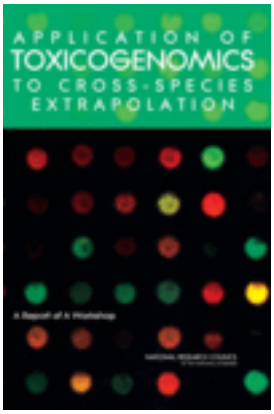

Application of Toxicogenomics to Cross-Species Extrapolation: A Report of a Workshop

Committee on Applications of Toxicogenomics to Cross-Species Extrapolation, Committee on Emerging Issues and Data on Environmental Contaminants, National Research Council

ISBN: 0-309-65613-3, 58 pages, 6 x 9, (2005)

APPLICATION OF TOXICOGENOMICS TO CROSS-SPECIES EXTRAPOLATION

Committee on Applications of Toxicogenomics
to Cross-Species Extrapolation

Committee on Emerging Issues and Data
on Environmental Contaminants

Board on Environmental Studies and Toxicology

Board on Life Sciences

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. N01-OD-4-2139 between the National Academy of Sciences and National Institute of Environmental Health Sciences. 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 0-309-10084-4

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. Wm. A. Wulf 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. Wm. A. Wulf are chair and vice chair, respectively, of the National Research Council.

www.national-academies.org

COMMITTEE ON APPLICATIONS OF TOXICOGENOMICS TO CROSS-SPECIES EXTRAPOLATION: A WORKSHOP

Members

N. LEIGH ANDERSON *(Chair)*, Plasma Proteome Institute, Washington, DC
JAMES S. BUS, Dow Chemical Company, Midland, MI
DAVID L. EATON, University of Washington, Seattle
SERRINE S. LAU, University of Arizona, Tucson
JOHN A. MOORE, Hollyhouse, Inc., Wicomico Church, VA
JOHN QUACKENBUSH, Harvard School of Public Health, Boston, MA
KENNETH S. RAMOS, University of Louisville, Louisville, KY

Staff

MARILEE SHELTON-DAVENPORT, Project Director
ROBERTA WEDGE, Project Director
NORMAN GROSSBLATT, Senior Editor
JENNIFER E. SAUNDERS, Research Associate
MIRSADA KARALIC-LONCAREVIC, Research Associate
ALEXANDRA STUPPLE, Senior Editorial Assistant
LUCY V. FUSCO, Senior Program Assistant
JORDAN CRAGO, Senior Program Assistant
SAMMY BARDLEY, Librarian

Sponsor

NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES

COMMITTEE ON EMERGING ISSUES AND DATA ON ENVIRONMENTAL CONTAMINANTS

Members

KENNETH S. RAMOS (*Chair*), University of Louisville, Louisville, KY
N. LEIGH ANDERSON, Plasma Proteome Institute, Washington, DC
PATRICIA A. BUFFLER, University of California, Berkeley
JAMES S. BUS, Dow Chemical Company, Midland, MI
JOSEPH DEGEORGE, Merck Pharmaceuticals, West Point, PA
GEORGIA M. DUNSTON, Howard University, Washington, DC
LINDA E. GREER, Natural Resources Defense Council, Washington, DC
ROBERT J. GRIFFIN, Marquette University, Milwaukee, WI
JOHN D. GROOPMAN, Johns Hopkins University, Baltimore, MD
CASIMIR A. KULIKOWSKI, Rutgers University, New Brunswick, NJ
AMY D. KYLE, University of California, Berkeley
PHILIP LEDER, Harvard Medical School, Boston, MA
PETER LORD, Johnson & Johnson, Raritan, NJ
WILLIAM B. MATTES, Gene Logic, LLC, Gaithersburg, MD
GEORGE ORPHANIDES, Syngenta, LLC, Cheshire, UK
FREDERICA P. PERERA, Columbia University, New York, NY
JOHN QUACKENBUSH, Harvard School of Public Health, Boston, MA
MARK A. ROTHSTEIN, University of Louisville School of Medicine, Louisville, KY
LEONA D. SAMSON, Massachusetts Institute of Technology, Cambridge
PETER S. SPENCER, Oregon Health Sciences Institute, Portland
LAWRENCE SUNG, University of Maryland, Baltimore
CHERYL L. WALKER, University of Texas, Smithville
RUSSELL D. WOLFINGER, SAS Institute, Inc., Cary, NC

Staff

MARILEE SHELTON-DAVENPORT, Project Director
KARL GUSTAVSON, Project Director
EILEEN ABT, Program Director
NORMAN GROSSBLATT, Senior Editor
JENNIFER E. SAUNDERS, Research Associate
LUCY V. FUSCO, Senior Program Assistant
JORDAN CRAGO, Senior Program Assistant

Preface

Toxicogenomics has been described as a discipline combining expertise in toxicology, genetics, molecular biology, and environmental health to elucidate the response of living organisms to stressful environments. It includes, but is not limited to, the study of how genomes respond to toxicant exposures and how genotype affects responses to toxicant exposures. As the technology rapidly develops, it is critical that scientists and the public communicate about the promises and limitations of this new field. Despite the dependence on animal models in toxicologic research for predicting human health effects in the regulatory arena, there can be important differences between how animals and humans respond to different chemicals. The Committee on Applications of Toxicogenomics to Cross-Species Extrapolation designed a workshop to consider using toxicogenomics in cross-species extrapolation from animals to humans. The workshop reflected on the promises and limitations of emerging data-rich approaches—such as genotyping (genomics), mRNA analysis (transcriptomics), protein analysis (proteomics), and metabolite analysis (metabolomics)—to inform cross-species extrapolation. Specifically, the workshop considered whether the data-rich technologies offer new ways of determining whether the effects of chemicals in test animals can be used to predict human responses.

This workshop report has been reviewed in draft form by persons chosen for their diverse perspectives and technical expertise in accordance with procedures approved by the National Research Council's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published workshop report as sound as possible and to ensure

that the report 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 wish to thank the following people for their review of this workshop report: Susan Sumner, RTI International; Jonathan H. Freedman, Duke University; Kevin W. Gaido, CIIT Centers for Integrated Genomics; and Frank A. Witzmann, Indiana University School of Medicine.

Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations, nor did they see the final draft of the workshop report before its release. The review of the workshop report was overseen by Rogene Henderson, of the Lovelace Respiratory Research Institute. Appointed by the National Research Council, she was responsible for making certain that an independent examination of the workshop report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of the workshop report rests entirely with the committee and the institution.

The committee gratefully acknowledges the following for making presentations at the workshop: John L. Butenhoff, 3M Company; Frank A. Witzmann, Indiana University School of Medicine; William H. Benson, Stephen Nesnow, and Kerry L. Dearfield, U.S. Environmental Protection Agency; Richard T. Di Giulio, Duke University; Donna Mendrick, Gene Logic Inc.; Susan Sumner, RTI International; and Russell Thomas, CIIT Centers for Health Research.

The committee is grateful for the assistance of the National Research Council staff in preparing this workshop summary: Marilee Shelton-Davenport, Roberta Wedge, and Karl Gustavson, project directors; James Reisa, director of the Board on Environmental Studies and Toxicology; Fran Sharples, director of the Board on Life Sciences; Jennifer Saunders and Mirsada Karalic-Loncarevic, research associates; Jennifer Roberts, postdoctoral research associate; Norman Grossblatt, senior editor; Lucy Fusco and Jordan Crago, senior program assistants; and Sammy Bardley, librarian.

Finally, I thank the members of the committee for their dedicated efforts throughout the development of this workshop summary.

N. Leigh Anderson
Chair, Committee on Application of
Toxicogenomics to Cross-Species Extrapolation

Contents

APPENDIXES

APPLICATION OF TOXICOGENOMICS TO CROSS-SPECIES EXTRAPOLATION

Summary of the Workshop

INTRODUCTION

Data on adverse effects of chemicals on humans can be acquired through epidemiologic studies and from occupational, inadvertent, accident-related, or other exposures, such as the airborne exposure that followed the World Trade Center attacks of September 11, 2001. However, intentional human testing of environmental chemicals is limited, and the available human data are generally insufficient for making regulatory decisions, because they do not shed enough light on relevant health issues. Reliance on experimental animal data is a cornerstone of toxicology and risk assessment, and responses observed in experimental animals are commonly assumed to be predictive of responses in humans. Regulatory agencies and industry rely on animal data to make health and safety decisions about exposure to and intake of chemicals from food, drugs, and the environment.

Although experimental animal data often constitute the only available predictor of human health effects, their predictive ability is limited. There are numerous differences between experimental animal and human responses to chemicals, including differences in the types of adverse effects experienced and the dosages at which they occur. The differences may reflect variations in the underlying biochemical mechanisms or in the distribution of the chemicals. It can be expensive or detrimental to public health if experimental animal models are not good predictors of

human health effects, so it is critical to select and validate animal models early in the regulatory process. Critical differences in how humans and experimental animals respond to chemicals may not be identified until after considerable testing has been conducted.

Toxicogenomics has been described as "an emerging discipline that combines expertise in toxicology, genetics, molecular biology, and environmental health to elucidate the response of living organisms to stressful environments" (Ramos 2003). Scientists in the field use new technologies to simultaneously assess the coordinated expression of genes in response to a particular chemical exposure ("transcriptomics"). They also look at how individual and species differences in the underlying DNA sequence itself can result in different responses to the environment ("genomics"). The "-omics"[1] part of "toxicogenomics" also encompasses several other types of profiling technologies including protein profiling (proteomics) and metabolite profiling in a cell or tissue (metabonomics). Toxicogenomics potentially can provide faster and less-expensive methods for predicting differences between experimental animal and human responses to chemicals.

The National Academies standing Committee on Emerging Issues and Data on Environmental Contaminants, sponsored by the National Institute of Environmental Health Sciences, provides a forum for communication among scientists and regulators in government, industry, environmental groups, the academic community, and the general public about topics at the forefront of toxicogenomics. The objective of the workshop reported here was to explore some of the scientific challenges and promises of applying toxicogenomic information to the extrapolation of animal data to humans. A workshop planning committee was formed to organize the August 12, 2004, workshop on Applications of Toxicogenomics Technologies to Cross-Species Extrapolation at the National Academies in Washington, DC.

This summary contains highlights of the workshop. Opinions expressed are those of the speakers, individual committee members, and other participants but do not represent the viewpoint of the National Academies or a consensus of any National Academies committee. PowerPoint presentations of the speakers are available at the standing committee's Web site (http://dels.nas.edu/emergingissues/index.asp). In

[1]The term "-omics" is used in this report to refer to various types of global analytical approaches, such as genomics, transcriptomics, metabolomics, and proteomics.

addition, recordings of speakers' talks (except those of Donna Mendrick and Susan Sumner) and other discussions are available at http://dels.nas.edu/emergingissues/index.asp. The workshop agenda and biosketches of the speakers and workshop planning committee members are included as appendixes.

David L. Eaton, of the University of Washington, chair of the standing committee and a member of the Application of Toxicogenomics to Cross-Species Extrapolation workshop planning committee, and N. Leigh Anderson, of the Plasma Proteome Institute, chair of the Application of Toxicogenomics to Cross-Species Extrapolation workshop planning committee, introduced the topic of cross-species extrapolation and explained the objectives of the workshop. The potential uses of toxicogenomic technologies in cross-species extrapolation were the topics of discussion. The objective of the workshop was to consider the promises and limitations of emerging data-rich approaches—such as genotyping (genomics), mRNA profiling (transcriptomics), protein profiling (proteomics), and metabolite profiling (metabolomics)—to inform cross-species extrapolation, particularly whether the effects of chemicals in test animals can be used to predict human responses.

A basic premise of toxicology and risk assessment is that experimental animals are generally appropriate models with which to identify potential chemical hazards to humans. However, the reliability of extrapolation for particular chemicals is often controversial. Despite the dependence on animal models for predicting human health effects in the regulatory arena, there can be important differences between how nonhuman animals and humans respond to chemicals. Much of the workshop discussion focused on mammalian species, but it is acknowledged that other toxicologic test species, such as zebrafish and the nematode *Caenorhabditis elegans*, can also provide a large body of information on many environmental toxicants.

Anderson introduced the question, "How good can cross-species extrapolation ever be?" The question could be attacked with two broad strategies. The first is understanding the biologic mechanisms of action of individual compounds from two perspectives: (1) whether identifying the dominant mechanism in one species, such as rats, will be sufficient to extrapolate results to another species, such as humans, and (2) whether all the potential toxicity mechanisms in a species can be characterized in enough detail to build a predictive model for humans. Anderson acknowledged that the latter concept might stretch the limits even of systems biology. The second, more practical strategy is to use in vitro sys-

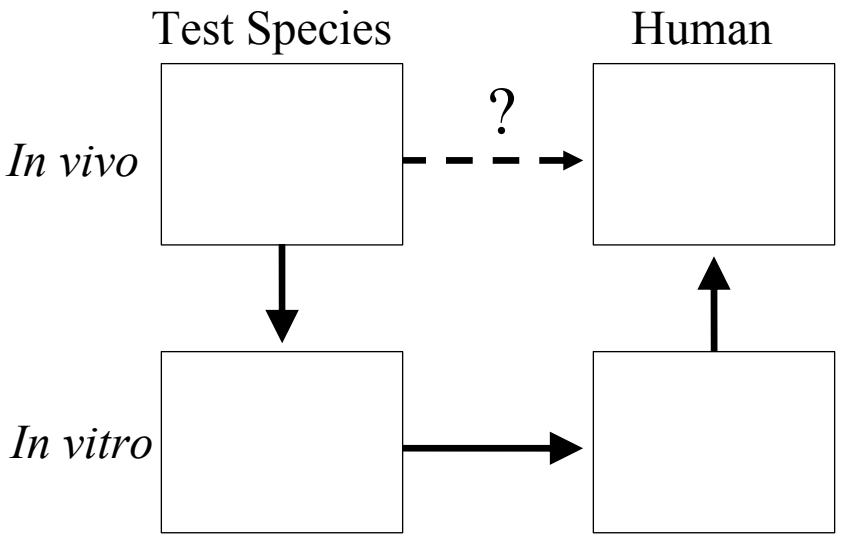

FIGURE 1 Extrapolation through in vitro systems.

tems. That strategy, illustrated in Figure 1 and discussed further below, uses in vitro systems for comparison of test species with humans. To function in this way, in vitro systems need to replicate sufficiently the in vivo characteristics of interest in the test species and in humans.

EMERGING MOLECULAR AND COMPUTATIONAL APPROACHES FOR CROSS-SPECIES EXTRAPOLATION

Richard Di Giulio, of Duke University, presented highlights of a 2002 report he and William H. Benson of the U.S. Environmental Protection Agency (EPA) edited, *Interconnections Between Human Health and Ecological Integrity* (Di Giulio and Benson 2002). The report drew several conclusions regarding the biologic bases of similarities and differences between humans and other animals: "1) Omic technologies will enhance understanding; 2) Extrapolations among levels of organization [are the] most critical and complete challenge; and 3) Advanced mathematics and modeling may provide a pivotal approach [to exploring the biologic bases of differences]."

A more recent workshop was organized by Di Giulio and Benson on the topic of cross-species extrapolation. The joint Society of Environmental Toxicology and Chemistry-Society of Toxicology Pellston (SETAC-SOT) workshop was held on July 19-22, 2004. The goals of the workshop were "to understand and enhance utility of -omics and computational biology in order to: 1) elucidate similarities and differences among species, 2) relate stressor-mediated responses to phenotype, 3) extend this science into innovative approaches for risk assessment and regulatory decision-making, and 4) develop the 'interconnections between human health and ecological integrity' paradigm."

The final workshop conclusions and recommendations on those topics will be released in the report of that workshop, expected in 2005. Di Giulio highlighted several overall workshop themes that may be apparent in the report: -omics technologies are not a replacement for traditional toxicology approaches in the foreseeable future, proof-of-concept studies are needed, more standardized approaches for conducting -omics assays and analyzing data are needed, -omics databases are needed for selected surrogate species, studies are needed to link -omics responses to adverse effects seen in experimental animals ("phenotypic anchoring"), and there is a need for enhanced training to produce cross-disciplinary scientists. In summary, SETAC-SOT workshop participants thought that genomic and computational approaches collectively will greatly enhance the ability to address many of the major issues in human and environmental toxicology. Specifically, the new technologies will provide unique approaches to address cross-species extrapolation in risk assessment in both human and environmental toxicology.

POTENTIAL IMPLICATIONS OF GENOMICS FOR RISK ASSESSMENT

Benson discussed potential implications of genomics for regulatory and risk assessment applications at EPA, focusing largely on a white paper on genomics and risk assessment that EPA released in March 2004 (Dearfield and Benson 2004; EPA 2004). The white paper describes how -omics may provide new approaches to old problems but notes that -omics data today are insufficient for risk assessment, although they may play a role in a weight-of-evidence analysis. It also explores different EPA perspectives on how the agency might use -omics data, providing

an overview of genomics for EPA risk assessors and managers on how -omics data will fit into their work. The document identifies anticipated regulatory and risk assessment applications and implications, provides an overview of current agency science activities in -omics that may support regulatory scenarios, and identifies scientific and research needs. -Omics data may be useful for screening and priority setting, especially if -omics responses are linked to adverse outcomes. Linking -omics data to exposure may also be helpful for biomonitoring—for example, tracking of pathogen sources. The white paper also discusses how -omics data could trigger reporting requirements.

Benson discussed how -omics might be useful in risk assessment in elucidating a chemical's mode of action (MOA), identifying and assessing effects on susceptible populations and life stages, and assessing mixtures. -Omics might be helpful with MOAs, for example, by elucidating pathways and contributing to predictive models. -Omics data might also increase the confidence in cross-species extrapolation if genes or patterns of gene expression are conserved between humans and test-animal species or support a conclusion of nonrelevance to humans if there is little or no similarity. Benson also asked when it makes sense to use wildlife data to improve human risk assessment.

Benson highlighted challenges specific to cross-species extrapolation. For example, there are differential dosimetry issues—different dose-response relationships. Animal physiology and environment will affect exposures to chemicals, and metabolic pathways can differ substantially among species. There are also various degrees of homology among species in genes, proteins, biochemistry, and physiology.

Benson next described EPA's Computational Toxicology Research[2] Program in the Office of Research and Development. The program is centered on what is referred to as the source-to-outcome continuum (Figure 2). The general objectives of the program are to improve the linkages in the source-to-outcome continuum, provide predictive models for screening and testing chemicals, and enhance quantitative risk assessment. The hope is that -omics can contribute to each of the boxes in the continuum and enhance quantitative risk assessment. The current effort is on endocrine-disrupting chemicals as a proof of concept for this approach to computational toxicology in general, including cross-species extrapolation.

[2]Computational toxicology is the application of mathematical and computer models for prediction of effects and the understanding of MOAs.

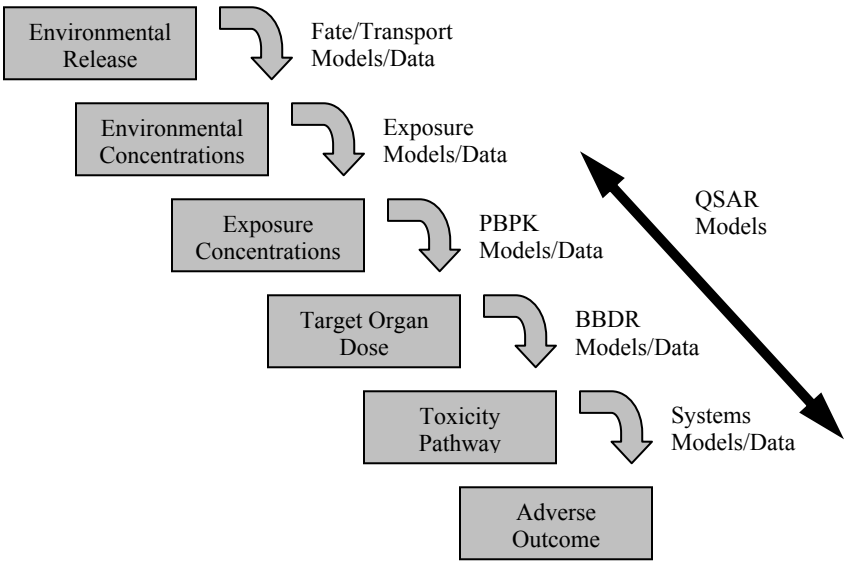

FIGURE 2 The source-to-outcome continuum. PBPK = physiologically based pharmacokinetics; QSAR = quantitative structure-activity relationship; BBDR = biologically based dose-response. Source: EPA 2003.

James S. Bus, of the Dow Chemical Company, and Di Giulio discussed how there are long-term opportunities to improve risk assessment, perhaps through different paradigms. Di Giulio's statement, that -omics will not replace traditional toxicologic approaches sparked discussion. Participants seemed to agree that not *all* traditional toxicologic testing could be replaced with -omics technologies, but they were hesitant to say that *none* would be replaced as the technology progressed. Studies that might not be amenable to -omics approaches include metabolic enzyme kinetics, for example. Kinetics are unlikely to be captured by current genomic or proteomic approaches, and pharmacokinetic studies in general might be difficult to replace with new -omic approaches, according to Eaton. The discussion concluded with Benson, Bus, and Di Giulio noting that -omics studies do not have to replace traditional studies to be useful but instead should be considered as "value-added" tests.

One participant pointed out that the use of toxicogenomic information would depend on whether this field of research is viewed from the perspective of the precautionary principle (that is, erring on the side of caution). Eaton agreed that that is important and could affect how in-

formation, such as a change in a transcription profile might be interpreted by the regulated community and the regulators. That is, if the precautionary principle were invoked, a given change in a transcription profile might be more likely to be considered an adverse effect.

TECHNOLOGICAL CHALLENGES OF CROSS-SPECIES EXTRAPOLATION USING PROTEOMICS

Frank A. Witzmann, of Indiana University, discussed technological challenges of cross-species extrapolation using proteomics. He explained that characterization of the proteome for a given target tissue and species is something that needs to be done first. Although proteomic toxicity testing needs to be done in the context of other -omics technologies, it is important to remember that most toxicologic studies include time-course and dose-response analyses, so sample numbers generally are high, and this places a burden on the proteomic technology. The types of proteomic responses to a toxicant that Witzmann discussed are quantitative changes in protein expression and differential posttranslational modification of proteins.

Different platforms, or technical approaches, are available for proteomic analysis. For example, two-dimensional gel electrophoresis (2DE) works well for several reasons. It has enormous resolving power: of the 5,000-10,000 proteins present in a cell, about 2,000 can be resolved in a single electrophoretic run. That allows detection of small changes in the concentrations or properties of proteins and the isolation of proteins in quantities sufficient for structural analysis. 2DE also works with the large number of samples that would be generated in a toxicology experiment with different exposure times and dosages. Witzmann emphasized that proper experimental design is needed to compare protein expression and posttranslational modification between species (for example, to extrapolate results from rat to human). Experiments could be designed to initially derive results from rat in vivo exposures, replicate them with cultured rat cells, and then attempt to replicate them in cultured human cells. Such an experimental design is frequently mentioned in this report (for example, see Figure 3).

As mentioned above, 2DE enables quantitative assessment of protein expression. Protein expression under different conditions can be compared with computational models. For example, Witzmann described how the protein profiles generated by exposure to structurally

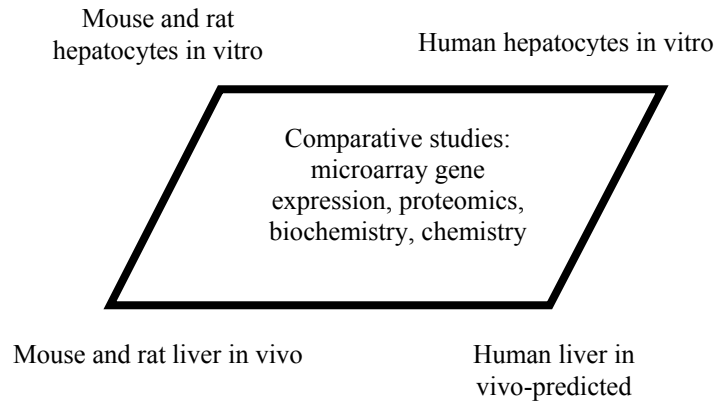

FIGURE 3 Interspecies comparisons and human relevance—a parallelogram approach. Source: Nesnow 2004. Reprinted with permission of the author.

diverse peroxisome proliferators and halocarbons can be compared with such computational modeling approaches as integrated quantitative structure-activity relationships (I-QSAR) analysis. He thinks that these modeling concepts could be applied to cross-species comparisons when homologous protein systems exist. A good portion of the challenge in doing this is bioinformatic. Accurate characterization of toxicologically relevant proteins—for example, with respect to homologous pathways, receptors, metabolism, and bioactivation—and determination of common toxic end points (such as oxidative stress and glutathione depletion) are required. That is, these techniques will work best when similar pathways are involved in similar or common toxic responses.

Witzmann was asked about the commonalities between the in vitro rat proteome and the human proteome. He said that "the 2D protein patterns seem very similar, but these expression profiles need to be analyzed more comprehensively and in a coherently designed way."

MODELING GENE-EXPRESSION DATA TO PREDICT HUMAN HEPATOTOXICITY AFTER INCONSISTENT ANIMAL RESPONSES

Donna Mendrick, of Gene Logic, outlined how her company has used toxicogenomic approaches to analyze the relevance of some tradi-

tional toxicologic data to human health. Specifically, pathologic results were seen in one laboratory animal species but not another. Gene Logic is trying to use -omics to learn whether the pathologic observations are specific to the one laboratory animal species—that is, whether they are relevant to human health. She emphasized that she was presenting the -omics findings as a case study of what can be learned from -omics technologies and hoped to hear participants' views on how much -omics data are needed to affect a conclusion about human health.

Mendrick reviewed the case study in which proprietary Compound X was being developed for clinical use by a pharmaceutical company. The compound did not suggest problems in traditional toxicity studies with rats. However, in dogs, liver fibrosis developed after 3 months of daily administration. Gene Logic's goal was to discover a mechanistic explanation for the species-specific toxicity observed in dogs to determine whether the toxicity is likely to occur in humans. The researchers used Affymetrix microarrays to measure changes in gene transcription in rat and canine livers exposed to Compound X in vivo.

It appeared that the dog genes dysregulated by exposure to Compound X were consistent with the fibrosis observed in dogs. However, that type of gene dysregulation was not observed in rat liver. For example, the fibrosis genes differentially dysregulated between dog and rat liver included INHBE (activin beta E).

To determine whether these canine genes might be relevant to human fibrosis, Gene Logic's BioExpress database of normal and diseased human tissue samples was used to compare genes dysregulated in the dog liver with genes dysregulated in humans who had liver fibrosis. Researchers found that some of the genes whose expression changed in the dog were changed in the diseased human samples as well. For example, the expression of INHBE is relatively liver specific in tissues from healthy rats and humans. However, the gene is also detectable in the dog *heart*, so researchers asked whether variations in INHBE expression suggested differences in regulation between species. Next, compound X was compared with phenobarbital, because phenobarbital was identified by the Gene Logic predictive models as similar to compound X in its effects on gene expression. Phenobarbital induces transcription of drug-metabolizing genes in rats and causes liver enlargement. Some reports indicate that phenobarbital may cause liver fibrosis in dogs, but it is generally considered nonhepatotoxic in humans and rats. (It does, however, raise flags because it has the potential to affect the metabolism of other compounds.) Mendrick compared the genes dysregulated in rat and dog

liver after phenobarbital treatment with those dysregulated after compound X treatment. Her conclusion was that compound X dysregulates many of the same genes as does phenobarbital in the rat and dog but that both affect few genes in common between species.

In summary, looking at the whole of what was learned with the phenobarbital comparisons and the liver fibrosis difference in humans, Mendrick concluded that the data might suggest that the gene dysregulation in the dog did not predict human health liabilities and that compound X might warrant further investigation. She pointed out that it would be useful to consider how much detail is needed to explain species differences; that is, what is sufficient to "make the case."

USING METABOLOMICS TO EXPLORE SPECIES DIFFERENCES IN METABOLISM AND DISTRIBUTION

Susan Sumner, of Paradigm Genetics,[3] discussed metabolism, metabolomics, and cross-species extrapolation. Metabonomics is defined as "the quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification"—in other words, assessing the changes in endogenous compounds as a function of which biologic indicator is being examined, such as toxicity or, in the case of a drug, efficacy (Nicholson et al. 1999). It involves assessing the changes in low-molecular-weight endogenous compounds as functions of the end point being examined. *Metabolomics*, *metabonomics*, and *metabolite profiles* are used interchangeably by many.

Investigators are using a number of analytical tools to assess endogenous metabolites, such as nuclear magnetic resonance spectroscopy and mass spectrometry coupled with chromatographic separation methods. Some methods look for particular classes of metabolites; others are less targeted. Methods looking at specific classes of metabolites, such as lipids, often focus the metabolomics effort toward specified pathways. The less-targeted methods are particularly useful for identifying new metabolic pathways and biologic mechanisms, because they enable investigators to discover events outside the proposed or known interactions.

Sumner began her presentation by noting that metabolism studies are advantageous for developing indicators of exposure, effect, or sus-

[3]Sumner is now at RTI International.

ceptibility, especially because low-molecular-weight metabolites can be found in urine and serum, two biologic fluids whose collection is relatively noninvasive in human studies. Biomarkers can be discovered directly in human biologic fluids or discovered in rodent models and applied to the study of human fluids. For example, physiologically based pharmacokinetic (PBPK) models developed with rodent data can be validated by using biomarkers in rodent fluids. After validation, the models developed from animal studies can be extrapolated to humans and then validated on the basis of markers measured in human fluids.

Sumner cautioned that metabolomics will not replace the examination of traditional end points in ADME (absorption, distribution, metabolism, and excretion) studies, nor will metabolomics determine the metabolic fate of a xenobiotic. Metabolomics is complementary to ADME studies, in that it provides information regarding how endogenous metabolite concentrations are altered as a function of a chemical exposure. We already know that chemicals can interact with endogenous compounds (for example, by forming conjugates with glutathione), so we can expect introduction of a chemical to result in alteration of the endogenous metabolite profiles. However, care has to be taken in interpretation of alterations in endogenous metabolite profiles, because an endogenous metabolite concentration may be affected by age, sex, time of sample collection, or dietary intake. Conducting studies at multiple concentrations and with multiple end points can help in deducing the subset of metabolites that are corollary to the effect being studied.

Examining biologic pathways is one approach to using metabolomics in cross-species extrapolation, because most mammalian biochemical pathways are conserved. Of course, not all pathways are fully conserved, and the flux through pathways can vary among test animal species. As an example of how cross-species comparisons work, Sumner discussed a study in which she and colleagues developed metabolite biomarkers of exposure to the compound hexachlorapropane (HCP). The study involved assaying the urine and expired breath of HCP-exposed rats for HCP metabolites, with the eventual goal of using those metabolites as biomarkers of human exposure. While conducting the study, her group also noted that concentrations of an endogenous metabolite were altered as a function of HCP exposure. Once they determined that the metabolite was ascorbic acid, they recognized that it may not be useful as a human biomarker, because, unlike rats, humans do not synthesize ascorbic acid. However, the identification of ascorbic acid as a rat biomarker does suggest that the glucuronic acid pathway might be important in

identifying biomarkers that do reflect human exposure or disease. This example illustrates how considering the pathways rather than just the individual metabolites themselves can be a useful approach.

A wealth of literature has begun to establish the usefulness of metabolomics in providing biomarkers for staging disease or predicting toxicity. The usefulness of metabolomics in developing biomarkers of a specific chemical- or disease-induced event is best understood. Its usefulness in mechanistic studies and particularly in cross-species extrapolation is not as well developed. The use of metabolomics in cross-species extrapolation will require a thorough understanding of the *metabolome* (all the low-molecular-weight metabolites in each specific sample) and the conservation of metabolomes across species. Given that differential metabolic profiles (such as those developed for urine) can be influenced by factors such as sex, age, strain, ethnic background, or even time of sample collection, it is likely that metabolomics can be used to develop models for extrapolating across species.

Understanding the metabolome will require going beyond the metabolites and their relationships described in conventional resources such as reference books and databases to include other metabolites that are not described in such resources. To that end, reference databases are now being developed to catalog metabolites on the basis of such factors as species, age, sex, strain, or even specific cell type. Linking these databases to analytical measurement tools and biologic outcomes will enable the identification of trends in metabolite profiles that can be associated with disease states or other effects of interest. The metabolites making up the specific profiles associated, for example, with a disease state can then be mapped to metabolic pathways for mechanistic association. However, mapping to metabolic pathways is not always straightforward, because a given metabolite can map to a number of pathways, and the specific pathways affected are not always known. Furthermore, some novel metabolites may not map to any currently defined pathway. Thus, there is a need to develop the mathematical tools for determining trends in the data that associate with response and also to determine the convergence of the trends in the context of biochemical mechanisms.

In summary, Sumner believes that the use of metabolomics in cross-species extrapolation will depend on many of the same factors that have been conventionally used to develop biomarkers of exposure, effect, and susceptibility. Studies will have to be designed to examine the effects of xenobiotics in multiple species for correlation to the effect of interest. For example, study designs may incorporate sensitive and non-

sensitive species, effect and no-effect doses, or transgenic models where susceptibility is understood. It is likely that the validation or understanding of a marker profile will be developed only after conducting such cross-species comparisons. She emphasized that in extrapolating across species, looking at specific metabolites may not provide the most informative biomarkers; rather, a comparison of metabolites from convergent pathways may provide better correlation with the response of interest. Finally, Sumner concluded her discussion by asking the audience to think about the possible advantages of using human urine and serum data to validate models of metabolism, pharmacokinetics, dynamics, MOA, and compartmentalization.

Eaton asked Sumner how the huge dietary variations in humans, compared with diet-controlled rodents, are taken into consideration in metabolism studies. Summer explained that there are two basic ways to handle the issue of human diet. The first is to do a human study with a controlled diet, to examine the effect of diet on metabolite profiles. The second is to assume that the disease state or adverse effect will overwhelm the effect of dietary differences on the metabolic profiles.[4] There are also data-reduction tools and knowledge bases of metabolites from dietary sources that can help to distinguish between exposure to a xenobiotic and a diet effect.

Sumner was also asked to comment on how human variability in metabolites should be factored into metabolomic studies. She replied that there is interindividual variability in metabolites, such as those found in urine, and intraindividual variability over time, but they have not received much consideration.

A SYSTEMS-BIOLOGY APPROACH TO CROSS-SPECIES EXTRAPOLATION

Russell Thomas, of the CIIT Centers for Health Research, discussed a systems-biology approach to cross-species extrapolation. To introduce why he believes that a systems approach is important for mechanism-based risk assessment, Thomas outlined two fundamental ways of determining how species can differ in their responses to a toxic

[4]After the workshop, a study was published on the feasibility of using metabolomics in clinical studies without dietary restrictions. It pointed to the need to carefully consider the influence of diet and culture when using metabolomics to interpret biomarkers (Lenz et al. 2004).

agent: pharmacokinetics, how much of a dose gets to a tissue, and pharmacodynamics, how the tissue responds once the dose reaches it. He explained how physiologically based models work relatively well for estimating pharmacokinetic differences between species but that there is not a good way to predict pharmacodynamic differences between species. Thomas proposed that our inability to predict pharmacodynamic differences among species is because of a lack of understanding of the cellular signaling networks involved and that a systems-biology approach could address this knowledge gap.

Thomas defines systems biology as "the quantitative description of an organism based on the assembly of individual components into subsystems of increasing complexity and organizational hierarchy," an approach that relies on integrating experimental and computational methods. Thomas described four steps that he believes are important in implementing a systems-biology approach in toxicology: identify the potential molecular targets of a toxic agent, determine how the targets and other molecules interact to form a signaling pathway, elucidate how the pathways interconnect to form a network, and describe the system quantitatively to provide information about the shape of the dose-response curve. Ultimately, understanding the molecules in the network and their quantitative interactions will provide insight into how well a given response is conserved across species.

In addressing the first of the four steps, Thomas described the application of large-scale gain-of-function and loss-of-function genomic screens to identify genes that play a functional role in a given pathway and may also represent potential targets of perturbation by a toxic agent. Cell-based assays are constructed by engineering cells to express a fluorescent or luminescent reporter gene when a specific signaling pathway is activated. In gain-of-function studies, robotic systems screen thousands of full-length genes to identify which ones, when overexpressed, alter the signaling in the pathway of interest. Similarly, in loss-of-function studies, the pathway is stimulated by using a ligand or other activator (for example, a MAP kinase pathway is stimulated by growth factor), and robotic systems screen thousands of individual RNA interference (RNAi) molecules[5] to identify which RNAi molecules, and thus which genes, alter the signaling in the pathway when they are not ex-

[5]RNAi is a tool where an RNA introduced into a cell ultimately causes the degradation of the complementary cellular mRNA (messenger RNA), leading to the depletion, or "knockdown," of the targeted gene's activity.

pressed—that is, when they are "knocked down." Together, the results from the gain-of-function and loss-of-function screens provide a comprehensive list of genes that represent potential targets of perturbation by a toxic agent.

To identify how the pieces fit together to form a signaling pathway, Thomas described how the genes identified in the gain-of-function and loss-of-function screens are analyzed combinatorially. Genes identified in the screens are examined together in binary combinations. If the action of the gene identified in the gain-of-function screen is reversed by the RNAi identified in the loss-of-function screen, the gain-of-function gene is acting upstream of the gene targeted by the RNAi. Conversely, if the activity of the gain-of-function gene is not diminished by the RNAi, the gain-of-function gene is operating downstream of the gene targeted by the RNAi. On the basis of the results of the combinatorial gain-of-function and loss-of-function screens, the data are filtered with bioinformatics tools to develop a putative pathway map that is then refined by comparing it with pathway information in the scientific literature.

The next step in implementing a systems-biology approach is to determine how different signaling pathways coordinate or interconnect to produce the toxic end point. The interconnecting signaling pathways are analogous to a communications network and can vary across species and result in dramatic pharmacodynamic differences. Thomas described how a combination of functional genomics tools using RNAi and time-course microarray analysis is being used to dissect the interconnections. After exposure to a toxic agent, the resulting gene-expression response typically occurs as a cascade. The primary wave of transcription contains critical regulatory genes that control the expression of genes in the secondary and tertiary waves. To dissect the gene-expression cascade, regulatory genes in the primary transcription response are systematically depleted with RNAi, and the time-course gene-expression response is reanalyzed. The RNAi treatment blocks the function of the regulatory gene and identifies dependent downstream changes in gene expression. By linking key regulatory genes at early points with secondary or tertiary gene-expression changes, the approach identifies how different signaling pathways interconnect to form a network.

The last step is to quantitatively describe the signaling network. The results of the functional genomic screens and microarray studies are integrated with bioinformatic tools to model the signaling network. The static diagram representing the signaling network is transformed into a dynamic computational model that uses rate equations to mathematically

describe each step in the pathway. Computational biologists are then able to modify the model on the basis of experimental data and pose additional hypotheses that can be tested in the laboratory.

Thomas discussed some of the practical challenges to the approach he had outlined. They include the high costs associated with large-scale biologic experiments and datasets, the current limitation of this large-scale approach to in vitro rather than in vivo systems, off-target effects of RNAi, and, most important, difficulties in obtaining accurate, quantitative kinetic data at the molecular level to describe parameters in the computational models. He hopes that eventually proteomic and metabolomic approaches can provide kinetic data, such as the phosphorylation rates or metabolites resulting from pathway activation. Thomas also described some philosophical challenges to systems biology, such as whether everything must be modeled before it is considered useful. He explained how even an incomplete model of a signaling network can provide important information. Finally, he emphasized that large-scale biology is inherently multidisciplinary and collaborative and requires that the contributions of all participants be acknowledged. In response to an audience question, Thomas acknowledged that a further consideration in the systems-biology approach is the large amount of time and resources required to develop the models, prepare the materials, and analyze the data; although, he said that the experiments themselves could be conducted relatively quickly.

An audience member asked Thomas about the accuracy of the "hits" in the models and about how many of the hits can be incorporated into the model (a hit is a detectable reporter change in response to gain or loss of function). Thomas replied that he can reliably address only false positives in the screens by verifying the hits with standard molecular biology approaches. False negatives cannot be addressed, because of practical and technical limitations. He went on to say that in a well-designed screen, there are not an overwhelming number of hits to follow up on (possibly 6-30), so the task is not insurmountable.

COMBINING TRANSCRIPTIONAL AND TOXICOLOGIC APPROACHES TO UNDERSTANDING THE BASIS OF SPECIES DIFFERENCES IN CONAZOLE CARCINOGENESIS

Stephen Nesnow, of EPA, described work being done at the National Health and Environmental Effects Research Laboratory in combin-

ing transcriptional and more-traditional toxicologic approaches as a basis for understanding species differences in conazole carcinogenesis. Conazoles are azole antifungal agents that are used both as pharmaceuticals and as pesticides. The conazoles used in these studies contained triazole; triazole is a five-membered aromatic ring that contains three nitrogen atoms. Some conazoles are hepatotoxic in both rats and mice but cause liver tumors only in mice; some are hepatotoxic in humans, and some cause thyroid tumors in rats but not in mice. Most conazoles do not appear to be genotoxic, but some can induce reproductive and developmental toxicity.

The mammalian cytochrome P450 family of enzymes metabolize drugs and other xenobiotics. Conazoles induce cytochrome P450 enzyme activities, and the overexpression of these enzymes has been associated with liver and thyroid tumorigenesis and hepatotoxicity. Conazoles also inhibit mammalian enzymes involved in steroid biosynthesis, and this activity has been linked to reproductive effects. Although P450 enzymes detoxify xenobiotics, the same oxidation processes involved in the detoxification process often involve the formation of reactive toxic and carcinogenic intermediates. Differences in the concentrations of P450 enzymes may contribute to the interspecies differences observed in the toxicity of chemicals and the varied susceptibility of different mammals to chemically induced cancer.

Nesnow and colleagues used traditional toxicologic methods combined with transcriptomics to study the MOA of carcinogenic conazoles and examine the toxicologic differences between rats, mice, and humans with the goal of determining whether modulation of P450 was the common underlying toxicologic event. They selected thyroid cancer in rats and liver cancer in mice as the end points of interest and studied a series of carcinogenic and noncarcinogenic conazoles. The livers from mice and rats were studied because altered liver function can contribute to both cancer types. The parallelogram in Figure 3 illustrates the approach to making interspecies comparisons of carcinogenicity. DNA microarray expression profiles are compared among human hepatocytes treated with conazoles in vitro, mouse and rat hepatocytes treated with conazoles in vitro, and livers of conazole-treated mice and rats. Information from those three profiles is used to predict the fourth corner of the parallelogram: effects on human liver in vivo. The study design included a number of variables to increase the interspecies comparisons that could be made. For example, time-course studies were done with three conazoles: one that induces liver tumors in mouse and thyroid tumors in rat, one that

induces liver tumors only in mice, and one that does not induce any tumors in either species. Each conazole was tested at three dosages (tumorigenic and nontumorigenic) with three treatment durations (4, 30, and 90 days) to improve internal comparisons. In addition to the microarray expression profiles, end points to be analyzed included histology, cell proliferation and apoptosis, clinical chemical measures, hormone concentrations, enzyme activity, and gene expression with real-time polymerase chain reaction (PCR).

The preliminary data obtained thus far do not include genomics data on cross-species differences. Most of the analyzed data instead are on traditional toxicologic measures (liver weight, P450 activity, and so on) for the different conazoles. However, Nesnow did share the gene-transcription results that his group had recently obtained with rat liver treated for 4 days at the high dosage. A Venn comparison was created to identify dysregulated genes that are common to all three compounds and genes that are peculiar to the particular compounds. Two of the compounds, myclobutanil and propiconazole, had about the same number of significantly changed genes as the control (62 and 68, respectively), whereas triadimefon had 316 significantly changed genes. When they mapped the dysregulated genes to pathways, they found that some were involved in enzyme induction and some also in enzyme regulation, transporters, signal transduction, and binding. Nesnow and his collaborators will be analyzing the gene-expression changes with other analytical and statistical approaches and verifying the microarray observations with quantitative real-time reverse-transcription polymerase chain reaction (RT-PCR). They will pursue gene-expression patterns and pathways that look interesting on the protein level with proteomics and functional assays. To sum up, in the results obtained so far, the group has not used genomics to make cross-species comparisons but will be using genomics to tease out which genes are uniquely dysregulated and perhaps responsible for the cross-species differences and differences observed between conazoles.

During the discussion, Eaton noted that one challenge to the parallelogram approach described is that in vitro experiments are acute exposures and in vivo exposures can be chronic. Nesnow responded that one approach for dealing with that is to look for toxicity biomarkers in vitro and early in the in vivo experiments and try to match them. He noted that in rats and mice, information was obtained for all four corners of the parallelogram, so some correlations can be made. His group is planning to conduct 24-hour and 4-day experiments to begin making comparisons.

SPECIES DIFFERENCES IN RESPONSE TO PERFLUOROOCTANOIC ACID

John L. Butenhoff, of 3M Corporation, discussed sex and species differences in response to perfluorooctanoate (PFOA) and touched on aspects of the differences that might be amenable to further study with -omics technologies. PFOA is a commercially used emulsifier and surfactant that is the subject of EPA review. It has been found in almost every sample of human serum examined by 3M and others at low parts-per-billion concentrations. It does not appear to be metabolized. Although there are not substantial differences in serum concentrations of PFOA between males and females or between different age groups or species, one puzzle regarding PFOA is that there are dramatic species and sex differences in its elimination. As illustrated in Table 1, elimination half-life can range from 2 to 4 hours in female rats to 4 years in humans.

How does that translate into toxicologic differences between species? In a two-generation study of reproduction, there was no direct effect on reproductive success or on rat pup mortality during the first 5 postnatal days at any dosage, but there was a statistically significant increase in postnatal mortality of F1 female rat pups at the highest dosage (30 mg/kg per day), particularly immediately after weaning. However, an unpublished study by Christopher Lau found that mouse pup mortality increased with dosage (up to 20 mg/kg per day) during the first 5 postnatal days. In a further study of adult male and pregnant female mice and rats given PFOA through gestation, male mice and rats had statistically higher serum PFOA concentrations than females, and both sexes of mice

TABLE 1 Species- and Sex-Related Differences in PFOA Elimination Half-Life

	Half-Life	
Species	Males	Females
Mouse	12 days	20 days
Rat	4-6 days	2-4 hours
Rabbit	5.5 hours	7.0 hours
Dog	20-30 days	8-13 days
Monkey	About 21 days	About 30 days
Human (retired 3M workers)	About 4 years	About 4 years

Source: Hanijärvi et al. 1988; Burris et al. 2002; Kemper 2003; Kudo and Kawashima 2003; Noker 2003.

had significantly higher serum concentrations than rats. Butenhoff reported on another study in which male and female rats received a single dose of PFOA 4, 5, 6, 7, and 8 weeks after birth and serum PFOA concentrations were measured 24 hours after dosing. At week 4, female serum PFOA dropped to half what it had been and remained there for the remainder of the test, but male serum PFOA rose dramatically in week 5 and remained high. Butenhoff said that those studies raised the question of whether the differences in pharmacokinetics, and thus retention and elimination of PFOA, during the maturation period are driving the sex and species differences seen in pup mortality.

Butenhoff went on to discuss the prepubertal and postpubertal expression of organic anion transporters (OATs). OATs are a family of proteins that facilitate the transport of organic anions, including drugs and endogenous compounds, across membranes. There are four classes of OATs, and they tend to occur in pairs (for example, OAT1 and OAT3 occur in the kidney); they also have a high gene-sequence homology. In rats, OAT1 and OAT3 expression was found to be about the same in males and females before puberty but expression increased much more in males than in females after puberty; their expression was stimulated by androgens and inhibited by estrogens (Ljubojevic et al. 2004). Different OATs are expressed differently in rats and mice and differently in males and females of each species, which might help to explain species differences in elimination (Corbin et al. 2002; Buist and Klaassen 2004). Understanding the significance of OAT expression for human health may be helped by looking at OAT expression in humans as well. Transcriptional analysis of human OAT1 isoforms was done recently (Bakhiya et al. 2003), but more needs to be done to understand human expression.

-Omics technologies may be useful for exploring protein expression as a basis of sex, species, and age differences in PFOA elimination and ultimately for informing risk assessment of this chemical. One group in Japan is already exploring the basis of sex differences in rats by looking at the mRNA expression of various OAT forms after castration or ovariectomy (Kudo et al. 2002). Butenhoff proposed several possible -omics approaches—such as inhibiting the expression of particular genes with gene-knockout experiments or increasing the expression of particular genes by transfecting with gene clones—that might be used to understand OAT expression. He opined that computational approaches might become possible when three-dimensional structures are known.

SUMMARY OF ROUNDTABLE DISCUSSION

After the individual presentations, the speakers and standing-committee members participated in a roundtable discussion. Anderson, chair of the workshop planning committee, led the discussion by asking about MOA and cross-species extrapolation. Other topics then arose as the presenters and audience engaged in a dialogue on issues surrounding the use of toxicogenomic data for improving cross-species extrapolations for toxicologic end points.

Mode of Action

Participants were asked to consider two questions: (1) When are data sufficient to conclude that an MOA established in one species, such as mice or rats, is relevant or not relevant to humans? and (2) Do the -omic technologies offer any advantages in defining the MOA of a chemical?

Kerry Dearfield, of EPA,[6] indicated that EPA has developed an MOA framework as part of its 2003 revisions to the "Guidelines for Carcinogen Risk Assessment." The MOA framework has a series of questions that should be asked about any set of data, including genomics information, to determine whether the hypothesized MOA and key events are appropriate for the end point of concern.

Yvonne Dragan, of the Food and Drug Administration (FDA), emphasized that it is important to remember that the identification of an MOA involved in a particular end point does not preclude the involvement of other MOAs—there can be more than one MOA for a given chemical, and experimental design influences the ability to examine target vs nontarget effects. But, according to Dearfield, even though regulatory agencies recognize that there may be multiple mechanisms, EPA typically regulates a chemical on the basis of a particular end point, such as cancer, and attempts to determine a plausible mechanism of action for that end point. In its risk assessments, however, EPA also describes other possible MOAs, and these may add to the weight of evidence for the hazard assessment. In the past, EPA used just one effect as the end point for its hazard assessment, but it recognizes that with the advent of the -omic technologies there may be many cellular activities that affect a

[6]Dearfield is now with the U.S. Department of Agriculture.

chemical's activity and MOA. Risk managers need to know all this information to make informed regulatory decisions.

In connection with whether -omics are advantageous in defining MOAs, Bing Ren, of the University of California, San Diego, explained how he thought that once a profile is understood in one species, it can be used to determine the MOA in another species more expeditiously than traditional toxicology methods. Addressing knowledge gaps might assist in developing hypotheses about transcriptional changes into knowledge about disruption of particular cellular pathways. Gaps might pertain to what pathways are involved in developmental states or are active in different tissues or to transcription factor binding in the genome.

John Leighton, of FDA, cautioned that it is necessary to validate the use of toxicogenomic data in one species—to move beyond the exploratory phase—before using them for regulatory risk assessments. The issue of validation and cross-species extrapolation is being addressed by the FDA guidance document for industry for submission of pharmacogenomics data (FDA 2003).

Similarity of Pathways Between Species

The use of biologic pathways to think about cross-species comparison was a theme that came up in Sumner's presentation and was highlighted by John Quackenbush,[7] of the Institute for Genomic Research. He noted that participants seemed to be thinking about pathways and signaling networks rather than looking at individual genes and proteins. Although his own work has sometimes focused on identifying orthologous genes between species, scientists have to go beyond orthologous genes and proteins and look at orthologous pathways and networks. For example, rather than seeing whether dysregulated genes are conserved across species, it is important to look at the pathway that the dysregulated genes suggest may be involved and ask whether similar pathways exist and are dysregulated in humans. Sumner discussed the importance of mapping metabolites of interest to metabolic pathways and comparing the pathways across species. For example, she found that ascorbic acid was increased in the urine of rats exposed to a toxicant, but the lack of rat-to-human pathway convergence makes the use of this marker ques-

[7]Quackenbush is now at the Dana-Farber Cancer Institute at the Harvard School of Public Health.

tionable. However, being able to map to the glucuronic acid pathway from rat to human may indeed be relevant for the development of a human marker.

Along the same lines of comparing possibly orthologous genes and proteins, Anderson asked what types of molecular targets might be looked at with toxicogenomic technologies to help with cross-species extrapolation. He mentioned that binding to receptor targets, for example, would be good to look at but that new approaches to measuring binding of chemicals to receptors appear to be in their infancy. He and Quackenbush suggested that it might be helpful to think about the types of chemical targets that are likely to be conserved across species. Possible examples are the generation of reactive oxygen species and other mechanisms of DNA damage.

Toxicogenomics for Other Extrapolations (Low vs High Dose)

The roundtable participants moved on from the discussion of toxicogenomics for cross-species extrapolation to how it might be used for other extrapolations in risk assessment, such as high- to low-dose extrapolations. Bus described the current risk assessment paradigm as flawed because of its reliance on testing chemicals at high dosages in nonhuman animals even though humans are typically exposed to much lower dosages. High-dosage testing does not provide necessary information about how an organism will respond to low dosages of a chemical. He indicated that a critical question in risk assessment is how to determine the MOA of an organism's response to low dosages of a chemical if no classical toxic effects are observed. That is, what biologic mechanisms are mounted to modulate expression of toxicity? He expressed hope that toxicogenomic approaches would offer a way out of the traditional risk assessment paradigm and provide a new tool for understanding how cells and organisms react to a new chemical. The theme of extrapolation from high to low dosages was echoed by several participants.

In response to the discussion about how toxicology can move beyond testing chemicals at high dosages to determining effects at actual, low dosages, Mendrick asked how scientists could determine which changes at a low dosage, that do not result in overt traditional pathologic effects, signal impending toxicity. She described Gene Logic's dose-escalation approach. Rather than use the same dosage of each compound, comparable concentrations of different compounds are determined by using a marker of mitochondrial damage as a phenotypic

benchmark—an amount that induces some mitochondrial toxicity but is less than what leads to a traditional pathologic end point.

Bus also emphasized the importance of examining the entirety of the comparative dose-response curves. If the comparative animal dose-response curves are not parallel, responses at high dosages may converge or even cross over at lower dosages, and this results in different implications for risk assessment. Another participant echoed the value of focusing on chemicals that had qualitative species differences at the low end of the dose-response curves rather than devoting efforts to chemicals that have the same effect in humans and other animals at low dosages.

Eaton followed this point, questioning what to do about qualitative differences in how species respond. For example, one species may respond to a chemical while another does not. That is a bigger issue than whether similar responses occur at different dosages, particularly at unrealistically high dosages. Bus agreed that quantitative differences in response (similar effects at different dosages, but with the same no-observed-effects level [NOEL]) do not have the same public-health implications as qualitative or more dramatic quantitative differences, for example, when different species receive environmentally realistic dosages of a chemical but the NOELs are dramatically different.

Dearfield thought that the biggest challenge would be sifting through the noise to figure out which of the expression changes in genes, proteins, or other markers would be precursors or biomarker of adverse events and which would not. He agreed that the promise of -omics technologies was in exploring toxic effects at lower dosages.

Use of Uncertainty Factors in Risk Assessment

The roundtable participants discussed how the uncertainty factors used to account for species differences might be affected by toxicogenomics. EPA approaches the use of uncertainty factors on a weight-of-evidence basis, according to Benson. Furthermore, some uncertainty factors depend on statutes, such as the Food Quality Protection Act, because they define what is legally considered safe. Dearfield asked some pragmatic questions faced by risk assessors who extrapolate from animal data to human health. Are risk assessors going to start changing how they think about reference doses[8] and uncertainty factors on the basis of

[8]Reference dose or concentration is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (in-

toxicogenomic information? Should the typical interspecies uncertainty factor of 10 be applied if genomics data suggest that the test species is more like the human in some way? Should a different uncertainty factor be applied for interspecies extrapolation if genomics data reveal that some pathway or metabolite is well conserved in humans? Although EPA does not have answers for those questions, it is starting to explore the use of toxicogenomics to better characterize such variability and to refine the use of uncertainty factors.

Using -Omics for Mode of Action vs Predictive Patterns

Quackenbush pointed out that it is important to distinguish between using -omics information to understand a mechanism and using it to make predictions about end points without necessarily understanding the underlying mechanism or biologic process responsible for the end point. He emphasized that mechanistic understanding is not necessary to gain useful information.

Ren weighed in on the issue of MOAs vs predictive patterns. He explained how predictive patterns might seem analogous to a criminal's fingerprint from a crime scene, but they are different. A detective who identifies a fingerprint at a crime scene can match it with a fingerprint in a database. Analogously, if a scientist identifies a particular -omics pattern, it might match with an -omics pattern associated with a specific disease. However, the difference is that organisms are dynamic, and it may be difficult to find the correct cell or tissue at the right moment. Given that it is not practical to do experiments across an entire human life span for every cell type, he thinks that the best way to make predictions is to understand mechanisms. Quackenbush thought that the issues of dynamic expression and interindividual variability could be resolved by collecting more data so that signals can be separated from noise.

Thomas thought that although it might be possible to develop a training set for making predictions in some arenas, such as cancer research and the pharmaceutical industry, it will be necessary to understand the mechanisms of toxicity to make a predictive model valuable not just from a hazard identification perspective but from a risk assessment perspective. Quackenbush did not agree, saying that even if -omics

---

cluding sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.

data did not help to elucidate an MOA, they could have predictive value. He used the example of developing a training set of common toxicogenomic profiles of 50 hepatotoxic compounds. If one knows the profile of those compounds, one can determine whether the 51st compound is hepatotoxic by comparing it with the other 50 profiles. These data might not identify an MOA, but the information may be valuable nonetheless.

Eaton agreed that even without an understanding of mechanisms, toxicogenomic tools may be useful as screening tools for priority setting, either among potential pharmaceutical leads in the drug world, or, at EPA, among new chemicals to test. That application of the technologies would not necessarily replace traditional toxicologic evaluation aimed at understanding mechanisms, but it is important not to sell the technologies short for their potential screening application. Eaton's comments were echoed by Leighton, who indicated that, from a pharmaceutical regulatory decision-making perspective, it is not necessary to understand an MOA—a predictive pattern is more valuable. He used the example of estradiol: even after 50 years of use and investigation, we are still unsure of how it works. However, MOA information is important because it allows extrapolation to untested populations.

In response, Mendrick noted that analyzing the MOA of a chemical might require complex time-course studies to identify all the genes or proteins that are being affected and ultimately expressed as phenotypic changes. If one is using genomics just to determine whether a chemical might be toxic, however, one needs to identify only the genes or proteins that reliably predict an adverse effect, not every gene in the pathway that leads to the effect.

Tiffany Tummino, of the Occupational Safety and Health Administration, reminded the group that MOA is helpful in attributing effects to one chemical vs another, that is, teasing out which effects are attributable to the chemical we are trying to regulate rather than to coexposures. John A. Moore, reminded participants of the traditional importance of understanding MOA in the assessment of environmental chemicals. When a chemical is tied to an adverse effect and the association is believed to be a false positive, the only way to demonstrate that the effect is indeed a false-positive effect is to explain the MOA. Eaton emphasized that without understanding the underlying mechanism, it is difficult to determine whether the different transcriptional signatures observed in different species are biologic because of differences or because of noise (that is, are nonpredictive responses).

Anderson pointed out that understanding the biologic basis of an adverse effect allows scientists to think about key critical steps in a bio-

logic pathway that distinguish between adverse and nonadverse effects. There is a continuum between information that provides mechanistic insight and information that is predictive without providing mechanistic insight. Casimir Kulikowski, of Rutgers University, thought that it might be useful to develop models for integrating different lines of evidence (mechanistic and nonmechanistic). Richard A. Canady, of the Executive Office of the President, and Eaton echoed that sentiment: scientists may need new ways of thinking about the data that are "mentally manageable."

Other Thoughts on Cross-Species Comparisons

Several other thoughts about cross-species comparisons were mentioned briefly in the roundtable discussion. Bus pointed out that -omics technologies may allow toxicologists to focus what they look at with traditional toxicologic methods. That is, to apply traditional toxicologic measures to end points or biologic processes that -omics data suggest may be important.

Sumner supported using -omics data as a signal of pathologic outcome but also for integrating them with traditional toxicology approaches, such as the use of the area under the curve and physiologically based pharmacokinetic modeling.

The parallelogram presented by Nesnow illustrated the experimental paradigm for identifying species differences in response to new chemicals or chemicals that are not well tested. The concept, described earlier, is that by having the information in three corners, one can extrapolate to predict what happens in the fourth corner—in vivo responses in humans. Eaton thought that it would be useful for scientists to generate data so this concept can be tested and the proof of principle demonstrated. Of course, this parallelogram approach to using transcriptomics may *not* be appropriate if the toxicologic effects would not be picked up by transcriptomics—such as enzyme activity changes via direct inhibition or other differences in pharmacokinetics or pharmacodynamics that may not be reflected in changes in the transcriptome.

CONCLUSION

This workshop examined the use of toxicogenomics for cross-species extrapolation. Such extrapolation is important in assessing risks

posed by exposure to chemicals because although toxicity testing is conducted in laboratory animals, it is human health that risk assessors are trying to protect. The workshop did not attempt to reach a consensus that any particular approach for using toxicogenomics technologies will expedite or facilitate cross-species extrapolation, but it identified several approaches that might be useful and several needs for more proof-of-principle research to demonstrate the utility of -omics technologies in enhancing cross-species extrapolation.

Insight may be gained as scientists try to use toxicogenomic data to hypothesize about possible MOAs of chemicals or to focus their chemical and pharmaceutical development programs. Insight may also be gained when more scientists who are using toxicogenomics to look at mechanisms of particular chemicals begin to study additional species, including humans. Such research will highlight similarities or dissimilarities of biochemical pathways and mechanisms between test species and humans.

References

Bakhiya, A., A. Bahn, G. Burckhardt, and N. Wolff. 2003. Human organic anion transporter 3 (hOAT3) can operate as an exchanger and mediate secretory urate flux. Cell Physiol. Biochem. 13(5):249-256.

Buist, S.C., and C.D. Klaassen. 2004. Rat and mouse differences in gender-predominant expression of organic anion transporter (Oat1-3; Slc22a6-8) mRNA levels. Drug Metab. Dispos. 32(6):620-625.

Burris, J.M., J.K. Lundberg, G.W. Olsen, C. Simpson, and J.H. Mandel. 2002. Determination of Serum Half-lives of Several Fluorochemicals. Interim Report No. 2. EPA Docket AR 226-1086. 3M Company, St. Paul, MN.

Corbin, I.R., R. Buist, V. Volotovskyy, J. Peeling, M. Zhang, and G.Y. Minuk. 2002. Regenerative activity and liver function following partial hepatectomy in the rat using (31)P-MR spectroscopy. Hepatology 36(2):345-353.

Dearfield, K.L., and W.H. Benson. 2004. Genomics implications for EPA regulatory and risk assessment applications. Emerging Issues in Environmental Health Sciences 6:2-3 [online]. Available: http://dels.nas.edu/emergingissues/docs/EI_issue6.pdf [accessed May 13, 2005].

Di Giulio, R.T., and W.H. Benson, eds. 2002. Interconnections Between Human Health and Ecological Integrity. Pensacola, FL: SETAC Press.

EPA (U.S. Environmental Protection Agency). 2003. A Framework for a Computational Toxicology Research Program in ORD. EPA 600/R-03/-65. Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC [online]. Available: http://www.epa.gov/

comptox/publications/comptoxframework06_02_04.pdf [accessed May 13, 2005].

EPA (U.S. Environmental Protection Agency). 2004. Potential Implications of Genomics for Regulatory and Risk Assessment Applications at EPA. Final Report. EPA 100/B-04/002. Prepared for the U.S. Environmental Protection Agency, by Genomics Task Force Workgroup. Science Policy Council, U.S. Environmental Protection Agency, Washington, DC [online]. Available: http://www.epa.gov/osa/genomics.htm [accessed May 13, 2005].

FDA (Food and Drug Administration). 2003. Guidance for Industry: Pharmacogenomic Data Submissions. Center for Drug Evaluation and Research, Center for Biologics Evaluation and Research, Center for Devices and Radiological Health, Food and Drug Administration. November 2003 [Online]. Available: http://www.fda.gov/cder/guidance/5900dft.pdf [accessed May 16, 2005].

Hanhijärvi, H., M.Ylinen, T. Haaranen, and T. Nevalainen. 1988. A proposed species difference in the renal excretion of perfluorooctanoic acid in the beagle dog and rat. Pp. 409-412 in New Developments in Biosciences: Their Implications for Laboratory Animal Science, A.C. Beynen, and H.A. Solleveld, eds. Dordrecht, the Netherlands: M. Nijhoff.

Kemper, R.A. 2003. Perfluorooctanoic Acid: Toxicokinetics in the Rat. Laboratory Project ID: DuPont-7473. EPA Docket AR-226-1350. DuPont Haskell Laboratories.

Kudo, N., and Y. Kawashima. 2003. Toxicity and toxicokinetics of perfluorooctanoic acid in humans and animals. J. Toxicol. Sci. 28(2):49-57.

Kudo, N., M. Katakura, Y. Sato, and Y. Kawashima. 2002. Sex hormone-regulated renal transport of perfluorooctanoic acid. Chem.-Biol. Interact. 139(3):301-316.

Lenz, E.M., J. Bright, I.D. Wilson, A. Hughes, J. Morrison, H. Lindberg, and A. Lockton. 2004. Metabolomics, dietary influences and cultural differences: A1H NMR-based study of urine samples obtained from healthy British and Swedish subjects. J. Pharm. Biomed. Anal. 36(4):841-849.

Ljubojevic, M., C.M. Herak-Kramberger, Y. Hagos, A. Bahn, H. Endou, G. Burckhardt, and I. Sabolic. 2004. Rat renal cortical OAT1 and OAT3 exhibit gender differences determined by both androgen stimulation and estrogen inhibition. Am. J. Physiol. Renal Physiol. 287(1):F124-F138.

Nesnow, S. 2004. A Transcriptional Analysis Approach to Understanding the Basis of Species Differences in Conazole Toxicology. Presentation at the Seventh Meeting on Emerging Issues and Data on Environmental Contaminants-Applications of Toxicogenomics to Cross-Species Extrapolation: A Workshop, August 12, 2004, Washington, DC.

Nicholson, J.K., J.C. Lindon, and E. Holmes. 1999. 'Metabonomics': Understanding the metabolic responses of living systems to

pathophysiological stimuli via multivariate statistical analysis of biologic NMR spectroscopic data. Xenobiotica 29(11):1181-1189.

Noker, P. 2003. A Pharmacokinetic Study of Potassium Perfluorooctanoate in the Cynomologus Monkey. Southern Research Institute Study ID: 99214. EPA Docket AR 226-1362.

Ramos, K.S. 2003. EHP Toxicogenomics: A publication forum in the post-genome era [editorial]. EHP Toxicogenomics 111(1T):A13.

Appendix A

WORKSHOP AGENDA

Applications of Toxicogenomics to Cross-Species Extrapolation: A Workshop

Despite the dependence on animal models in toxicologic research for predicting human health effects in the regulatory arena, there can be significant differences between how animals and humans respond to different chemicals. This workshop will consider promises and limitations in using emerging high-throughput approaches, such as genotyping (genomics), mRNA analysis (transcriptomics), protein analysis (proteomics), and metabolite analysis (metabolomics), to inform cross-species extrapolation.

Thursday, August 12th 2004

9:00 am Welcome and Overview of the Workshop, including what is meant by "Cross-Species Extrapolation"—Leigh Anderson/ David Eaton

9:10 am Richard Di Giulio, Duke University
Highlights from a Recent Pellston Workshop on Emerging Molecular and Computational Approaches for Cross-Species Extrapolation

9:35 am William Benson, U.S. Environmental Protection Agency
Potential Implications of Genomics for Regulatory and Risk Assessment Applications at EPA

10:00 am Discussion of Issues Raised by Di Giulio and Benson

10:30 am *BREAK*

10:40 am Frank Witzmann, Indiana University
Technological Challenges of Cross-Species Extrapolation Using Proteomics

11:10 am Donna Mendrick, Gene Logic
Modeling Gene Expression Data to Predict Human Hepatotoxicity Following Inconsistent Animal Responses

11:40 am Discussion of Talks

12:00 pm *LUNCH*

1:00 pm Susan Sumner, Paradigm Genetics
Using Metabolomics/-omics to Explore Species Differences in Metabolism and Distribution

1:30 pm Russell Thomas, CIIT Centers for Health Research
A Systems Biology Approach to Cross-Species Extrapolation

2:00 pm Discussion of Talks

2:20 pm *BREAK*

2:30 pm Stephen Nesnow, U.S. Environmental Protection Agency
Combining Transcriptional and Toxicologic Approaches to Understanding the Basis of Species Differences in Conazole Carcinogenesis

3:00 pm John Butenhoff, 3M Co.
Species Differences in Response to Perfluorooctanoic Acid

3:30 pm Discussion of Talks

3:50 pm *BREAK*

4:00 pm Roundtable Discussion—questions such as:

- "Once a molecular basis for understanding a species differences has been established, what are the challenges to incorporating -omics information about species differences into the regulatory framework?"
- "What are the advantages to using -omics compared to other approaches for detecting or explaining cross-species differences?"
- "How much data are sufficient for arguing that a particular mode of action is most relevant to humans?"

5:00 pm *ADJOURN*

Appendix B

BIOGRAPHICAL INFORMATION ON WORKSHOP SPEAKERS

William H. Benson is director of the National Health and Environmental Effects Research Laboratory's Gulf Ecology Division within the U.S. Environmental Protection Agency (EPA), Office of Research and Development. Dr. Benson obtained a BS in biology from the Florida Institute of Technology and his MS and PhD in toxicology from the University of Kentucky. In graduate school, he was the first recipient of the Society of Environmental Toxicology and Chemistry (SETAC) Pre-Doctoral Fellowship sponsored by the Procter & Gamble Company. Dr. Benson has published over 100 scientific publications on environmental toxicology and chemistry. His research activities have been directed toward assessing the influence of environmental stressors on health and ecologic conditions. He has conducted research in metal and pesticide bioavailability, reproductive and developmental effects in aquatic organisms, endocrine-disrupting chemicals, and the use of indicators in environmental monitoring and assessment. Dr. Benson is a past president of SETAC and has served on its International Council. He was elected as a Fellow of the American Association for the Advancement of Science and is active in several other professional societies. He serves as chair of Dow Chemical Company's Technical Advisory Board for Toxicology and as cochair of the Coordinating Committee for Development of a Technical Framework and Training for Genomics in EPA.

John L. Butenhoff is a corporate scientist in the Medical Department of 3M. He is responsible for the toxicology and health risk assessment activities associated with legacy perfluorinated alkyl acids that were produced by 3M before 2002. Dr. Butenhoff has been an employee of 3M since 1976 and has held technical and management positions in industrial hygiene, toxicology, and corporate product responsibility. He received his AB in biology from Franklin and Marshall College and his MS in occupational health and PhD in toxicology from the University of Minnesota. He holds an adjunct faculty position in the graduate program in toxicology at the University of Minnesota through the Department of Biochemistry and Molecular Biology, School of Medicine, Duluth. Dr. Butenhoff holds professional board certifications by the American Board of Toxicology and the American Academy of Industrial Hygiene.

Richard Di Giulio is professor of environmental toxicology in the Nicholas School of the Environment and Earth Sciences at Duke University. At Duke, he also serves as director of the Integrated Toxicology Program (a doctoral and postdoctoral training program), director of the Superfund Basic Research Center, and associate director of the Center for Comparative Biology of Vulnerable Populations (all are supported principally by the National Institute for Environmental Health Sciences, NIEHS). He received a BA in comparative literature from the University of Texas at Austin (1972), an MS in wildlife biology from Louisiana State University (1978), and a PhD in environmental toxicology from Virginia Polytechnic Institute and State University (1982). His research in aquatic and comparative toxicology emphasizes mechanistic studies of chemical toxicity and adaptation, emphasizing metabolism, oxidative stress, and gene interactions. Current studies include an investigation of mechanisms of adaptation, fitness costs, and genetic consequences in a population of killifish (*Fundulus*) that inhabit a polluted estuary in Virginia; mechanisms by which selected chemicals perturb cardiovascular development in *Fundulus*; and effects of chemicals on gene expression and regulation in this model. He has organized symposia and workshops and written on the broader subject of interconnections between human health and ecologic integrity. Dr. Di Giulio serves as an adviser for the Scientific Advisory Board of the U.S. Environmental Protection Agency (EPA) and for the Canadian Network of Toxicology Centres. He has served on the Board of Directors of the Society of Environmental Toxicology and Chemistry, is active in the Society of Toxicology , and is on the editorial boards of *Human and Ecological Risk Assessment* and *Toxi-*

cologic Sciences. Dr. Di Giulio's current and recent research has been supported by NIEHS, EPA, and the Office of Naval Research.

Donna Mendrick is the vice president of toxicogenomics at Gene Logic Inc. She was on the Editorial Board of the *Journal of Histochemistry and Cytochemistry* for 8 years, a member of the National Institute of Health Small Business Initiative Research Immunology Study Section for 8 years, and a member of the Board of Directors of the National Kidney Foundation of Massachusetts for 4 years. Before joining Gene Logic in 1998, Dr. Mendrick was a group leader in pharmacology at Human Genome Sciences, Inc., where she planned and directed acute and chronic toxicity, developmental, and ADME (absorption, distribution, metabolism, and excretion) studies for investigational new drug submissions; performed in-house pharmacology experiments; and directed project teams. Before joining Human Genome Sciences in 1995, she was an assistant professor in the Department of Pathology at Harvard Medical School, where her research focused on renal immunopathology and endothelial biology. Dr. Mendrick received her PhD from the State University of New York at Buffalo in immunopathology.

Stephen Nesnow is a senior scientist in the Environmental Carcinogenesis Division of the U.S. Environmental Protection Agency (EPA) National Health and Environmental Effects Research Laboratory. Dr. Nesnow received his BS in chemistry from Bucknell University and his MS and PhD degrees in organic chemistry from New York University. In graduate school, he received a New York University Graduate School of Arts and Science Predoctoral Fellowship. After two postdoctoral fellowships at the Sloan-Kettering Institute for Cancer Research and at the McArdle Laboratory for Cancer Research, he joined the faculties of the University of Wisconsin and the University of North Carolina. Dr. Nesnow joined EPA in 1977. He served as the branch chief of the Biochemistry and Pathobiology Branch for 20 years. Dr. Nesnow has published more than 214 scientific publications in chemical carcinogenesis, with emphasis on metabolism, tumorigenesis, DNA adducts, and complex mixtures. He has received a number of awards from EPA, including a Bronze Medal and nine Scientific and Technological Achievement Awards. Dr. Nesnow is a member of the Editorial Boards of *Chemical Research in Toxicology*, *Cancer Letters,* and *The Journal of Environmental Science and Health* and has served as a member of the Aspen Cancer Conference Advisory Committee, as a member of the Board of

Governors of the International Symposium on Polynuclear Aromatic Hydrocarbons, and on the International Agency for Research on Cancer Working Groups. Dr. Nesnow has been an invited speaker at many national and international symposia and has served as organizer and session chair at many of them. He serves as an adjunct professor in the School of Medicine, University of North Carolina at Chapel Hill.

Susan Sumner, who was with Paradigm Genetics at the time of this workshop, is now taking a lead role at RTI International in the development of a biomarker discovery program in metabolomics. She was trained (1982-1986) as a physical chemist with a specialty in spectroscopy at North Carolina State University (NCSU) after completion of undergraduate studies in the NCSU Department of Chemistry (1979-1982). She completed a 2-year staff fellowship (1987-1989) in biologic applications of spectroscopy at the National Heart, Lung, and Blood Institute. Dr. Sumner was employed by the Chemical Industry Institute of Toxicology for 13 years, where she served as a principle investigator and study director. Her research focused on mechanisms of chemical-induced toxicity, cross-species extrapolation, and the relevance of animal models in assessment of human health risk. Dr. Sumner continued developing and applying spectroscopic methods for biomarker discovery and elucidation of biochemical mechanisms while employed at Paradigm Genetics, Inc. (2002-2004). She has directed both nuclear magnetic resonance and mass spectrometry facilities for metabolite characterization and metabolomic analysis. She joined the Health Sciences Division of RTI International in August 2004.

Russell Thomas is an associate investigator at CIIT Centers for Health Research in the Division of Computational Biology. He is the director of the Functional Genomics Research Program and the Gene Expression Core Facility. Dr. Thomas completed his PhD in Toxicology at Colorado State University and focused on constructing pharmacokinetic and pharmacodynamic models of the effects of chlorinated benzenes. After his doctoral studies, Dr. Thomas performed postdoctoral research in molecular biology and genomics at the McArdle Cancer Research Laboratory at the University of Wisconsin. In addition to the doctoral degree, he has earned a master's degree in radiation ecology and an undergraduate degree in chemistry. Before coming to CIIT, Dr. Thomas worked in bioinformatics in the biotechnology industry and was the head of the genomics and toxicology groups at a biopharmaceutical company. Dr.

Thomas's research focuses on integrating genomics, bioinformatics, and computational biology into a systems-biology approach to specific toxicologic responses.

Frank A. Witzmann received his PhD in physiology from Marquette University and now serves as professor of cellular and integrative physiology at Indiana University School of Medicine. He has applied large-scale two-dimensional electrophoretic analyses in a variety of paradigms since the middle 1980s and currently directs the use of gel-based proteomics approaches in projects concerning various aspects of toxicology, pharmacology, vascular biology, and central nervous system physiology. He has published over 90 refereed manuscripts, book chapters, and technical reports. He regularly participates in grant and program reviews for the U.S. Environmental Protection Agency, the National Institute of Environmental Health Sciences, and the Department of Defense; is a past president of the Electrophoresis Society (USA); and serves as associate editor of *Briefings in Functional Genomics and Proteomics* and on the editorial board of *Analytical Biochemistry*.

Appendix C

BIOGRAPHICAL INFORMATION ON WORKSHOP PLANNING COMMITTEE

N. Leigh Anderson (*Chair*) is chief executive officer at the Plasma Proteome Institute (PPI) in Washington, DC. He earned a PhD in molecular biology from Cambridge University, England. Before founding PPI, Dr. Anderson was chief scientific officer at the Large Scale Biology Corporation (LSBC), whose proteomics division he founded. At LSBC, he developed the first automated two-dimensional electrophoresis technology platform for proteomics research, including the measurement of large numbers of proteins in human serum and tissues, and pioneered an array of applications in drug discovery, toxicology, and surrogate markers. Dr. Anderson's research interests have included the investigation of gene-expression effects of pharmaceutical agents, both in vivo and in vitro, and the development of systematic databases describing the regulation of complex gene-expression systems. Other interests include mass spectrometry, bioterrorism detection and response, and conceptual development of drug-discovery systems.

James S. Bus is director of external technology at Dow Chemical Company. He received his PhD in pharmacology from Michigan State University in 1975. His research interests include the mechanism of super-

oxide radical-mediated paraquat toxicity, the relationship between benzene metabolism and toxicity, metabolic pathways as defense mechanisms against toxicant exposure, and mode-of-action considerations in the use of transgenic animals for mutagenicity and carcinogenicity evaluations. He is a member of several professional societies, including the Society of Toxicology (serving as president in 1996-1997), the American Society for Pharmacology and Experimental Therapeutics, the American Conference of Governmental and Industrial Hygienists, and the Teratology Society, and he is a diplomate of the American Board of Toxicology. Dr. Bus serves on the U.S. Environmental Protection Agency Scientific Advisory Board.

David L. Eaton is professor of environmental health, associate dean of research, and director of the Center for Ecogenetics and Environmental Health at the University of Washington. He received a PhD in pharmacology from the University of Kansas Medical Center. Dr. Eaton's research interests include the molecular basis of environmental causes of cancer and how human genetic differences in biotransformation enzymes may increase or decrease individual susceptibility to chemicals in the environment. He has served on numerous boards and committees, including the Board of Directors and as treasurer of the American Board of Toxicology (1990-1994), and was recently the president of the Society of Toxicology. Dr. Eaton has also served on the National Research Council Board on Environmental Studies and Toxicology and Subcommittee to Update the 1999 Arsenic Report.

Serrine S. Lau is a professor at the College of Pharmacy and director of the Southwest Environmental Health Sciences Center at the University of Arizona at Tucson. She earned a PhD in pharmacology from the University of Michigan. The focus of Dr. Lau's research involves coupling the metabolic activation of chemicals to their target-organ toxicity. The major subjects of research in Dr. Lau's laboratory include mass spectrometric approaches to proteomics, the mechanism of hydroquinone-mediated carcinogenicity, and prostanoid-mediated cytoprotection.

John A. Moore received his DVM from Michigan State University, is a board-certified toxicologist, and has primary interests in risk assessment and developmental and reproductive toxicology. Dr. Moore has held a number of senior positions in the U.S. Government, including assistant administrator for pesticides and toxic substances and acting deputy ad-

ministrator of the U.S. Environmental Protection Agency; deputy director of the National Toxicology Program (NTP); and director of toxicology research and testing at the National Institute of Environmental Health Sciences. He served for 10 years as head of the not-for-profit Institute for Evaluating Health Risks and recently completed a 5-year term as principal scientist at the NTP Center for the Evaluation of Risks to Human Reproduction. Dr. Moore has served on several National Research Council committees, including being chair of the Subcommittee on the Toxicity of Diisopropyl Methylphosphonate and a member of the Subcommittee on Reproductive and Developmental Toxicology.

John Quackenbush is a professor in the Department of Biostatistics and Computational Biology, Dana-Farber Institute and Department of Biostatistics, Harvard University School of Public Health. His primary research areas are functional genomics and bioinformatics, and his work has focused on the integration of diverse data types to provide insight into biologic systems. He and his group have been investigating gene-expression patterns in animal models with the goal of identifying mechanisms underlying a range of human diseases. They have also used microarrays to look for diagnostic and prognostic expression fingerprints in human breast and colon cancer, and he has been active in using plant models to develop methods for integrating functional genomics and metabolomics approaches. He earned a PhD in theoretical particle physics from the University of California, Los Angeles.

Kenneth S. Ramos is professor and chair of the Department of Biochemistry and Molecular Biology at the University of Louisville Health Sciences Center. He also serves as director of the Center for Genetics and Molecular Medicine. He received a PhD in biochemical pharmacology and toxicology from the University of Texas at Austin. His research focuses on the study of molecular mechanisms of environmental disease and redox-regulated transcriptional control. Dr. Ramos has served on numerous National Research Council committees including the Committee for a Review of Evidence Regarding Link between Exposure to Agent Orange and Diabetes, Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides: First Biennial Update, Howard Hughes Medical Institute Predoctoral Fellowships Panel on Neurosciences and Physiology, and the Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides: Second Biennial Update.