

Emerging Issues

in Environmental Health Sciences

Issue 10
June 2006

The Newsletter of the
Committee on Emerging Issues and
Data on Environmental Contaminants

Toxicogenomics and Early-Life Exposures: A Workshop

The February 8, 2006 meeting of the Committee on Emerging Issues and Data on Environmental Contaminants featured a workshop on toxicogenomics and early-life exposures. This workshop explored the role of toxicogenomics in elucidating the impact of early-life exposures to toxic chemicals on disease occurrence later in life.

The workshop included three sessions: the first on new research in developmental toxicology and the use of toxicogenomic approaches to evaluate the impact of early-life exposures; the second on considering early-life exposures in epidemiology and risk assessment; and, finally, a roundtable discussion among workshop participants to consider the future role of toxicogenomic research in developmental toxicology and children's health protection.

The first session included a series of presentations focused on chemical exposures that alter the methylation of DNA and the potential of this effect to produce disease later in life. Dr. Cheryl Lyn Walker, University of Texas M.D. Anderson Cancer Center, introduced the topic with an

overview of developmental programming and the impact that early-life exposures can have on incidence and severity of adult diseases such as cardiovascular disease, diabetes and cancer. In her presentation entitled "Early-Life Exposures and Prostate Cancer Risk: Using Toxicogenomics to Interrogate the Epigenome", Dr. Shuk Meicho, University of Cincinnati, presented research indicating that transient developmental exposure of rats to low, environmentally relevant doses of Bisphenol A or estradiol increases prostate susceptibility to cancer and permanent alterations in DNA methylation. Dr. Michael Skinner, Washington State University, also evaluated the transgenerational epigenetic actions of endocrine disruptors. He presented observations from research with toxicogenomic microarray assays demonstrating that altered DNA methylation from exposure to endocrine disruptors was heritable, resulting in defects passed down generations.

continued on page 2

About the Committee...

The Standing Committee on Emerging Issues and Data on Environmental Contaminants (the Standing Committee) was constituted by the National Academies at the request of the National Institute of Environmental Health Sciences to provide a public forum for communication among government, industry, environmental groups, and the academic community about emerging issues in the environmental health sciences. At present, the scope of the Standing Committee is focused on toxicogenomics and its applications in environmental and pharmaceutical safety assessment, risk communication, and public policy.

IN THIS ISSUE

- 1 *Toxicogenomics and Early-Life Exposures: A Workshop*
- 3 *Applications of Genomic Signatures: A Workshop*
- 4 *Recent Workshop Summary*
- 7 *June 29, 2006, Workshop on Intellectual Property Concerns for Toxicogenomics*

Tox. and Early-Life Exposures

continued from page 1

Randy Jirtle of Duke University presented on “Imprinted Genes and Transposons: Epigenomic Targets Linking Prenatal Nutrition to Adult Disease Susceptibility” and outlined research in his lab that used mouse experiments to investigate the importance of maternal nutrition in determining the susceptibility of offspring to diseases. His research showed that maternal dietary supplementation during pregnancy with either methyl donating substances (e.g. folic acid and vitamin B12) or genistein, a phytoestrogen present in soy products, alters DNA methylation and reduces the risk of developing obesity, diabetes, and cancer in the offspring.

The next two presentations in this session addressed challenges associated with testing for developmental toxicity and the potential role for toxicogenomics. Theodore Slotkin of Duke University discussed the difficulties of testing for adverse outcomes from developmental neurotoxicants. These compounds can exert their toxic effects in different developmental time frames, and the same compound can have different dose-effect relationships, targets, and neurobehavioral outcomes, depending on the time of exposure. His presentation highlighted the shortcomings of currently-used standardized animal tests and critically examined the potential use of toxicogenomic microarray technologies. Robert Brent of Thomas Jefferson University presented on “Extrapolating Data on Early-Life Exposures from Animals to Humans” and discussed the use of animal models for indicating potential human effects of developmental toxicants. Through a series of case studies and examples, Dr. Brent highlighted developmental differences between animals and humans, emphasizing the general benefit of animal model use, but with the recognition that they may not always provide accurate insight to human toxicology. His presentation also highlighted the use of toxicogenomics for delineating the genetic diversity of humans and the modifying effect these differences will have on toxic responses.

The second session examined broader efforts in the fields of epidemiology, risk assessment, and children’s environmental health and the potential role of toxicogenomics in these fields. Drs. Frederica Perera and Rachel Miller, from the Columbia Center for Children’s Environmental Health presented on molecular epidemiologic studies conducted on groups of children before and after birth. The research described the approaches for measuring exposures of women and children to pollutants and allergens (e.g., from cockroaches, dust, and mice) and monitoring biomarkers of effect (including the

use of toxicogenomic microarrays). The results to date indicate that prenatal exposure to multiple toxicants (e.g., polynuclear aromatic hydrocarbons, tobacco smoke, residential pesticides) is common and that the same pollutants are associated with adverse effects on fetal growth, cognitive development and/or asthma. Peter Scheidt of the National Institutes of Health also presented an update on the proposed National Children’s Study, a longitudinal study of environmental influences on child health and development that is being considered by the National Institute of Child Health and Human Development and a consortium of Federal agencies.

Deborah Rice of the Maine Center for Disease Control and Prevention presented on the neurotoxic effects of developmental exposure to environmental chemicals and evidence that these exposures are producing behavioral deficits in children. In a series of case studies on various chemicals (e.g. lead, PCBs and methylmercury), she highlighted the influence that genetic polymorphisms have on the differential susceptibility to toxic effects and

the need to address gene-chemical interactions in a coordinated manner with experimental and epidemiological studies. The final speaker was Tracey Woodruff of the U.S. Environmental Protection Agency (EPA). She discussed incorporating early-life susceptibility into risk assessment and EPA’s “Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens” to describe how

data on early-life susceptibility can be incorporated into risk assessment. She also commented on the potential for toxicogenomics and other new methods to provide important data to inform risk assessment and the policy process.

Amy Kyle of the University of California - Berkeley moderated a panel discussion on applying the advances in toxicogenomic research to better understand and address early-life exposures that contribute to dis-

continued on page 4

Download the agenda, audio, and presentations from the February 8th workshop at http://www.dels.nas.edu/emergingissues/toxicogenomics_meet12.shtml. A workshop summary will be available in the future.

This newsletter as well as additional information about the committee and its activities can be found at <http://dels.nas.edu/emergingissues>. The newsletter of the Committee on Emerging Issues and Data on Environmental Contaminants, is published to keep you informed of committee activities. This is a joint project of the National Research Council’s Board on Environmental Studies and Toxicology and Board on Life Sciences. The views expressed in the articles in this newsletter are those of the individual authors and do not reflect the findings or conclusions of The National Academies.

Applications of Genomic Signatures: A Workshop

A workshop on “Applications of Genomic Signatures” was held December 7, 2005 in Welches, Oregon as part of the 11th meeting of the committee on Emerging Issues and Data on Environmental Contaminants. This workshop, chaired by Peter Spencer of Oregon Health & Science University, was part of a wider state-of-the-science conference focused on genotype-to-phenotype correlations in health and disease that was hosted by the Center for Research on Occupational and Environmental Toxicology at the Oregon Health & Science University. The workshop will be summarized in a forthcoming National Research Council publication; this article describes highlights from the workshop.

The workshop focused on potential uses of gene expression “signatures” for describing a chemical’s toxicity in regulatory and other applications.

Currently, genomic signatures are not widely used in regulatory settings, so discussion was only possible because the speakers generously subjected their data and approaches to critique and because participants were willing to comment on data they may not have seen in advance. The meeting included academic scientists (many from the NIEHS Toxicogenomics Research Consortium and the NIEHS Comparative Mouse Genomics Centers Consortium) and participants from regulatory agencies, regulated industries, and companies that generate proprietary toxicogenic information.

Introduction

Peter Lord, of Johnson & Johnson (J&J), provided an overview of toxicogenomic information used by pharmaceutical companies, as well as questions regarding regulatory submission. In drug development, toxicogenomics is used in an effort to investigate a compound’s potential safety liabilities as early in the drug development process as possible, for more economical decision making.

Lord explained how differences in gene expression between animals dosed with a compound and control animals are generally considered in two ways. First, scientists compare patterns of gene expression “signatures” from novel compounds to signatures of compounds whose toxicity is better understood to gain insight on the novel compound’s effects. Second, “pathway analysis” is used to examine individual gene expression changes to gain insight into the affected biological pathways. J&J has developed a database on over 200 compounds to facilitate identification of gene expression signa-

tures consistent with six types of toxicity: macrophage activation, peroxisome proliferation, oxidative stress and reactive metabolites, cholestasis, phospholipidosis, and non-genotoxic carcinogenicity.

Lord is also working with a group called HL7/CDISC/13C Pharmacogenomics Data Standards Track 1 Toxicogenomics Working Group to debate formats and quantity of toxicogenomic data supporting the regulatory submission of drug safety claims. Lord described how a claim’s conclusions should be supported with enough information (including data analysis steps) that regulators can critique the assessment and conclusion.

Acetaminophen Data

Richard Paules, of the NIEHS, described toxicogenic data on acetaminophen generated by the NIEHS National Center for Toxicogenomics. Paules’s group is particularly interested in identifying gene expression signatures that can be used to assess the extent of liver injury following acetaminophen poisoning. In this workshop, the acetaminophen data were considered to illustrate the type of toxicogenomic data that may be generated on a compound.

Acetaminophen-affected genes are typically involved in stress responses, including those involved in generating energy for the cell. The gene expression data appear to include two components: a continuum of quantitative changes, with the most sensitive genes responding first; and a series of qualitative changes with thresholds above which additional pathways are affected. The latter observation led Paules to speculate that there may be a point at which the gene expression predicts a pending severe toxicity.

The panel (Richard Brennan, Iconix; Judith Graham, American Chemistry Council; Robert Kavlock, Environmental Protection Agency; John K. Leighton, Food and Drug Administration; William B. Mattes, Gene Logic; and Nigel Walker, National Toxicology Program) discussed the potential regulatory challenges created by the increasing ability of toxicogenomics to detect effects at lower chemical levels. There was also debate about whether there are minimum chemical concentrations (or thresholds) below which gene expression changes do not exist or cannot be detected. Some participants believe that any apparent threshold reflects the technology’s detection limits rather than the underlying biology.

Don’t take our word for it, listen for yourself! Audio files and PowerPoint files of this workshop are available at http://dels.nas.edu/emergingissues/toxicogenomics_meet11.shtml.

continued on page 4

Tox. and Early-Life Exposures

continued from page 2

ease in children and adults. Panel members included Jerry Heindel, National Institute of Environmental Health Sciences; Gail McCarver, Medical College of Wisconsin; John Quackenbush, Dana Farber Cancer Institute; and Anthony Scialli, Sciences International Incorporated. The discussion covered a range of topics, including what aspects of early-life exposures and subsequent disease need to be understood better; which techniques and research avenues appear most promising; how to move toward broader implementation of these techniques and findings; and the institutional and legal issues surrounding early-life exposures. Closing remarks were given by Linda Greer, Natural Resources Defense Council, who commented on the scientific findings outlined in the presentations and potential policy implications.

The workshop planning committee is currently preparing a summary of the workshop discussions and presentations. This summary will be published as part of the series of workshop reports generated by the Committee on Emerging Issues and Data on Environmental Contaminants (see http://dels.nas.edu/emergin-gissues/workshop_reports.shtml).

WORKSHOP PLANNING COMMITTEE Toxicogenomics and Early-Life Exposures

Cheryl Walker (Chair)
M.D. Anderson Cancer Center, University of Texas

Linda Greer
Natural Resources Defense Council

Robert Griffin
Center for Mass Media Research,
College of Communication, Marquette University

Amy Kyle
School of Public Health,
University of California – Berkeley

George Orphanides
Syngenta Central, Toxicology Laboratory

Frederica Perera
Mailman School of Public Health and Columbia Center
for Children's Environmental Health, Columbia University

Mark Rothstein
University of Louisville, School of Medicine

App. of Genomics Signatures

continued from page 3

Endocrine Disrupter Data

George Daston, of Procter & Gamble, described his group's efforts to develop signatures to identify chemicals that may disrupt the endocrine system years after an exposure. Daston hopes that using toxicogenomics will lead to the development of a screening test that is more specific (does not generate too many false positives) than other screening tests that may be developed while still being adequately sensitive (not too many false negatives).

Daston's group identified a putative gene expression signature using 66 genes that respond to estrogenic compounds. The response of these genes was related to the dose, and specificity was observed with the compounds tested to date. These gene expression changes affected several targets and biological pathways: cell growth and differentiation, metabolic enzymes, cell death/apoptosis, and structural proteins.

Workshop participants discussed whether the gene expression changes described by Daston are sufficient for identifying potential endocrine disrupting compounds. They also discussed whether toxicogenomics can really be used to predict latent adverse effects. In this discussion, participants noted both the importance of considering end points other than gene expression, such as pathologic and physiologic changes, and that more data and analysis are needed to assess the value of toxicogenomics in predicting latent effects.

Antifungal Data

David Dix, of the Environmental Protection Agency's National Center for Computational Toxicology, discussed a project with Iconix Pharmaceuticals that sought to evaluate the ability of genomic signatures to predict toxicity and to identify affected biological pathways. Specifically, they were looking for information to identify similarities and differences in the toxicity of three antifungal compounds.

In the studies, each compound was administered to test animals at one dose, and gene expression was analyzed at several time points. For comparison, more conventional toxicity assessments were conducted in parallel. The gene expression signatures of three antifungal compounds were compared with the hundreds of other chemicals in the Iconix Drug Matrix database. It was predicted that these antifungals would lead to several effects: liver enzyme increases indicative of injury, hepatomegaly, inflammation, pregnane X receptor (PXR) stimulation, and an acute phase response (for 2/3 compounds). These predicted effects were consistent with in-vivo observations in the same animals.

continued on page 5

App. of Genomics Signatures

continued from page 4

In addition to comparing signatures of these compounds to other compounds in the database, a pathway analysis was conducted to assess the mechanisms by which such toxicities might occur. This pathway analysis suggested involvement of pathways consistent with hepatomegaly and inflammation. Both the signature- and pathway- based analyses of the gene expression data from this study grouped the three antifungals with other azole antifungals in the Iconix Drug Matrix database, though differences were noted between the signatures of these agrichemicals and antifungal drugs in the database.

In the discussion, some participants said they would have liked to see a range of doses analyzed to generate a dose-response relationship. Others emphasized that the results could be impacted by how the doses of test compounds were selected (selection was based on body weight effects or hepatomegaly, in this case).

Other Highlights

Peter Spencer asked about the value of using a “negative control” to isolate, or “subtract” compound- specific effects on gene expression. That is, should the effects of compounds that have similar structures but do not cause the toxicity of interest be compared to isolate gene expression changes specific to the toxicity of interest? Some participants suggested that, in some cases, removing common responses may indeed account for effects not due to a particular toxicity. However, subtracting other responses may not be appropriate due to their potential interactive effects.

Participants discussed the research community’s apparent comfort level with traditional toxicology endpoints such as histopathology and toxicogenomics. While some participants noted that histopathology is currently the “gold standard” for assessing toxicity, others pointed out its limitations (for example, in the number of cells examined) and that the comfort with it is due largely to familiarity. Along these lines, William Mattes of Gene Logic thought the comfort level with toxicogenomics may increase as more biologists look at gene expression experiments.

Some participants thought reaching regulatory and scientific community acceptance will depend on subjecting signatures to public comment. One participant commented on the inability to scrutinize the proprietary signatures developed by companies such as Iconix and Gene Logic that conduct toxicogenomic data analysis.

Some participants suggested organizing efforts to develop agreement on what defines a signature for a particular application and whether a given signature is appropriate for use in that application. In

a related point, James Bus of Dow Chemical Company thought it would be useful to set priorities on which signatures (for example: kidney toxicity, liver toxicity) should be generated first.

Participants also discussed the concept of “validating” or “verifying” a gene expression signature. These terms can have a range of meanings to different scientists (to some, validation refers to the lengthy, complex process of reaching interagency agreement outlined by the Interagency Coordinating Committee on the Validation of Alternative Methods, see <http://iccvam.niehs.nih.gov>), and the requirements for verification will depend on the intended use of the signature (for example, internal use versus regulatory use).

Joseph DeGeorge, of Merck Research Laboratories, explained that a distinction should also be made between the use of signatures for pharmaceutical and environmental chemicals. That is, there are fewer ques-

WORKSHOP PLANNING COMMITTEE Applications of Genomic Signatures

Peter S. Spencer (Chair)
School of Medicine, Oregon Health &
Science University

James S. Bus
The Dow Chemical Company

Joseph J. DeGeorge
Merck Research Laboratories

Amy D. Kyle
School of Public Health, University of
California, Berkeley

Kenneth S. Ramos
Department of Biochemistry and Molecular Biology,
University of Louisville

Cheryl Lyn Walker
M.D. Anderson Cancer Center, University of Texas

Save the date:

November 14, 2006

**Workshop on Improving and Protecting
Public Health Through the Use
of Toxicogenomics**

Washington, DC

continued on page 6

App. of Genomics Signatures

continued from page 5

tions and concerns about using signature data to qualitatively identify potential hazards of pharmaceuticals during lead development. Similarly, Robert Kavlock of the EPA Office of Research and Development pointed out that when using signatures for prioritizing environmental chemicals, false negatives could be problematic. This is because environmental chemicals whose signatures do not indicate adverse effects may not be subject to further toxicity testing, unlike pharmaceuticals that would undergo traditional animal testing regardless.

When asked to consider specific signatures, such as those described by Daston, DeGeorge said the breakout group he led thought scientists' interpretation of signatures is limited by a currently insufficient body of knowledge to place the toxicogenomic data into a biological context. While toxicogenomic data might reinforce traditional observations, at this point in time, the toxicogenomic data would probably be insufficient to stand alone as a predictor of toxicity. Mattes and Daston remarked that in the future, history and context will make scientists more comfortable evaluating how toxicogenomic data translate into disease state information. Noting progress to date, Kenneth Ramos of the University of Louisville Health Sciences Center and several other participants described how the community has come a long way since issues of data variability and platform independence dominated conversation.

Finally, several participants stated that additional toxicogenomic data, integrated with traditional types of toxicological data in a database, are necessary to provide the biologic context for regulatory interpretation of toxicogenomic information, especially for environmental chemicals. While often referred to as a need for a database, Ramos and others described how the real need is for strategic and systemic efforts to generate data to populate such a database—an expensive effort. Ramos, Bus and others suggested it is time for the

community to describe the most important questions to be answered and formulate a plan for generating the data.

STAFF

Board Directors

James Reisa (BEST)

Fran Sharples (BLS)

Project Officers

Marilee Shelton-Davenport

Karl Gustavson

Project Assistants

Lucy Fusco

Anne Jurkowski

Newsletter

Radiah Rose

Recent Workshop Summary



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

Based on a workshop held in August 2004, this report explores how toxicogenomics might improve the prediction of human responses to chemicals. This report is available free online at http://dels.nas.edu/emergingissues/workshop_reports.shtml.

Intellectual Property Concerns for Toxicogenomics: A Workshop

Washington, DC

Thursday, June 29, 2006

This workshop will provide a forum for discussing current and evolving intellectual property laws and practices as they relate to toxicogenomics, particularly their intended or unintended effects on the sharing of toxicogenomic data and on application development. Additionally, the workshop will explore intellectual property mechanisms and database management policies that enable the sharing and access of toxicogenomics data and applications.

MORNING SESSION

- 8:15 Welcome and Introduction to Workshop
- 8:30 **Primer on Intellectual Property**
Christopher M. Holman, University of Missouri-Kansas City School of Law
- 9:00 **Cutting-Edge Issues in Intellectual Property Litigation**
Jeffrey A. Lindeman, Nixon Peabody LLP
- 9:30 **Cutting-Edge Practices and Considerations in Technology Transfer**
Brian R. Stanton, Office of Technology Transfer, National Institutes of Health
- 10:00 **Review of the 2006 NRC report "Reaping the Benefits of Genomic and Proteomic Research: Intellectual Property Rights, Innovation, and Public Health"**
Roderick R. McKelvie, Covington & Burling
- 10:15 Break
- 10:30 Question and Answer Session
- 11:15 **Intellectual Property Impact on Toxicogenomics Research and Application Development**
Paul C. Kimball, Gene Logic (invited)

AFTERNOON SESSION

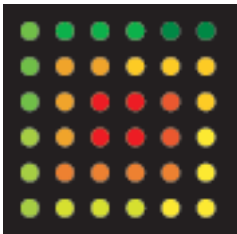
- 1:00 **Intellectual Property Concerns in the Development of a Community Resource Project**
Claire T. Driscoll, Technology Transfer Office, National Human Genome Research Institute

- 2:00 **Panel 1: Incentives for Contributors to Community Resource Projects**
Claire T. Driscoll, Technology Transfer Office, National Human Genome Research Institute
Robert L. Strausberg, J. Craig Venter Institute
Edward M. Yoshida, Merck & Co., Inc.
- 2:45 **Panel 2: Intellectual Property Protection of Database Outputs**
Roderick R. McKelvie, Covington & Burling
George Elliott, United States Patent and Trademark Office
Arti K. Rai, Duke University
- 3:30 Break
- 3:45 **Panel 3: Access to Patented Toxicogenomics Applications**
Joann A. Boughman, American Society of Human Genetics
Jon F. Merz, University of Pennsylvania School of Medicine
Timothy Holbrook, Chicago-Kent College of Law
- 4:30 Question and Answer Session
- 5:00 Closing Remarks

For more information, please email Anne Jurkowski at ajurkowski@nas.edu or call (202) 334-1442

First Class
U.S. Postage
PAID
Permit No. 0123
Capitol Heights, MD

Committee on Emerging Issues and Data on Environmental Contaminants
Board on Environmental Studies and Toxicology
Board on Life Sciences
THE NATIONAL ACADEMIES
500 Fifth Street NW
Washington, DC 20001



Workshop announcement

Intellectual Property Concerns for Toxicogenomics: A Workshop

Thursday, June 29, 2006

National Academy of Sciences Building
Washington, DC

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

The nation turns to the National Academies—National Academy of Sciences, National Academy of Engineering, Institute of Medicine, and National Research Council—for independent, objective advice on issues that affect people's lives worldwide.

www.national-academies.org