR-null mice can strengthen the weight of evidence for mode-of-action analysis of environmentally relevant chemicals. We tested this hypothesis by examining gene expression by Affymetrix arrays in the livers of wild-type mice and mice nullizygous for CAR, PXR, or PPAR-α exposed to chemicals of a number of classes, including perfluoroalkyl acids, conazole fungicides, and a phthalate ester plasticizer. A comparison of gene expression in exposed wild-type and nullizygous mice revealed the extent of the involvement of the receptors in mediating the effects of the chemicals. Three major conclusions can be drawn from these studies. (1) Many chemicals activate more than one receptor in the mouse liver. Perfluorooctanoic acid (PFOA) and di-2-ethylhexyl phthalate (DEHP) exhibit features of activation of PPAR-α and CAR in wild-type mice. In the absence of PPAR-α, perfluorooctane sulfonate (PFOS) activates CAR. (2) Chemicals exhibit differences in receptor dependence. Approximately 99%, 92%, and 85% of the genes regulated by WY-14,643 (a PPAR-α agonist), PFOS, and PFOA, respectively, were dependent on PPAR-α for altered expression. These results indicate that PPAR-α plays a dominant role in mediating the effects of these chemicals despite minor differences in the extent of receptor dependence. (3) In the absence of the receptor that mediates the majority of effects, some chemicals exhibit greater induction of alternative pathways. PFOA and PFOS activate a CAR-like signature to a greater extent in PPAR-α-null mice than in wild-type mice. Because PPAR-α and CAR exhibit antagonistic effects, greater CAR-like effects may occur through loss of repression by PPAR-α. These findings demonstrate that PPAR-α plays a necessary

role in mediating the effects of PFOA, PFOS, and DEHP. In conclusion, coupling genomewide transcript profiling in different genetic backgrounds can be valuable in determination of the mode of action of liver-tumor induction by environmentally relevant chemicals.

Disclaimer: This abstract does not represent U.S. Environmental Protection Agency policy.

Issues in using human variability distributions to estimate low-dose risk

Kenny S. Crump,[1] Weihsueh Chiu,[2] and Ravi P. Subramaniam[2]
[1]Louisiana Tech University, Ruston, LA; [2]U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment, Research Triangle Park, NC

Low-dose extrapolation and accounting for human variability in susceptibility remain among the key issues in implementing the recommendations of the National Research Council report *Toxicity Testing in the 21st Century: A Vision and a Strategy* (NRC 2007). In *Science and Decisions: Advancing Risk Assessment* (NRC 2008), a separate committee made numerous recommendations on addressing these issues in the current context of toxicity testing that may also be applicable to the envisioned 21st century toxicity-testing paradigm. Among the recommendations is a proposal to estimate low-dose risks by using models derived from human variability distributions (HVDs). In the existing approaches to HVD modeling, log-normal distributions are estimated from data on various pharmacokinetic and pharmacodynamic parameters that impact individual sensitivities to the toxic response. These distributions are combined into an overall log-normal distribution for the product of the individual parameters by adding their variances. The log-normal distribution is transferred to the dose axis by centering it at a point-of-departure (POD) dose usually estimated from animal data. The resulting log-normal distribution is used to quantify low-dose risk.

This poster examines the implications of various assumptions in this approach:

- Existing approaches to HVD modeling generally assume that the distribution of individual threshold doses determined by dichotomizing a continuous apical response is log-normal. This assumption is incompatible with an assumption that the apical responses themselves are log-normal (except under highly specialized conditions). However, the two assumptions generally lead to very different risk estimates.
- The assumption that risk can be expressed as a function of a product of independent parameters lacks phenomenological support. A demonstration is provided that shows that this assumption is generally invalid.
- Even if these problems were not present, such modeling would be unreliable because of model uncertainty. As demonstrated herein, distributions other

than the log-normal distribution can describe the available data on the parameters affecting sensitivity equally well but provide low-dose risk estimates that differ by orders of magnitude from those obtained by using the log-normal distribution.

- These issues remain whether the end point of interest is an apical toxic response or a specified degree of toxicity-pathway perturbation.

In view of these problems, we recommend caution in the use of HVD modeling as a general approach to setting exposure standards for human exposures to toxic chemicals.

Disclaimer: The views expressed in this poster represent those of the authors and do not reflect the views or policies of the U.S. Environmental Protection Agency.

Major challenges to biologically based dose-response modeling for estimating low-dose human risk using molecular toxicology data

Kenny S. Crump,[1] Chao Chen,[2] Weihsueh A. Chiu,[2] Thomas A. Louis,[3] Ravi P. Subramaniam,[2] and Christopher J. Portier[4]
[1]Louisiana Tech University, Ruston, LA; [2]U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment, Research Triangle Park, NC; [3]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; [4]National Institute of Environmental Health Sciences, Research Triangle Park, NC

The strength of recent advances in molecular toxicology arises from the potential to provide information on proximal markers of dose and on early markers of contributions from multiple pathways to diseased states. Biologically based dose-response (BBDR) modeling can incorporate data on biological processes at the cellular and molecular levels to link external exposure to an adverse effect, and such modeling has therefore been suggested by some as a viable link between data generated on toxicity-pathway perturbations and estimates of risk of adverse responses at doses of concern to humans. This poster presents the point of view that there are likely serious impediments to developing BBDR models for this specific purpose. Such models have been used profitably to evaluate proposed mechanisms of toxicity and to identify data gaps. However, the application of these models to the quantitative predictions of low-dose human risk (limited so far to clonal growth modeling for predicting cancer) has not improved the reliability of such predictions despite the extensive effort expended. That is because BBDR models do not eliminate the need for empirical modeling of the relationship between dose and effect but only move it from the whole organism to a lower level of biological organization. Moreover, in doing this, BBDR models introduce significant new sources of uncertainty. Quantitative inferences from the data are limited by inter- and intra-individual heterogeneity that cannot be eliminated with available or rea-

sonably anticipated experimental techniques. BBDR modeling also does not avoid uncertainties in the mechanisms of toxicity relevant to low-level human exposures. We are not recommending against research to develop BBDR models, which have many potential uses. However, we recommend that before a BBDR model is used to set human exposure standards, an evaluation of the robustness of the model predictions in light of limitations and sources of uncertainty described above needs to be performed. Furthermore, efforts to develop BBDR models for risk assessment should consider their resource and time requirements vis-à-vis their potential benefits.

Disclaimer: The views expressed in this poster represent those of the authors and do not reflect the views or policies of the U.S. Environmental Protection Agency.

One vision of the future use of in vitro data in setting human exposure standards—familiar problems and familiar solutions

Kenny Crump[1] and Tom Louis[2]
[1]Louisiana Tech University, Ruston, LA; [2]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

Detailed mathematical models of biological processes have not proved capable of providing useful quantitative information regarding dose-response curve shapes at very low doses. Because we expect that this state of affairs will continue in the foreseeable future, we support the vision of the National Research Council committee that produced *Toxicity Testing in the 21st Century: A Vision and a Strategy* of not recommending development of quantitative estimates of human risk from in vitro data. Rather, efforts should focus on estimating human exposure levels that would not be expected to cause perturbations in normal physiology that could result in adverse health consequences in humans. In considering how this approach is likely to play out in practice, we consider the following idealized situation:

A critical toxic pathway has been identified for a chemical, and dose-response data exist for an in vitro assay for this pathway. A physiologically based pharmacokinetic (PBPK) model is also available that relates human exposure to delivery of the chemical to the critical cells in humans. How should this information be used to set an exposure standard?

This problem is conceptually very similar to what is currently faced in using data from whole-animal tests, and we expect the way forward to be similar. We envision that it will necessarily involve the rather low-technology approach of defining a point of departure (POD), applying safety factors to the POD to arrive at a cell concentration expected not to cause adverse perturbations, and using a PBPK model to relate the resulting concentration at the cell level to human exposure. Setting these factors will involve a considerable dose of scientific judgment. Application of the approach may also require changes in environ-

mental laws. On the basis of experience with attempts to use biologically based dose-response (BBDR) models to quantify dose-response shapes at low doses, we predict that detailed mechanistic models developed with computational systems biology will impact the process qualitatively (perhaps through deciding among broad categories of dose responses that could influence safety factors) rather than provide direct numerical input.

To achieve the goal of protecting sensitive subgroups, we recommend testing many human cell lines from representative populations and from targeted populations that may be especially sensitive to perturbations of specific toxicity pathways.

Strategic direction and application of computational models, -omics, and HTS approaches for the risk assessment of industrial and pesticide chemicals

V. Dellarco, T. Henry, N. Kramek, J. Mclain, P. Sayre, J. Seed, and S. Bradbury
U.S. Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances, Washington, DC

Significant advances have been made in human health and ecological risk assessment over the last decade. Substantial challenges, however, remain in providing credible scientific information in a timely and efficient manner to support risk assessment and risk-management decisions under various statutes (such as the Toxic Substances Control Act, the Federal Insecticide Fungicide and Rodenticide Act, and the Food Quality Protection Act) that the Office of Prevention, Pesticides, and Toxic Substances (OPPTS) is responsible for implementing. A major challenge confronting OPPTS is the need for critical information to address risk uncertainties in large chemical inventories (such as high- and medium-production-volume industrial chemicals and pesticide inert ingredients); these uncertainties result from limited knowledge across chemical classes and their adverse outcomes. That information gap needs to be addressed in a reliable way, yet in a time- and cost-effective manner. Solutions to meeting this challenge include not just the generation of more data faster but also the determination of which chemical- and exposure-specific effects data are essential for managing and assessing the most likely risks. From a strategic and tactical view, elucidating the initiating and key events in critical toxicity pathways is necessary to reduce uncertainties associated with the use of in vitro assays, high-throughput screening (HTS), and in silico approaches. This need is articulated in the 2007 National Research Council report *Toxicity Testing in the 21st Century: A Vision and a Strategy*. Addressing the need and establishing libraries of critical toxicity pathways will provide a substantial basis for using in silico and HTS approaches to predict toxicity credibly, to support priority-setting and screening assessments, and to provide more robust scientific foundations for quantitative risk assessment. This poster describes the knowledge bases and computational

tools OPPTS currently uses in its regulatory program, where the program aims to be in the short term and the long term, and our views of the potential risk-assessment and risk-management applications of computational toxicology.

Disclaimer: The views expressed in this article are those of the authors and do not necessarily reflect those of the U.S. Environmental Protection Agency.

Toxicity-pathway-based mode-of-action modeling for risk assessment

Stephen Edwards,[1] Julian Preston,[1] Andrew Geller,[1] Annie Jarabek,[1,2] Douglas C. Wolf,[1] Elaine Cohen Hubal,[3] Gerald Ankley,[1] Hisham El-Masri,[1] Imran Shah,[3] John R. Fowle III,[1] Jeffrey Ross,[1] John Nichols,[1] Kevin Crofton,[1] Mike Devito,[1] Ram Ramabhadran,[1] Robert Kavlock,[3] Rory Conolly,[3] Sid Hunter,[1] Tom Knudsen,[3] and William Mundy[1]
[1]U.S. Environmental Protection Agency, National Health and Environmental Effects Laboratory, Research Triangle Park, NC; [2]U.S. Environmental Protection Agency, National Center for Environmental Assessment, Research Triangle Park, NC; [3]U.S. Environmental Protection Agency, National Center for Computational Toxicology, Research Triangle Park, NC

In response to the 2007 National Research Council report on toxicity testing in the 21st century, the U.S. Environmental Protection Agency (EPA) has entered into a memorandum of understanding with the National Human Genome Research Institute and the National Toxicology Program to jointly pursue ways to incorporate high-throughput methods into hazard identification and risk characterization. In collaboration with these organizations, EPA researchers are coordinating in vitro, laboratory animal, human, and ecological field studies with computational methods for data analysis and modeling to establish a path forward.

For toxicity-pathway-based risk assessment to become a reality, research must be done to estimate exposure at the cellular level as well as to link perturbations of that toxicity pathway quantitatively to an adverse outcome (an apical end point as currently established in regulatory applications). The first task requires linking environmental concentrations to internal doses at which the toxicity pathways are active and defining the relationship between those internal doses and in vitro concentrations used for high-throughput screening. The second task requires the determination of the key events linking perturbations in the toxicity pathway to the resulting adverse outcome and quantitative in vivo parameter estimates for these key events relative to in vitro toxicity-pathway measurements. This poster describes EPA efforts to address the second task through the establishment of interdisciplinary teams to identify novel toxicity pathways, establish a mode of action (MOA) linking perturbation of a toxicity pathway to adverse outcomes, establish bioindicators of key events in the MOA, collect quantitative in vivo dose-response and time-course data for bioindicators to enable quantitative modeling, and create quantitative models for both human and

ecological risk assessment. Every effort will be made to obtain a subset of bio-indicators from biological samples that can be obtained noninvasively (such as exhaled breath, blood, and urine) for evaluating the MOA in humans and ecologically important species. Quantitative information obtained from the latter studies can also be used to better calibrate the quantitative models for the species of interest. Initial computational models will likely be biologically based dose-response (BBDR) models, which focus on toxicity pathways rather than specific chemicals. However, efforts to create virtual tissues with emergent properties are also under way. Both modeling efforts will build on a network-based MOA framework that can incorporate information from global-biology (-omics) measurements. Finally, special considerations for extending these network-based MOA models to ecological risk assessment will be addressed.

Disclaimer: This abstract does not reflect U.S. Environmental Protection Agency policy.

Tetramethoxystilbene (TMS), an inhibitor of CYP1B1, delays and does not protect MCF-7 cells against benzo[a]pyrene (BaP)-DNA adduct formation

Tracey L. Einem, Miriam C. Poirier, and Rao L. Divi
National Institutes of Health, National Cancer Institute, Center for Cancer Research, Lung Cancer Biomarkers Group, CDI Section, Bethesda, MD

Exposure to carcinogenic polycyclic aromatic hydrocarbons (PAHs) induces cytochrome P450s (CYPs) 1A1 and 1B1, which biotransform PAHs, including benzo[a]pyrene (BaP), into metabolites that form PAH-DNA adducts. We hypothesized that tetramethoxystilbene (TMS), an analogue of resveratrol and a potent CYP1B1 inhibitor, may modulate BaP-DNA adduct levels. At 2- to 12-h intervals during 96 h of exposure, we measured BaP-DNA adducts with chemiluminescence immunoassay (CIA), CYP1A1 and 1B1 gene-expression changes with relative real-time polymerase chain reactions (PCR), and CYP1A1 and 1B1 enzyme activity with ethoxyresorufin-*O*-deethylase (EROD) assay in MCF-7 breast-cancer cells exposed to 1 μM BaP with or without 1 μM or 4 μM TMS. Peak BaP-DNA adduct levels for BaP alone, BaP + 1 μM TMS, and BaP + 4 μM TMS were 1,572, 1,658, and 1,718 adducts/10^8 nucleotides, respectively, and were observed at 16, 24, and 36 h, respectively. Thus, TMS induced a right shift in BaP-DNA adduct formation. Area-under-the-curve ($AUC_{4\text{-}96h}$) values for BaP-DNA adducts were 69,549, 81,603, and 76,778 adducts/10^8 nucleotides for BaP alone, BaP + 1 μM TMS, and BaP + 4 μM TMS, respectively. Fold-increase values for peak CYP1A1 expression observed at 16, 24, and 36 h were 758, 1,713, and 2,995 for BaP alone, BaP + 1 μM TMS, and BaP + 4 μM TMS, respectively. Similarly, fold-increase values for peak CYP1B1 expression were 50, 60, and 83 for BaP alone, BaP + 1 μM TMS, and BaP + 4 μM TMS, respectively, with peaks occurring at the same times as maximum CYP1A1 expression. Peak BaP-induced EROD activities for BaP alone, BaP + 1 μM TMS,

and BaP + 4 μM TMS were 2,443, 3,596, and 6,987 fmoles/min/mg protein, respectively, with peaks observed at 24, 48, and 60 h, respectively. Overall, addition of TMS to BaP-exposed MCF-7 cells slowed BaP-DNA adduct formation, CYP1A1 and CYP1B1 gene expression, and BaP-induced EROD activity. However, compared with BaP exposure alone, total levels ($AUC_{4\text{-}96h}$ values) of BaP-DNA adducts, CYP1A1 and CYP1B1 gene expression, and EROD activity were all increased with the addition of TMS ($p < 0.05$). Although TMS is being considered as a potential chemopreventive agent for estrogen-mediated carcinogenesis, our results here suggest that TMS may increase PAH-induced genotoxicity. Therefore, caution should be exercised when considering TMS or other CYP1B1 inhibitors for chemoprevention.

An approach to using genomic data in risk assessment

Susan Y. Euling,[1*] Lori White,[2] Meric Ovacik,[7] Susan Makris,[1] Banalata Sen,[2] Ioannis P. Androulakis,[3] Susan Hester,[4] Kevin W. Gaido,[5] Andrea S. Kim,[1#] Robert Benson,[6] Vickie S. Wilson,[4] Channa Keshava,[7] Nagalakshmi Keshava,[7] Paul M. Foster,[2] L. Earl Gray Jr.,[4] Chad M. Thompson,[1*] and Weihsueh Chiu[1]
[1]U.S. Environmental Protection Agency, National Center for Environmental Assessment, Washington, DC; [2]National Institute of Environmental Health Sciences, Research Triangle Park, NC; [3]Rutgers University of Medicine and Dentistry of New Jersey, Environmental Bioinformatics and Computational Toxicology Center, Piscataway, NJ; [4]U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Research Triangle Park, NC; [5]The Hamner Institute for Health Research, Research Triangle Park, NC; [6]U.S. Environmental Protection Agency, Region 8, Denver, CO; [7]U.S. Environmental Protection Agency, National Center for Environmental Assessment, Research Triangle Park, NC
**Current affiliation: ToxStrategies, Katy, TX; [#]Current affiliation: Allergan, Inc., Irvine, CA*

Currently, the U.S. Environmental Protection Agency provides no guidance for evaluating and incorporating genomic data into risk assessment. Approaches for using genomic data in risk assessment are needed. A multidisciplinary team of scientists developed an approach that includes evaluating the genomic data for information, examining both genomic and toxicity datasets, defining a set of questions to direct the evaluation, and performing new analyses of genomic data when available. A case study of male reproductive developmental effects of dibutyl phthalate (DBP) was performed and focused on the qualitative use of the available genomic data in risk assessment. The male reproductive developmental toxicology dataset was assessed for low-incidence findings and end points with and without a known mode of action (MOA). Two previously described MOAs for DBP that explain a number of the male reproductive developmental effects observed after in utero exposure in rats are reduced fetal tes-

ticular testosterone production and *Insl3* gene expression. But the DBP toxicology dataset suggests that additional MOAs may be operative. The DBP genomic dataset, composed of data from nine microarray rat studies and other gene-expression studies, was evaluated. The genes whose expression was altered and the direction of effect were fairly consistent across all expression studies. Reanalysis of one microarray study of the testis was performed with the purpose of identifying all pathways affected in the testis after in utero DBP exposure. Preliminary results identified additional pathways in a number of biological processes, including cell signaling, cell adhesion, and growth and differentiation. The newly identified pathways may be associated with testis outcomes that currently lack a known MOA. The DBP genomic dataset elucidated the mechanism and mode of toxicity. Methods to elucidate interspecies differences in toxicodynamics were explored, and preliminary results indicate a relatively high degree of conservation for the steroidogenesis genes, which underlie the reduced testicular testosterone MOA. Research needs, recommendations, and methods for analyzing genomic data for risk-assessment purposes were also identified. The approach for incorporating toxicogenomic data in risk assessment may be used in future chemical assessments. The draft report of this project is currently undergoing external peer review.

Disclaimer: The views expressed are those of the authors and do not represent the policies or endorsement of the U.S. Environmental Protection Agency.

Adverse effects of a common plasticizer on cardiac function

Nikki Gillum,[1] Zaruhi Karabekian,[1] Luther Swift,[1] Ron Brown,[2] Matthew Kay,[1] and Narine Sarvazyan[1]
[1]George Washington University, Washington, DC; [2]U.S. Food and Drug Administration, Center for Devices and Radiological Health, Silver Spring, MD

Di(2-ethylhexyl) phthalate (DEHP) is a widely used plasticizer that is found in a variety of polyvinyl chloride (PVC) medical products. DEHP leaches from PVC tubing and has been linked to cancer and adverse reproductive effects. Currently, little is known of the impact of phthalates on the heart. The effects of DEHP were examined by conducting in vitro experiments with confluent layers of rat neonatal cardiomyocytes. Cells were exposed to 1-50 μg/mL DEHP for 5 min to 4 days. A decrease in conduction velocity was noted as early as 24 h after DEHP treatment at concentrations as low as 1 μg/mL; however, this effect was not attributed to a decrease in cell viability. Prolonged exposure resulted in a further decrease in conduction velocity, cell uncoupling, and asynchronous contractions. The mechanism behind DEHP-induced changes was a loss of junctional connexin-43 documented with western blot analysis, dye-transfer assay, and immunofluorescence. The data suggest that DEHP modifies connexin-43 trafficking and protein assembly into functional gap junctions and

impairs the electrical behavior of a cardiac cell network. Applicability of these findings to human patients remains to be established.

Systems-biology analysis suggests insulin/AKT signaling in compound-induced hemangiosarcoma

Mark M. Gosink, Petra H. Koza-Taylor, Michael P. Lawton, and Jiri Aubrecht
Pfizer Inc., Groton, CT

Systems biology is a young field that attempts to study biological processes in terms of gene networks and pathways. We used a systems-biology technique, geneset enrichment analysis, and the Jackson Laboratory's mutation database to analyze the 16 genes whose mutation results in increased hemangiosarcomas. The analyses indicated a key role of insulin/AKT signaling in hemangiosarcoma development in mice. Activation of AKT signaling has been shown to have a central role in angiogenesis and to be important in the formation of hemangiosarcomas in chickens. In addition, many of the compounds that induce hemangiosarcomas in mice are also linked to the insulin signaling pathway, and some have been shown to activate AKT. Furthermore, the involvement of the AKT signaling pathway in hemangiosarcoma formation points to an explanation of the disparity in response to various compounds seen between species. Previous work has established that phosphoinositide (PI) signaling in mice is distinct from that seen in rats (and humans), with mice having sustained high PI levels. These high levels may result in prolonged AKT signaling in mice with a subsequent increase in the probability of hemangiosarcoma formation. Here we propose a general model linking hemangiosarcomas to the AKT pathway and report how many compounds that are known to induce hemangiosarcomas in mice are tied into this model. We also present data from microarray experiments in mice treated with 2-butoxyethanol (2-BE). 2-BE is a solvent known to induce hemangiosarcomas in mice, but not rats, and is used as a model of compound-induced nongenotoxic hemangiosarcoma. In our model, 2-BE may induce AKT signaling through a local inflammatory mechanism. We present data showing that 2-BE induces the expression of a number of inflammatory and angiogenic markers. Overall, we show that 2-BE induces inflammatory-response genes and propose that this response induces hemangiosarcomas through the AKT signaling pathway.

Improving prediction of chemical carcinogenicity by considering multiple mechanisms and applying toxicogenomic approaches

Kathryn Z. Guyton,[1] Amy D. Kyle,[2] Jiri Aubrecht,[3] Vincent J. Cogliano,[4] David A. Eastmond,[5] Marc Jackson,[6] Nagalakshmi Keshava,[1] Martha S. Sandy,[7] Babasaheb Sonawane,[1] Luoping Zhang,[2] Michael D. Waters,[6] and Martyn T. Smith[2]

[1]U.S. Environmental Protection Agency, National Center for Environmental Assessment, Office of Research and Development, Washington, DC; [2]University of California, School of Public Health, Berkeley, CA; [3]Pfizer Global Research and Development, Drug Safety Research and Development, Groton, CT; [4]International Agency for Research on Cancer, Lyon, France; [5]University of California, Environmental Toxicology Graduate Program and Department of Cell Biology & Neuroscience, Riverside, CA; [6]Integrated Laboratory Systems (ILS), Inc., Research Triangle Park, NC; [7]California Environmental Protection Agency Office of Environmental Health Hazard Assessment, Oakland, CA

Experimental methods for predicting the carcinogenicity of environmental agents have not been substantially updated in decades. These screening methodologies aim to identify genotoxicants under the premise that such agents would most likely pose cancer risk in humans. The emphasis on distinguishing genotoxic from nongenotoxic carcinogens is also motivated by assumed implications for risks at low doses; it is purported that, in contrast with nongenotoxic agents, genotoxicants would lack a threshold in the low-dose region and therefore pose a risk at all exposure levels. However, few data on large, diverse human populations exposed to carcinogens are available to discern the general nature of the dose-response relationship for these two classes of compounds. As an initial step in evaluating current methods and exploring future approaches, we undertook a broad review of recent advances in cancer biology, focusing on scientific evidence of health significance that support regulatory decisions. To provide insight into the mechanisms especially relevant for chemical carcinogenesis in humans, data on eight known human carcinogens (a subset of the 105 agents currently classified by the International Agency for Research on Cancer [IARC] in Group 1—carcinogenic to humans) were examined to assess the potential contribution of a range of possible mechanisms of carcinogenicity. The analysis considered whether chemical carcinogens may act through multiple mechanisms and found that a simple dichotomous characterization regarding whether a chemical is "genotoxic" or "nongenotoxic," although often part of risk assessment approaches, is overly simplistic and does not adequately reflect the range of mechanisms that are likely to contribute to the carcinogenicity of these agents. We also examine the extent to which in vitro, in vivo, and human biomarker studies have the potential to provide mechanistic insights relevant to chemical carcinogenesis. We consider these findings in the context of current approaches to using mechanistic information, including those of IARC and the U.S. Environmental Protection Agency. Our findings underscore the need for methods that better reflect current knowledge, characterize a wider array of hazard traits, improve the ability to predict the potential carcinogenicity of chemicals, and are less time-consuming and costly.

Disclaimer: The views expressed are those of the authors and do not necessarily reflect the policy or endorsement of their affiliated institutions, the California Environmental Protection Agency or the U.S. Environmental Protection Agency.

Opportunities for progress in using mechanistic information in risk assessment: the PPAR-α activation mode-of-action hypothesis revisited

Kathryn Z. Guyton, Weihsueh A. Chiu, Thomas F. Bateson, Jennifer Jinot, Cheryl Siegel Scott, Rebecca C. Brown, and Jane C. Caldwell
U.S. Environmental Protection Agency, National Center for Environmental Assessment, Office of Research and Development, Washington, DC

Data on a chemical's mode of action (MOA) can be critical in decisions in evaluating human health risks. However, there are several challenges in evaluating and applying MOA data for carcinogen risk-assessment purposes. These challenges include determining whether an MOA causes cancer and estimating the likely human sensitivity to an MOA. These issues are elaborated in the present review of the peroxisome proliferator-activated receptor alpha (PPAR-α) activation MOA for the induction of rodent hepatocarcinogenesis by certain chemicals. PPAR-α activation has been posited as a sole causative factor in rodent hepatocarcinogenesis. On the basis of a hypothesized lower human sensitivity to this MOA, it also has been concluded that rodent hepatocarcinogenesis by PPAR-α activators is irrelevant to human carcinogenic risk. Herein we review recent studies that experimentally challenge those suppositions, providing evidence that the plasticizer di(2-ethylhexyl) phthalate is hepatocarcinogenic in PPAR-α-null mice and that the MOA but not hepatocarcinogenesis is evoked by PPAR-α activation in a transgenic mouse model. Our review and analyses raise questions about the hypothesized PPAR-α activation MOA as a sole explanation of rodent hepatocarcinogenesis by PPAR-α agonists and therefore the utility of this hypothesized MOA as a primary basis for assessing human carcinogenic risk from the diverse compounds that activate PPAR-α. These findings suggest opportunities for progress in how MOA hypotheses are developed, tested, and applied in human health risk assessment.

Disclaimer: These views are those of the authors and do not necessarily reflect U.S. Environmental Protection Agency policy or opinion.

Potential role of toxicity-pathway analysis in understanding multiple modes of action in asbestos-induced adverse respiratory health outcomes

Maureen R. Gwinn, Babasaheb Sonawane, and Danielle DeVoney
U.S. Environmental Protection Agency, National Center for Environmental Assessment, Office of Research and Development, Washington, DC

Asbestos-containing materials may release asbestos fibers into the air during product use, demolition work, or building or home maintenance, repair, and remodeling. Adverse health outcomes of asbestos exposure are evident at the molecular level (DNA damage, lipid peroxidation, and so on) and at the tissue and whole-organism level (fibrosis, mesothelioma, lung cancer, and so on). An

overview of the literature was undertaken to identify and explore the potential modes of action of asbestos-induced disease, many of which involve reactive oxygen species (ROSs). Asbestos-induced ROS production potentially from chronic inflammation, surface reactivity, and so on, is associated with multiple modes of action in carcinogenicity (such as genotoxicity and cytotoxicity). We examined evidence on multiple mechanisms of asbestos and how they may contribute to the overall response to asbestos exposure to understand how these mechanisms may lead to the resulting adverse health outcomes. This analysis also examined the response to specific fiber types as an aid to elucidating the key determinants of fiber toxicology. The effect of the mineral form is discussed in terms of type and relative magnitude of biological response. Overall, our analysis finds that chrysotile and amphibole asbestos may contribute to ROS production differentially in terms of both magnitude and potential mechanisms of response. However, the findings highlight the data gaps in determining how different fibers lead to variable downstream molecular events that are likely to result in asbestos-induced disease. These limitations in the available data need to be addressed to understand key differences between fiber types that lead to varied health effects. Defining a toxicity signature profile for particular fiber types with new methodologies, particularly genomics and proteomics, would supply information on signaling pathways involved in response to asbestos exposure. Alternatively, the methodologies may also be used to define signature profiles for specific asbestos-induced disease end points. Information obtained from toxicity-pathway-based analyses of multiple fiber types, target tissues, and adverse health end points will aid in defining determinants of the toxicity of specific fiber types.

Disclaimer: The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.

In vitro screening for chemical toxicity in a genetically diverse human model system

Shannon Hatcher,[1] Oksana Kosyk,[1] Pamela Ross,[1] Fred Wright,[2] John Schwarz,[2] David Dix,[3] and Ivan Rusyn[1]
[1]University of North Carolina at Chapel Hill, Department of Environmental Sciences and Engineering, Chapel Hill, NC; [2]University of North Carolina at Chapel Hill, Department of Biostatistics, Chapel Hill, NC; [3]U.S. Environmental Protection Agency, Research Triangle Park, NC

Immortalized human lymphoblastoid cell lines have been used to demonstrate that genetic polymorphisms control gene expression, that it is possible to use cell lines from related and unrelated individuals to identify the factors that affect the phenotypes in response to xenobiotic treatment, and that there is heritability of gene-expression traits in segregating human populations. Our re-

search aims to extend the application of such studies to investigative toxicology by assessing interindividual variability and heritability of chemical-induced toxicity phenotypes in cell lines from the Centre d'Etude du Polymorphisme Humain (CEPH) trios assembled by the HapMap Consortium. Our goal is to aid in the development of predictive in vitro genetics-anchored models of chemical-induced toxicity. In our initial screen, we treated 87 cell lines from the CEPH trios with 14 environmental chemicals. We applied each chemical in three doses with a replicate of each dose on a 96-well plate, including wells for background and controls. We measured adenosine triphosphate (ATP) production, a measure of cell viability, and caspase-3/7 activity, a marker of apoptosis, 24 h after treatment. We also produced biological replicates for a subsample of the cell lines to evaluate reproducibility of both assays. This experiment demonstrated that variability of response across the chemicals exists for some, but not all, agents, with perfluorooctanoic acid and phenobarbital exhibiting the greatest degree of interindividual variability. At the same time, an appreciable degree of interindividual variability in susceptibility of cell lines to the chemicals was also observed. Although our preliminary assessment of the data shows no significant heritability of toxicity-response phenotypes across these cell lines, genetic factors controlling wide variability in response to some agents need to be addressed. In summary, we show that the approach of screening chemicals for toxicity in a genetically defined, yet variable in vitro system is potentially useful for identification of both agents and individuals that may be at highest risk.

Disclaimer: This work was supported, in part, by U.S. Environmental Protection Agency grant RD-83382501. This work was reviewed by the U.S. Environmental Protection Agency and approved for publication but does not necessarily reflect official agency policy.

Application of an integrated bioinformatics system for pathway-based analysis of toxicity data

S. Isukapalli,[1] D.Gerecke,[1] Y.C. Chang,[1] M. Chen,[2] I. Androulakis,[3] W. Welsh,[4] W. Tong,[2] and P.G. Georgopoulos[1]
[1]Environmental and Occupational Health Sciences Institute, A Joint Institute of the University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School and Rutgers University, Piscataway, NJ; [2]U.S. Food and Drug Administration, National Center for Toxicological Research, Jefferson, AK; [3]Rutgers University, Department of Chemical and Biochemical Engineering, Piscataway, NJ; [4]University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School and Rutgers University, Department of Pharmacology, Piscataway, NJ

The ebTrack system is being developed as an integrated bioinformatics system for toxicological research and consistent analysis of environmental and toxicological data from diverse sources. It is a modular "multitool" platform based on

enhancements to the U.S. Food and Drug Administration's ArrayTrack system. Enhancements include additional modules for pathway-based analysis of gene-expression data, linkages to external systems for analysis of toxicity pathways, and modules for the analysis of proteomic and metabolomic-metabonomic data. An application of ebTrack is presented that focuses on pathway analysis of gene expression after exposure to sulfur mustard (HD or SM), a chemical-warfare agent. In the first phase (Gerecke et al. 2009), ebTrack was applied to study the progression of SM-induced blistering through differential pathway analysis of the time-course data that spanned periods up to 172 h. Pathway analysis using the KEGG library and Ingenuity Pathway Analysis (IPA) indicated that cytokine-cytokine receptor interaction, cell-adhesion molecules (CAMs), and hematopoietic cell lineage are common pathways affected at different times. Gene ontology analysis identified the most significantly altered biological processes as the immune response, inflammatory response, and chemotaxis; these findings are consistent with other reported results for shorter periods. A second phase of analysis, reported here, focuses on additional microarray experiments that were conducted to assess the impact of different inhibitors (MMP-2/MMP-9 inhibitor and ilomastat) on the response to SM exposure. In the case of MMP-2/MM-9 inhibitor pretreatment, subtle but clearly identifiable differences in gene expression were noted, whereas substantial differences were noted in the case of ilomastat pretreatment. Furthermore, significant variability in gene expression profiles was noted in different mice that were of the same sex, age, and body weight. Ongoing analysis focuses on characterizing interindividual variability in time-course profiles of pathway activities in the presence of different candidate countermeasures.

Disclaimer: Support for this work was provided by U.S. Environmental Protection Agency grant GAD R 832721-010, National Institute of Arthritis and Musculoskeletal and Skin Disease grant U54AR055073, and National Institute of Environmental Health Sciences grant P30ES005022. This work has not been reviewed by and does not represent the opinions of the funding agencies.

Metabolic flux analysis: understanding mechanisms of conazole toxicity in cultured hepatocytes

Vidya V. Iyer,[1] Ioannis Androulakis,[1,2] Charles M. Roth,[1,2] and Marianthi G. Ierapetritou[1]
[1]Rutgers University, Department of Chemical and Biochemical Engineering, Piscataway, NJ; [2]Rutgers University, Department of Biomedical Engineering, Piscataway, NJ

The liver, being the major site of xenobiotic metabolism, plays a critical role in xenobiotic biotransformation and clearance. Quantifying the links between central hepatic and xenobiotic metabolism is critical for understanding the systemic response to xenobiotic exposure and for an overall better understanding of the pathogenesis of toxicant-induced hepatic injury. Metabolic flux, the flow

of carbon through metabolic networks, is a crucial indicator of cell physiology. Metabolic-flux analysis (MFA) has found widespread application to characterizing intracellular fluxes and describing cellular states. We propose to use MFA along with extreme pathway analysis to explore the effect of the conazoles (triadimefon, myclobutanil, and propiconazole) on the interaction between central hepatic metabolism and detoxification pathways.

Hepatocytes are plated at a density of 1 million cells in a collagen sandwich configuration that mimics the organization of the liver sinusoid, allowing the cultured hepatocytes to maintain structural integrity and stable, differentiated functions for periods up to 2 months. The medium is changed daily for 3-6 days of stabilization. At this time, they are challenged with media containing conazoles. Following exposure, medium is collected daily for 3 days, and cells are sacrificed each day for viability and damage. Further, concentrations of various metabolites (such as conazole, glucose, urea, albumin, amino acids, fatty acids, and cholesterol) in the media and supernatant are measured to determine the extracellular fluxes. Preliminary dosing experiments have shown that with 0.5 mM triadimefon, cell viability is not affected, but there is a steady drop in urea production from day 1 to day 3 compared with the control. Urea production is an important marker of hepatic function. A drop in urea production indicates active interaction of central metabolism with detoxification pathways. Further, with 1.0 mM triadimefon, the drop in urea production was enhanced and was accompanied by cell death.

By using a metabolic network that depicts hepatic metabolism and conazole detoxification pathways, extracellular measurements, and stoichiometric balances on each metabolite in an MFA framework, intracellular fluxes with varied levels of conazoles can be evaluated. In particular, knowing which pathways respond the most between various treatments could elucidate the involvement of a particular subset responsible for the observed phenotype. Taken together, the findings are expected to have significant impact on our understanding of hepatocellular exposure. Specifically, they will help to validate a novel in vitro and in silico approach to cellular toxicology.

Development of in vitro-based QSAR-PBPK models for prediction of inhalation and dermal kinetics of organic chemicals in humans

E. Kamgang and K. Krishnan
University of Montreal, School of Engineering Sciences and Technology, Montreal, QC, Canada

Quantitative structure-activity relationship (QSAR) models of in vitro data on metabolism rates and partition coefficients (PCs) can potentially facilitate rapid construction of in vivo physiologically based pharmacokinetic (PBPK) models. The objective of the present project was to develop QSAR-PBPK models based on in vitro data on metabolism and PCs and use them as a basis for

predicting the pharmacokinetics of some volatile organic chemicals (VOCs) in humans. For this purpose, data on rat blood:air (Pb) and fat:air (Pf) PCs and intrinsic metabolic clearance (CLint) obtained by using rat liver slices for some C5-C10 VOCs were compiled from the literature. Multilinear additive QSAR models for Pf, Pb, and CLint were developed on the basis of the number and nature of molecular fragments (CH3, CH2, CH, C, C=C, H, benzene ring, and H in benzene ring structure) in these VOCs. By accounting for the difference in the content of neutral lipids between fat and other tissues, the liver:air and muscle:air PCs of the compounds investigated in this study were computed. The predicted partition coefficients were within a factor of 0.77-1.15 of the experimental values. The skin permeability coefficients (Kp) were also calculated on the basis of the octanol:water partition coefficient (log P_{ow}) and molecular weight, whereas the CLint for humans was obtained by fractional body-weight scaling of QSAR-derived CLint in rats. The QSAR algorithms and the equations that constitute the PBPK model were incorporated in a Microsoft EXCEL® spreadsheet. The integrated QSAR-PBPK model adequately simulated the pharmacokinetics of *m*-xylene in humans on the basis of molecular structure for both inhalation exposure (33 ppm, 7 h) and dermal exposure (1 mmol/L air, 20 min). The integrated QSAR-PBPK modeling approach developed in this study would be potentially useful as a tool for predicting pharmacokinetics of organic chemicals in humans in data-poor situations.

Biological profiling of endocrine-related effects of chemicals in ToxCast

R.J. Kavlock, D. Dix, K. Houck, R. Judson, D. Reif, T. Knudsen, and M. Martin
U.S. Environmental Protection Agency, Office of Research and Development, National Center for Computational Toxicology, Research Triangle Park, NC

The Food Quality Protection Act of 1996 mandates that the U.S. Environmental Protection Agency (EPA) implement a validated screening program for detecting estrogenic chemicals and other endocrine targets deemed appropriate by the administrator. EPA's Endocrine Disruptor Screening Program (EDSP) has been developing and validating screening assays for disruption of estrogen (E), androgen (A), and thyroid (T) signaling pathways. The EDSP includes in vitro and in vivo assays for detecting E, A, or T activity; and 73 chemicals have been proposed for initial screening. ToxCast™ is an EPA research program using a broad range of high-throughput screens to profile the bioactivity of chemicals and develop predictive signatures of toxicity on the basis of modeling in vitro assay data to in vivo toxicity phenotypes. ToxCast profiled 56 of the 73 EDSP chemicals, using in vitro assays that characterized receptor binding, activation, inhibition, and target-gene regulation and provided biological fingerprints relevant to E, A, T, and other endocrine-related activities. Of the over 600 ToxCast assays, five assess E, and four each are related to A and T receptor signaling. In addition to E, A, and T end points, ToxCast measured interactions

with progesterone, glucocorticoid, and peroxisome proliferator-activated receptors; aromatase activity; and other nuclear receptors—including AhR, CAR, FXR, LXR, and PXR—that may modulate endocrine metabolism. Many assay targets were human proteins, but in some cases rodent or other species were targeted, affording cross-species comparisons. Results for the prototypic xenoestrogen bisphenol A and the antiandrogen vinclozolin support the ability of ToxCast to identify potential endocrine disruptors, while screening other end points beyond E, A, and T offers broader insights into the bioactivity of the EDSP chemicals.

Disclaimer: Although this work was reviewed by the U.S. Environmental Protection Agency and approved for publication, it may not necessarily reflect official agency policy.

Physiologically based pharmacokinetic and pharmacodynamic model of 4-hydroxyphenylpyruvate dioxygenase inhibition by mesotrione

David Kim and Timothy Pastoor
Syngenta Crop Protection, Greensboro, NC

Purpose: Mesotrione, a member of the triketone family, is a selective herbicide used in the control of broad-leaf weeds. The mechanism of toxicity of this class of compounds in mammals is the specific inhibition of 4-hydroxyphenylpyruvate dioxygenase (HPPD), which can result in a reversible dose- and species-specific increase in plasma tyrosine concentration. The U.S. Food and Drug Administration (FDA) considers plasma tyrosine levels not to be toxicologically significant if below 500 nmol/mL. The aim of this study was to develop a physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model of mesotrione and tyrosine for use in human risk assessments.

Methods: The PBPK model consists of compartments representing the gastrointestinal (GI) tract, liver, kidney, slowly perfused tissues, and central plasma. The PD model for inhibition of HPPD by mesotrione is based on the Jusko et al. (1998) description of the indirect pharmacodynamic response. Time-course data for validation of the PBPK/PD model were obtained from a previously published human volunteer study (Hall et al. 2001).

Results: The PBPK model was optimized by adjusting the parameters for GI absorption, fecal excretion, metabolic clearance, and urinary clearance to fit the plasma and urine time-course data. The PD model was optimized by fitting the zero-order rate constant for production of tyrosine with the first-order rate constant for loss of tyrosine against plasma tyrosine data. Mesotrione's inhibition of HPPD was sigmoidal, from which values of I_{max} (maximal inhibition factor) and IC_{50} (concentration eliciting 50% of the maximum inhibition) were determined. The final PBPK/PD model was used to simulate tyrosine kinetics in children. Mesotrione exposure inputs to the model were calculated by using the U.S. Environmental Protection Agency's default methodology for residential

risk assessment; conservative parameter values were used to calculate exposure. Monte Carlo simulations were performed to examine heterogeneity in plasma tyrosine levels. The simulations, using upper-bound exposure estimates, demonstrated that tyrosine levels in children would be below the FDA threshold of 500 nmol/mL.

Conclusion: We present a mathematical model for mesotrione pharmacokinetics and tyrosine pharmacodynamics that is coupled via inhibition of HPPD. This investigation revealed that rigorous application of PBPK/PD modeling, combined with qualitative understanding of the mode of action, can be used to evaluate potential risks associated with mesotrione exposures in human populations. The use of PBPK/PD models in quantifying perturbations of toxicity pathways is a robust science that can be applied, as appropriate, to human health risk evaluations.

Predictive modeling of developmental toxicity using the U.S. Environmental Protection Agency's virtual embryo

Thomas B. Knudsen,[1] Richard S. Judson,[1] Michael Rountree,[1] Richard M. Spencer,[2] and Amar V. Singh[2]
[1]*U.S. Environmental Protection Agency, Office of Research and Development, National Center for Computational Toxicology, Research Triangle Park, NC;*
[2]*Lockheed Martin, contractor to the National Center for Computational Toxicology, Research Triangle Park, NC*

Standard practice in prenatal developmental toxicology involves testing chemicals in pregnant laboratory animals of two species, typically rats and rabbits, that were exposed during organogenesis and evaluating for fetal growth retardation, structural malformations, and prenatal death just prior to term. Phenotypic heterogeneity that often follows from disruption of molecular function, cellular processes, and signaling pathways in the embryo poses a major challenge to understanding mechanisms. The compendium of biological signatures mined from the U.S. Environmental Protection Agency (EPA) ToxCast™ high-throughput screening assays can be mapped to in vivo end points in prenatal developmental-toxicity studies (ToxRefDB). An initial evaluation of about 300 chemicals and about 500 assays returned well over 400 significant associations with developmental end points. We observed an increase in the number of predictive associations for developmental end points in the general rank order of fetal weight reduction to skeletal defects to soft tissue abnormalities to prenatal losses. The associations included 70-75 canonical pathways (Ingenuity, KEGG) having at least five significant ($p \leq 0.05$) assay-end point predictors within a pathway. The associative pattern was evident despite the fact that initial ToxCast™ in vitro assays were not run on embryonic systems. Furthermore, the diversity of perturbed pathways signifies complex downstream sequelae that must be connected to embryogenesis. EPA's new virtual embryo (v-Embryo™)

project is providing this framework, using cell-based computational models of morphogenesis that accept data on biological pathways and relevant knowledge of the developing system to simulate dysmorphogenesis. Successful computational (in silico) models will eventually be useful to explore which of the diverse biological pathways, signaling networks, and morphogenetic processes best characterize sensitive systems at susceptible stages of pregnancy.

Disclaimer: This work has been reviewed by the U.S. Environmental Protection Agency and approved for publication but does not necessarily reflect official agency policy.

Dose-response pathway analysis for gene-expression microarrays

Zhen Li,[1,2] Ivan Rusyn,[2,3] and Fred A. Wright[1,2]
[1]University of North Carolina at Chapel Hill, Department of Biostatistics, Chapel Hill, NC; [2]University of North Carolina at Chapel Hill, The Carolina Environmental Bioinformatics Center, Chapel Hill, NC; [3]University of North Carolina at Chapel Hill, Department of Environmental Sciences and Engineering, Chapel Hill, NC

When performing dose-response studies in which transcriptional response is measured by using expression microarrays, it is of interest to test the coordinated involvement of transcripts from known biological pathways or functional categories. Existing gene-set testing methods are not well suited to dose-response studies, in which it is desirable to make a global summary of transcriptional response of an entire pathway while preserving false-positive rates for testing. We propose an extension of the Significance Analysis of Function and Expression (SAFE) approach applied to dose-response studies. Dose-response modeling of individual transcripts is applied and used to build a summarized response curve across a pathway or category. Hypothesis testing for pathway involvement is performed by using permutation and bootstrapping and requires fast methods to assess the statistical significance of individual transcripts. We describe extensive evaluation of a number of fast curve-fitting techniques with the goal of preserving false-positive rates, even for small samples sizes, while maintaining reasonable power for large sample or effect sizes. Summarized dose-response profiles for entire pathways are described with bootstrap-based confidence envelopes. Applications to simulated and real datasets are used to show the value of our approach.

Development of in vitro toxicogenetic models for hepatotoxicity

Stephanie Martinez,[1] Blair Bradford,[1] Todd Stewart,[2] Valerie Soldatow,[2] Kirsten Amaral,[2] Stephen Ferguson,[2] Chris Black,[2] Edward LeCluyse,[2] and Ivan Rusyn[1]

[1]University of North Carolina at Chapel Hill, Department of Environmental Sciences and Engineering, Chapel Hill, NC; [2]CellzDirect/Invitrogen Corporation, Durham, NC

Numerous studies support the fact that a genetically diverse mouse population may be useful as an animal model to understand and predict toxicity in humans. We hypothesized that cultures of hepatocytes obtained from a large panel of inbred mouse strains can produce data indicative of interindividual differences in in vivo responses to hepatotoxicants. To test the hypothesis and establish whether high-throughput in vitro studies using cultured hepatocytes from genetically distinct mouse strains are feasible, we aimed to standardize cell isolation and culture conditions, determine whether the near-physiological maintenance of the cells isolated from different mouse inbred strains can be achieved, and assess whether the reproducibility of functionality can be attained in a given strain over subsequent isolations. Hepatocytes were isolated from 15 strains of mice and cultured for up to 7 days in traditional 2D culture. The cells were assessed for viability and functionality on a daily basis by measuring production of lactate, pyruvate, and urea and leakage of lactate dehydrogenase. We also used calcein and ethidium fluorescence staining to assess cell viability at 1, 3, 5, and 7 days of culture. Our data show that high yield (48-87 million hepatocytes/mouse) and viability (86-98%) can be achieved across a panel of strains. Total RNA was isolated from the cells harvested on days 1 and 3 of culture and reverse transcription polymerase chain reaction (RT-PCR) analysis was carried out to evaluate mRNA levels representative of liver-specific genes. Furthermore, we conclude that cell function of hepatocytes isolated from different strains and cultured under standardized conditions is comparable and that cells remain viable and metabolically active as indexed by lactate, pyruvate, and urea production. These experiments open new opportunities for high-throughput and low-cost in vitro assays that may be used for studies of toxicity in a genetically diverse population.

Disclaimer: This work was supported in part by National Institute of Environmental Health Sciences grant R01-ES015241 and U.S. Environmental Protection Agency grant RD-833825.

Using the ToxMiner™ database for identifying disease-gene associations in the ToxCast™ dataset

Holly Mortensen, David Dix, Keith Houck, Robert Kavlock, and Richard Judson
U.S. Environmental Protection Agency, National Center for Computational Toxicology, Office of Research and Development, Research Triangle Park, NC

The U.S. Environmental Protection Agency (EPA) ToxCast™ program is using in vitro high-throughput screening (HTS) to profile and model the bioactivity of environmental chemicals. The main goal of the ToxCast program is to generate predictive signatures of toxicity that ultimately provide rapid and cost-

effective methods to set priorities among chemicals for targeted in vivo testing and thus improve the efficiency of the use of animals in those bioassays. The chemicals selected for phase I are composed largely of a diverse set of pesticide active ingredients, whose EPA registration process included sufficient supporting in vivo data. These were supplemented with a number of nonpesticide, high-production-volume chemicals of environmental concern. Application of HTS to environmental toxicants is a novel approach to predictive toxicology and differs from what is required for drug-efficacy screening in several ways. Biochemical interaction of environmental chemicals is generally weaker than that seen with drugs and their intended targets. Additionally, the chemical diversity space covered by environmental chemicals is much broader than that of pharmaceuticals. The ToxMiner™ database was created to link biological, metabolic, and cellular pathway data to genes and in vitro assay data for the chemicals screened in the ToxCast phase I HTS assays. Also included in ToxMiner was human disease information, which correlated with ToxCast assays that target specific genetic loci. We have implemented initial pathway inference and network analyses, which allow linkage of the types of adverse health outcomes with exposure to chemicals screened in phase I. This approach permits exploration of disease at a higher level of cellular and organismal organization, focusing on multiple, related disorders, and may aid in the understanding of common disease outcomes (such as cancer and immune disorders) that are characterized by locus heterogeneity. Through the use of the ToxMiner database and the analysis framework presented here, we hope to gain insight into relationships between potential disease states in humans and environmental chemicals and to contribute to the larger goals of toxicogenomics by clarifying the role of gene-environment interactions in pathobiology.

Disclaimer: Although this work was reviewed by the U.S Environmental Protection Agency and approved for publication, it may not necessarily reflect official agency policy.

Computational xenobiotic metabolism prediction system for toxicity assessment

Fangping Mu and Helen H. Cui
Los Alamos National Laboratory, Los Alamos, NM

Biotransformation is the process whereby a substance, usually a foreign compound (xenobiotic), is chemically transformed in the body to form a metabolite or a variety of metabolites. Chemical transformations can activate a xenobiotic, rendering it toxic, or can alter a xenobiotic to a nontoxic species.

Expert systems represent state-of-the-art xenobiotic metabolism prediction systems. These systems are rule-based systems designed to identify functional-group transformations that occur in known reactions and then, by generalizing, to formulate reaction rules for global application. The rules can provide reason-

able prediction of all possible metabolite formation. However, they commonly predict many more metabolites than are observed experimentally. Ranking of the possibility of metabolite formation is still not consistently available.

To overcome the significant number of false positives in rule-based systems for metabolism prediction, we investigated machine-learning technology for xenobiotic metabolism prediction. We collected human xenobiotic reactions from Elsevier MDL's Metabolite Database and classified reactions according to rules based on functional-group biotransformations. For each reaction rule, the reaction center can be well defined and is represented as a molecular substructure pattern by using SMARTS, which is a language for describing molecular patterns. Using the SMARTS patterns, we identified potential reaction centers for each reaction class by using the identified metabolites in Elsevier MDL's Metabolite Database. Each set of potential reaction centers was divided into negative and positive examples. More than 23 atomic properties were used to model the topological, geometric, and electronic and steric environment of the atoms in the molecule. More than 42 molecular properties were used to model the shape, surface, energy, and charge distribution of the molecule. Support vector machines were used to separate the positive and negative examples in each reaction class. A total of 36 biotransformations were modeled. Results show that the overall sensitivity and specificity of classifiers are around 87%.

To demonstrate the relevance of metabolism to toxicity, we used epoxide formation as an example. Epoxide hydrolase detoxifies molecules that have an epoxide moiety by hydrolyzing the epoxide to a diol. However, some stable epoxides are unsuitable substrates for this enzyme. We collected stable epoxides from Toxnet (http://toxnet.nlm.nih.gov/) and found 489 chemicals that contain epoxide moieties. The metabolism prediction model for epoxide hydrolysis predicted that only 24, or 4.9%, would be hydrolyzed enzymatically. Prediction of metabolism with this method can enhance the accuracy of toxicity assessment.

A comparison of multiple methods to evaluate biphasic (hormetic) dose responses in high-throughput in vitro toxicology screens

Marc A. Nascarella[1,2,*] and Edward J. Calabrese[2]
[1]*Gradient Corporation, Cambridge, MA;* [2]*University of Massachusetts, Division of Environmental Health Sciences, Amherst, MA*
[*]Presenting author

We describe several methods for evaluating the low-dose response in in vitro drug screens. This presentation focuses on previous work in which the response of yeast exposed to over 2,100 putative anticancer agents in a high-throughput drug screen was studied (Calabrese et al. 2006, 2008; Nascarella et al. 2009). We describe a methodology to evaluate the fundamental shape of the dose-response curve to determine whether there is nonrandom biological activity below the toxic threshold (Calabrese et al. 2006). We also show how the toxic

threshold using a benchmark-dose (BMD) procedure was estimated and then how the distribution of responses at concentrations below the estimated toxic threshold was evaluated. This approach is followed by the description of a second methodology that uses a complementary but separate evaluation to determine the average magnitude of response and the distribution of mean responses for the putative anticancer agents (Calabrese et al. 2008). We selected concentration-response studies that had concentrations below the estimated BMD to determine the average magnitude of response below a toxic threshold. This analysis is novel in that we use a linear mixed model to predict the average response in the low-concentration zone for each anticancer agent. We describe how we used the average response for each of the anticancer agents using the best linear unbiased prediction (BLUP) or empirical Bayes approach (presented with prediction intervals). This assessment provides a more accurate prediction of the true chemical mean response than a simple mean (because the regression toward the mean affects only chemicals whose predictor differs from the mean, and not the mean itself). In a third line of inquiry, we demonstrate how we quantified the individual hormetic concentration responses by measuring the width of the hormetic zone, the interval from the maximum stimulatory concentration to the toxic threshold, and the amplitude of the maximum stimulation (Nascarella et al. 2009). We describe the advantages and disadvantages of using these multiple evaluation schemes in determining biological activity below the toxic threshold.

Application of toxicogenomics to develop a mode of action for a carcinogenic conazole fungicide

S. Nesnow,[1] J. Allen,[1] C. Blackman,[1] P.-J. Chen,[2] Y. Ge,[1] S. Hester,[1] L. King,[1] P. Ortiz,[1] J. Ross,[1] S.-F. Thai,[1] W. Ward,[1] W. Winnik,[1] and D. Wolf[1]
[1]U.S. Environmental Protection Agency, National Heath and Environmental Effects Research Laboratory, Research Triangle Park, NC; [2]National Taiwan University, Department of Agricultural Chemistry, Taipei, Taiwan

Conazoles are a common class of fungicides used to control fungal growth in the environment and in humans. Some of these agents have adverse toxicological outcomes in mammals as carcinogens, reproductive toxins, and hepatotoxins. We coupled the results of genomic analyses with traditional laboratory investigations (in toxicology, molecular biology, and biochemistry) to propose a mode of action (MOA) for the carcinogenic activity of propiconazole in mouse liver. A key element of the approach was to use activity-inactivity pairs of conazoles. This allowed the sequestration of the genomic results toward the toxicologic end points and provided a rapid method to identify genes, pathways, and networks that could be responsible for the observed toxic effects. Conazoles are designed to inhibit CYP51; this is a central step in the biosynthesis of ergosterol in fungal systems and of ergosterol, cholesterol, vitamin D, and the sex steroids

in mammalian systems. Conazoles are pleiotropic—they can both induce and inhibit mammalian CYPs, and these characteristics help to explain their varied toxic activities. We performed both dose-response and time-course studies in mice to develop and characterize key events in the MOA that can describe the propiconazole-induced carcinogenic process. These studies provided data on the following series of key events in the carcinogenic MOA of propiconazole: nuclear receptor activation, CYP induction, decreases in hepatic retinoic acid levels, increased oxidative stress, decreases in serum cholesterol levels, increases in mevalonic acid levels, increased cell proliferation, decreased apoptosis, and induction of in vivo mutagenicity. Those key events have been synthesized into an MOA that describes the carcinogenic process induced by propiconazole in mouse liver.

Disclaimer: This abstract does not represent U.S. Environmental Protection Agency policy.

Use of toxicogenomic data at the U.S. Environmental Protection Agency to inform the cancer assessment of the fungicide propiconazole

N. McCarroll,[1] E. Reaves,[1] M. Manibusan,[1] D. C. Wolf,[2] S. D. Hester,[2] J. Allen,[2] S-F. Thai,[2] J. Ross,[2] Y. Ge,[2] W. Winnik,[2] L. King,[2] C. Blackman,[2] W.O. Ward,[2] and S. Nesnow[2]
[1]U.S. Environmental Protection Agency, Office of Pesticide Programs, Washington, DC; [2]U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Office of Research and Development, Research Triangle Park, NC

The U.S Environmental Protection Agency (EPA) Office of Pesticide Programs (OPP) routinely uses mode-of-action (MOA) data, when they are available, for pesticide cancer risk assessment. An MOA analysis incorporates data from required toxicology studies and supplemental mechanistic data. These data are evaluated to identify a set of key events, quantifiable and critical steps, in the pathway to tumor development. EPA has considered genomic data as part of the weight of the evidence (WOE) in support of an MOA. However, to expand this effort, standard approaches are being developed to include toxicogenomic data and data from other new technologies into the risk-assessment process. Conazoles are antifungal pesticides used for the protection of fruit, vegetable, and cereal crops and as pharmaceuticals for the treatment of fungal infections. Antifungal activity is exerted through inhibition of a specific cytochrome, CYP51, a critical step in the biosynthesis of ergosterol, a steroid required for formation of fungal cell walls. Many conazoles induce hepatotoxicity and liver tumors. A toxicogenomic dataset has been developed for the mouse liver tumorigen propiconazole. The objective of this study was to determine how toxicogenomic data could inform MOA analysis and the interpretation of human relevance. Toxicogenomic data, supplemental tissue-response information, molecular and bio-

chemical studies, and traditional registration studies were used to determine the value of applying genomic data to the MOA analysis. Postulated key events based on genomic and experimental studies include nuclear receptor activation, CYP induction, cholesterol inhibition, oxidative stress, altered retinoic acid and mevalonic acid levels, and in vivo mutagenicity. Those key events were organized into a hypothesized MOA that explains the tumorigenic response to propiconazole. The EPA cancer risk assessment guidance was used to integrate genomic data into the risk assessment. This study shows how toxicogenomic data can inform our understanding of cancer and increase the efficiency and accuracy of a risk assessment.

Disclaimer: The views expressed in this abstract do not necessarily reflect those of the U.S. Environmental Protection Agency.

Prediction of in vivo dose-response relationship from in vitro concentration-response relationship using cellular-level PBPK modeling

Thomas Peyret and Kannan Krishnan
Département de Sante Environnementale et Sante au Travail, Université de Montréal, Montréal, Québec, Canada

There is still a lack of appropriate tools to extrapolate the results of in vitro toxicity tests to in vivo conditions, particularly for risk-assessment purposes. In this study, multicompartmental models describing the in vitro and in vivo systems were developed and evaluated by using toluene as the model substrate. The in vivo and in vitro models consist of four components (cell and interstitial space for the tissue and plasma and erythrocytes for the vascular components) and two components (cell and culture medium), respectively. Cell:culture medium (EMEM), cell:blood, interstitial:blood, and tissue:blood partition coefficients (PCs) used in the in vitro or in vivo models were derived from medium (EMEM, cell, interstitial space, plasma, and erythrocyte):water PCs. The medium:water PCs in turn were calculated on the basis of the fractional content and extent of toluene uptake into the neutral lipid, phospholipid, water, and protein components of the media. The free concentration of toluene in neutral lipids, neutral phospholipids, and water was calculated on the basis of its solubility (oil:water PC). The toluene binding to hemoglobin was calculated from the free concentration in the microenvironment and hemoglobin:microenvironment PC (derived from blood:air data). The in vitro toluene concentration in the neuroblastoma culture system was calculated by multiplying the EMEM concentration (McDermott et al. 2007. Toxicol in vitro 21:116-124) by the cell:EMEM PC. The in vivo brain cell concentration of toluene was calculated from the cell:blood and the concentration in blood in the human PBPK model adopted from Tardif et al. (1997. Toxicol appl pharmacol 144:120-134). The PBPK model was used to extrapolate the toluene concentration-response relationship from an in vitro SH-SY5Y cell-viability study to in vivo conditions. The EMEM:air PC predicted by the in vitro model was 78% of the value measured

by McDermott et al. (2007). The human exposure concentrations of 132, 231, 350, 647, and 1,011 ppm provided the same brain-cell concentrations at steady state as the in vitro concentrations of 5.64 μM (no-observed-adverse-effect level), 13.1 μM (lowest observed-adverse-effect level), 22.5 μM, 46.6 μM, and 76.3 μM.

This work was supported by a research grant from AFSSET—L'Agence française de sécurité sanitaire de l'environnement et du travail.

Dissecting enzyme regulation by multiple allosteric effectors using the random sampling-high dimensional model representation algorithm

Joshua Rabinowitz,[1,2] Jennifer Hsiao,[1] Kimberly Gryncel,[3] Evan Kantrowitz,[3] Xiaojiang Feng,[1] Genyuan Li,[1] and Herschel Rabitz[1]
[1]Princeton University, Department of Chemistry, Princeton, NJ; [2]Princeton University, Lewis-Sigler Institute for Integrative Genomics, Princeton, NJ; [3]Boston College, Department of Chemistry, Merkert Chemistry Center, Chestnut Hill, MA

The random sampling-high dimensional model representation algorithm (RS-HDMR) serves to extract complex relationships within multivariable systems. It is a completely data-driven algorithm that can reveal linear, nonlinear, independent, and cooperative relationships from random sampling of the target variables with favorable scalability. RS-HDMR has been applied to a variety of systems in chemistry, physics, biology, engineering, and environmental sciences. In this study, it is used to dissect the combinatorial allosteric regulation of the enzyme aspartate transcarbamoylase (ATCase, EC 2.1.3.2 of *Escherichia coli*).

ATCase catalyzes the committed step of pyrimidine biosynthesis and is allosterically regulated by all four ribonucleoside triphosphates (NTPs) in a nonlinear manner. In this work, ATCase activity was measured in vitro at 300 random NTP concentration combinations, each involving (consistent with in vivo conditions) all four NTPs being present. These data were then used to derive an RS-HDMR model of ATCase activity over the full four-dimensional NTP space. The model accounted for 90% of the variance in the experimental data. Its main elements were positive ATCase regulation by adenosine triphosphate (ATP) and negative regulation by cytidine triphosphate (CTP), with the negative effects of CTP dominating the positive ones of ATP when both regulators were abundant (a negative cooperative effect of ATP x CTP). Strong sensitivity to both ATP and CTP concentrations occurred in their physiological concentration ranges. Uridine 5'-triphosphate (UTP) had only a slight effect, and guanine triphosphate (GTP) had almost none. These findings support a predominant role of CTP and ATP in ATCase regulation. The general approach provides a new paradigm for dissecting multifactorial regulation of biological molecules and processes.

Parallel analysis of activation of the cellular stress-response system for rapid evaluation of environmental toxicants

Steven Simmons,[1] David Reif,[2] and Ram Ramabhadran[1]
[1]U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Research Triangle Park, NC; [2]U.S. Environmental Protection Agency, National Center for Computational Toxicology, Research Triangle Park, NC

Determining the toxic potential and mode of action (MOA) of environmental chemicals is a cost- and labor-intensive endeavor that has traditionally involved the use of laboratory animals. These considerations have necessitated the development of efficient in vitro, cell-based approaches that can permit the rapid analysis of chemical toxicities in a high-throughput mode as envisioned in the 2007 National Research Council report *Toxicity Testing in the 21st Century: A Vision and a Strategy*. Although assay technologies developed in the drug-discovery field provide the appropriate tools for this approach, a strategy for toxicity evaluation based on screening against specific cellular targets is impractical because of the large numbers of such putative targets and the large numbers of chemicals (and other products, such as nanomaterials) that require screening. To address those issues, we are developing a small ensemble (fewer than 10) of rapid and inexpensive reporter-gene assays based on the well-characterized cellular stress-response pathways. These adaptive pathways are activated in a coordinated fashion on exposure to environmental insults in an attempt by the cell to maintain or re-establish homeostasis. Although the pathways are activated at very low doses of toxicants, they also trigger terminal events, such as apoptosis and other adverse effects, when the cell is damaged irreversibly. Those characteristics make them ideal sentinels for rapid and sensitive in vitro screening of toxicants that is amenable to the high-throughput modality.

We are in the process of characterizing the integrated response of this stress assay ensemble to chemical toxicants to validate its use as a means of grouping chemicals that produce similar biological responses and also to infer their MOA. Luciferase-based reporter-gene assays consisting of promoters derived from the stress-pathway target genes or artificial promoters based on specific stress-response elements have been incorporated into lentiviral vectors that allow their rapid and stable delivery to a wide variety of cell types, both primary and established. Using this approach, we have determined the stress signatures of a set of toxic and nontoxic metals and those of other compounds and developed an approach for graphic representation of the signatures to facilitate visual comparison. We anticipate that this approach will be useful for determining the MOA of environmental toxicants.

Disclaimer: This is an abstract of a proposed presentation and does not necessarily reflect U.S. Environmental Protection Agency policy.

The application of cellular systems biology to create a "safety risk index" for potential drug-induced adverse events

D. Lansing Taylor
Cellumen, Inc., Pittsburgh, PA

The purpose of a cellular systems biology (CSB™) approach to safety profiling is to address the complexity of "systems" responses to perturbations that involve multiple cellular pathways, organelles, and mechanisms of action. Not only does this approach begin to define the biology of potential adverse events, but it enables improved prediction of subsequent effects in vivo. A systems approach also facilitates early target selection and optimization of chemistry around both safety and efficacy end points by using very small amounts of precious drug substance early in candidate selection. The handoff from discovery to development is consequently more effective and, not surprisingly, results in overall reduced attrition. The CellCiphr® approach involves (1) the use of the relevant cells representing the major rodent and human organs, (2) panels of organ-specific functional biomarkers multiplexed by using fluorescence detection with high-content screening readers, (3) a growing database of reference drugs on which there are both safety data and CellCiphr® profile data, and (4) the safety risk index generated with classifier software. Profiles are generally performed at three time points to establish 10-point dose-response curves using over 10 cellular functional biomarkers in a 384-well format. Currently, there are separate panels of human HepG2 cells, primary rat hepatocytes, and rat cardiomyocytes. A human primary hepatocyte panel is being completed. In addition, a variety of both human and rodent stem-cell-derived cell systems are being evaluated for the major organs, including liver, heart, kidney, brain, immune system, and gastrointestinal tract in 2-D and 3-D architectures.

Early studies have demonstrated that a CellCiphr profile shows predictive power not evident in simpler cell-based assays using the standard "ROC" curve analyses based on over 230 compounds in the database. The database has now grown to over 500 compounds. The safety risk index was used retrospectively to set priorities among lead series, such as the "glitazones" that demonstrated the value of CellCiphr for priority-setting. The ability to define mechanisms of action has also been demonstrated in profiling a group of non-steroidal anti-inflammatory drugs. When the biologically rich data are used, key signaling pathways are flagged that define the mechanisms of action that are responsible for potential toxic liabilities and adverse events. Finally, it has been demonstrated that the CellCiphr can flag as "high risk" those drugs that have been withdrawn or marked with a "black box" warning.

Using the CellCiphr approach will result in improved early safety profiling, improved and powerful human predictivity, mode-of-action and biomarker identification, and, most important, overall reduction of safety-related attrition.

Metabolomics in risk assessment

Suryanarayana V. Vulimiri
U.S. Environmental Protection Agency, National Center for Environmental Assessment, Office of Research and Development, Washington, DC

Exposure to a toxic chemical is often reflected in the perturbation of several cellular events in which biochemicals of a given metabolic pathway are upregulated, downregulated, or unaffected. Cellular responses exhibited by both experimental animals and humans to toxic exposure are complex, but they undergo similar types of metabolic change that lead to adverse outcomes. Metabolomics is an emerging technology that uses high-throughput methods to simultaneously identify, quantify, and characterize low-molecular-weight (<1800 Da) biochemicals from numerous metabolic pathways. Several modes of action (MOAs) of toxic chemicals—such as oxidative stress, inflammation, cell proliferation, and cell damage or cytotoxicity—can be studied with the metabolomic approach. For example, oxidative stress, a common cellular perturbation caused by exposure to environmental insults, could be measured by the changes in the ratios of reduced glutathione (GSH) and oxidized glutathione (GSSG) in the GSH biosynthesis pathway. Other MOAs—such as inflammation, cell proliferation, and cytotoxicity—are identified by measuring the levels of arachidonate, ornithine, and *o*-phosphoethanolamine, respectively. The MOAs can be identified by the biochemical profile of the toxic response in vitro and in vivo. In population-based studies, the metabolomic approach can also be used for analyzing the putative biomarkers in body fluids, such as blood, plasma, serum, and urine. A comprehensive mechanism-based interpretation of information obtained through the metabolomics approach can be used to improve understanding and to advance the MOA-based assessment of environmental risks to human health. Information from the metabolomics approach can also be combined with genomics information in molecular characterization of the hazards that cause perturbations in different toxicity pathways. Overall, the metabolomic approach is used as a noninvasive method to identify metabolic profiles and putative biomarkers as early predictors of toxicity and disease.

Disclaimer: The views expressed are those of the author and do not necessarily reflect the U.S. Environmental Protection Agency's opinion or policy.

Formaldehyde and leukemia: epidemiology, potential mechanisms, and implications for risk assessment

Luoping Zhang,[1] Laura Beane-Freeman,[2] Jun Nakamura,[3] Stephen S. Hecht,[4] John Vandenberg,[5] Martyn T. Smith,[1] and Babasaheb R. Sonawane[6]
[1]University of California, Berkeley, School of Public Health, Berkeley, CA; [2]National Institutes of Health, National Cancer Institute, Division of Cancer Epidemiology and Genetics, Bethesda, MD; [3]University of North Carolina at

Chapel Hill, Department of Environmental Sciences and Engineering, Chapel Hill, NC; [4]University of Minnesota, Masonic Cancer Center, Minneapolis, MN; [5]U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessments, Research Triangle Park, NC; [6]U.S. Environmental Protection Agency, Washington, DC

Formaldehyde is widely used in commerce. Although it is regulated in many countries, occupational and environmental exposure to formaldehyde can remain quite high. Formaldehyde is an established human carcinogen (nasopharyngeal cancer) and may be associated with an increased risk of leukemia in exposed individuals. However, risk assessment of formaldehyde and leukemia has been challenging because of inconsistencies in human and animal studies and the lack of a known mechanism for leukemia induction. As part of the 39th Annual Environmental Mutagen Society Meeting in October 2008, a special symposium was held at which an up-to-date review of the epidemiology and potential mechanisms of formaldehyde and leukemia and their implications for risk assessment were presented. Updated results of two of the three largest industrial cohort studies of formaldehyde-exposed workers have shown positive associations with leukemia, particularly myeloid leukemia. A more recent meta-analysis of studies also supports the association. Recent mechanistic studies show the formation of formaldehyde-DNA adducts after formaldehyde exposure and the need for specific DNA repair (Fanconi Anemia-BRCA) pathways to protect against formaldehyde toxicity. Together, the updated findings suggest the need for future studies that more effectively assess the risk of leukemia arising from formaldehyde exposure. It was recognized that increased communication among scientists who practice epidemiology, toxicology, biology, and risk assessment could enhance the design of such studies, and specific recommendations that could lead to greater understanding of the issue were made. A toxicogenomic approach in experimental models and human exposure studies, with the measurement of biomarkers of internal exposure, such as formaldehyde-DNA and protein adducts, should prove fruitful. Currently, only limited tools are available to measure such adducts in blood, bone marrow, and other target tissues specifically, so their development is urgently needed. Adducts and relevant DNA-repair pathways should be examined in the bone marrow of mice treated with formaldehyde-generating chemicals and in human progenitors exposed to formaldehyde in vitro. Use of transgenic mice deficient in DNA-repair genes could further facilitate the exploration of this area. In conclusion, there are many opportunities to incorporate knowledge from multiple disciplines that could be used to reduce uncertainty in the risk assessment of formaldehyde and leukemia.

Disclaimer: The views expressed are those of the authors and do not necessarily reflect the views, policies, or endorsement of their affiliated institutions, the National Cancer Institute, or the U.S. Environmental Protection Agency.