**UNITED STATES ENVIRONMENTAL PROTECTION AGENCY**
**WASHINGTON D.C. 20460**

**OFFICE OF THE ADMINISTRATOR**
**SCIENCE ADVISORY BOARD**

DATE

EPA-SAB-13-XXX

The Honorable Lisa P. Jackson
Administrator
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, N.W.
Washington, D.C. 20460

Subject: SAB Advice on Advancing the Application of Computational Toxicology Research for Human Health Risk Assessment

Dear Administrator Jackson:

The Science Advisory Board (SAB), as well as the National Academy of Sciences, has encouraged the EPA to improve its risk assessment practices and to modify its single-chemical approach. Tens of thousands of chemicals are currently in commerce and hundreds more introduced every year, yet only a small fraction have been adequately assessed for potential hazard. To meet this challenge, the agency established the Computational Toxicology (CompTox) Research Program to explore ways to exploit modern advances in molecular biology, chemistry, exposure science and computer science to more effectively and efficiently assess chemical hazards and ultimately their risks. The SAB previously has underscored the importance of this research program and has been interested in the successful application of CompTox data to advance the EPA's hazard assessment and, in combination with exposure data, risk assessment.

In addition to permitting more rapid evaluation of individual chemicals, the CompTox research program also has the potential to provide the agency with a means of shifting its traditional focus on single stressors, endpoints, sources, pathways and environmental media to evaluate, more broadly, multiple factors simultaneously. To assist the EPA in this process, the SAB asked its Exposure and Human Health Committee to evaluate how the products from the CompTox research program are being used by EPA, whether the program outputs align with the needs of the EPA's programs and whether limitations or challenges to using CompTox hazard and exposure data in decision-making for risk assessment can be identified and addressed. The SAB committee, along with two members of the EPA Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel, received briefings from EPA representatives regarding the use of CompTox research program outputs as one component into EPA risk assessments. In the enclosed report, the SAB provides its analysis and advice regarding the issues that the agency should consider as they move forward with implementation. This letter highlights the SAB's major recommendations.

The SAB commends the EPA for undertaking the immense effort of establishing the CompTox research program. The program is still in the development stage and the agency has not yet begun to incorporate the information derived from it into various applications (e.g., prioritization, screening or

risk assessment). However, the program already has contributed to the EPA's efforts to conduct a rapid response evaluation of chemicals. For example, EPA's ability to employ high-throughput screening (HTS) assays to test for endocrine activity in the eight candidate oil dispersants for use during the Gulf of Mexico Deepwater Horizon accident was possible, in part, due to the existence of the CompTox research program. While the agency was able to obtaining such test data in a short timeframe, the crisis highlights the need to develop a structured approach for utilizing the CompTox information in emergency situations. Specifically, in the case of the Deepwater Horizon accident, a very limited subset of assays was used to evaluate the dispersants. Were the data derived from this limited set of assays the most appropriate? Were they sufficient? The need to obtain data quickly to inform decisions in a crisis emphasizes the importance of developing a structured approach beforehand in the form of data use guides (DUGs). These DUGs should be developed after a thorough characterization of programmatic needs –the intended goals of a prioritization effort, screening or a risk assessment – together with the identification of examples of where CompTox information appears to add value.

The challenges that the EPA faces regarding the various applications of CompTox data are substantial and are well known to the agency. These include, but are not limited to, detailed characterization of each individual assay, determining the accuracy of the assays against traditional *in vivo* studies, determining how the data generated predict effects on apical endpoints or empirically verifiable outcomes of exposure (e.g., developmental anomalies) employed in validated guideline studies and the ways in which these patterns of data predict the risk of human disease. The latter issue is the most difficult. As the agency elucidates the Adverse Outcome Pathways (AOPs) that would link these patterns to human disease, there are new methodologies using new cell and tissue types that may prove useful to link early gestational exposures or environmental insults to many common disorders. The EPA would be well-served to partner with professional societies and research institutes whose mission is to understand the diseases under investigation to gain further insight.

Exposure science also will be critical to prioritize chemicals for screening and further assessment. While the CompTox research program is currently focused on understanding AOPs, a similar effort for incorporating metabolism and other toxicokinetic factors, and understanding exposure (e.g. through the CompTox ExpoCast effort) is needed before these approaches can be fully applied in decision-making. Methods for incorporating biomonitoring data, exposure pathways, chemical source information, and information on human activity patterns also need to be developed and incorporated into risk assessments. A clear explanation of the limitations of the models, the reliability of the assay systems, the certainty associated with an AOP and knowledge of the metabolism of the chemical being assessed are only some of the important considerations that should be addressed when screening untested chemicals or developing the hazard or exposure component of a risk assessment.

Demonstrating the predictive value of CompTox data and its utility for the EPA's decisions is needed to overcome barriers to its acceptance within and outside the agency. This will require a combination of research to develop reliable methods and experience in using them to predict hazard and risk, relative to more traditional methods. Through incremental change to the current approaches for assessing risk, first in supplementing and later by replacing existing methodologies, the EPA likely will be able to demonstrate the value of these new technologies, which will lead to greater confidence in the use of CompTox and ExpoCast as predictive tools to understand hazard and risk.

Outreach, training and communication also are vital to effective implementation of CompTox outputs and advancing EPA risk assessment. Efforts to reach out to EPA program offices that could benefit from CompTox data and engaging stakeholders to communicate the value and utility of the research

program are laudable and should continue. We commend EPA for establishing the Computational Toxicology Communities of Practice which is composed of more than 300 people from over 50 public and private sector organizations interested in the application of computational toxicology and exposure science to EPA's risk assessments. We also support the agency's goal of transparency, publishing all the data online so that the public can view and interpret these data. In fact, these data will likely be the source of numerous Ph.D. dissertations in the near future. However, the website (http://www.epa.gov/ncct/) is somewhat difficult to navigate and it would be useful for the agency to redouble their efforts to ensure that the public can access the data with relative ease.

In summary,

- The SAB applauds the work of the CompTox research program, and recommends the continued development of CompTox outputs to lead to a better understanding and expansion of the potential utility of this technology.
- EPA should explore partnerships with clinical and research societies whose members represent the experts in mechanisms of disease to help the agency develop AOPs.
- EPA should develop data use guidelines for information generated by CompTox, including ExpoCast, for the various purposes to which it is intended.
- EPA should increase its efforts to understand chemical exposure, including determining how and where the chemicals are used and activity patterns of people that will result in exposure and not just the chemicals' movement through the environment based on fundamental chemical properties.
- We encourage the agency to continue to engage stakeholders and provide easy access to data on the CompTox website.

As the EPA gains more experience and expertise in the use of CompTox outputs in risk assessment, along with the development of ExpoCast, we look forward to future opportunities for providing advice to EPA on this important effort.

Sincerely,

# NOTICE

This report has been written as part of the activities of the EPA Science Advisory Board, a public advisory group providing extramural scientific information and advice to the Administrator and other officials of the Environmental Protection Agency. The Board is structured to provide balanced, expert assessment of scientific matters related to the problems facing the agency. This report has not been reviewed for approval by the agency and, hence, the contents of this report do not necessarily represent the views and policies of the Environmental Protection Agency, nor of other agencies in the Executive Branch of the Federal government, nor does mention of trade names or commercial products constitute a recommendation for use. Reports of the EPA Science Advisory Board are posted on the EPA website at http://www.epa.gov/sab.

# U.S. Environmental Protection Agency
# Science Advisory Board
# Exposure and Human Health Committee and SAP Liaisons

**CHAIR**

**Dr. R. Thomas Zoeller**, Professor, Department of Biology, University of Massachusetts, Amherst, MA

**MEMBERS**

**Dr. Claude Emond**, Adjunct Clinical Professor , Department of Environmental and Occupational Health , Faculty of Medicine, University of Montreal, Montréal, QC, Canada

**Dr. Anna Fan-Cheuk**, Chief, Pesticide and Environmental Toxicology Section, California Environmental Protection Agency, Oakland, CA

**Dr. Alfred Franzblau**, Professor, Department of Environmental Health Sciences, School of Public Health, University of Michigan, Ann Arbor, MI

**Dr. Maida Galvez**, Assistant Professor, Department of Community and Preventive Medicine, Department of Pediatrics, Mount Sinai School of Medicine, New York, NY

**Dr. Chris Gennings**, Professor, Department of Biostatistics, Virginia Commonwealth University, Richmond, VA

**Dr. Gary Ginsberg**, Toxicologist, Environmental & Occupational Health, Connecticut Department of Public Health, Hartford, CT

**Dr. Robert L. Goble**, Research Professor, George Perkins Marsh Institute, Clark University, Worcester, MA

**Dr. Russ Hauser**, Frederick Lee Hisaw Professor , Department of Environmental Health, Harvard School of Public Health, Boston, MA

**Dr. Laurie Haws**, Principal Health Scientist, ToxStrategies, Inc., Austin, TX

**Dr. Darryl Hood**, Professor, Department of Neuroscience and Pharmacology, Center for Environmental Health Disparities and Medicine, Meharry Medical College, Nashville, TN

**Dr. Kirsten Moysich**, Professor of Oncology , Department of Cancer Prevention and Control, Roswell Park Cancer Institute , Buffalo, NY

**Dr. Gloria Post**, Research Scientist, Office of Science, New Jersey Department of Environmental Protection, Trenton, NJ

**Dr. Virginia Rauh**, Professor and Deputy Director, Department of Population and Family Health, School of Public Health, Columbia University, New York, NY

**Dr P. Barry Ryan**, Professor of Exposure Science and Environmental Chemistry, Environmental and Occupational Health, Rollins School of Public Health, Emory University, Atlanta, GA

**Dr. John Vena**, Professor and Department Head, Department of Epidemiology and Biostatistics, College of Public Health, University of Georgia, Athens, GA

**Dr. Clifford Weisel**, Professor, Department of Environmental and Occupational Medicine , University of Medicine and Dentistry of New Jersey, Piscataway, NJ

**Dr. Robert Wright**, Associate Professor, Pediatrics, Division of Environmental Health, Harvard School of Public Health, Boston, MA

## SAP LIAISONS

**Dr. Janice Chambers**, William L. Giles Distinguished Professor and Director, Center for Environmental Health Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS

**Dr. Daniel Schlenk**, Professor of Aquatic Ecotoxicology & Environmental Toxicology, Department of Environmental Sciences, University of California, Riverside, CA

## SCIENCE ADVISORY BOARD STAFF

**Dr. Suhair Shallal**, Designated Federal Officer, U.S. Environmental Protection Agency, Science Advisory Board (1400R), 1200 Pennsylvania Avenue, NW, Washington, DC

# U.S. Environmental Protection Agency
# Science Advisory Board
# BOARD

**CHAIR**

**Dr. David T. Allen**, Gertz Regents Professor of Chemical Engineering and the Director of the Center for Energy and Environmental Resources, The University of Texas, Austin, TX

**MEMBERS**

**Dr. George Alexeeff**, Director, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Oakland, CA

**Dr. Pedro Alvarez**, Department Chair and George R. Brown Professor of Engineering, Department of Civil & Environmental Engineering, Rice University, Houston, TX

**Dr. Joseph Arvai**, Svare Chair in Applied Decision Research, Institute for Sustainable Energy, Environment, & Economy, Haskayne School of Business, University of Calgary, Calgary, Alberta, Canada

**Dr. Thomas Burbacher**, Professor, Department of Environmental and Occupational Health Sciences, School of Public Health, University of Washington, Seattle, WA

**Dr. Ingrid Burke**, Director, Haub School and Ruckelshaus Institute of Environment and Natural Resources, University of Wyoming, Laramie, WY

**Dr. Thomas Burke**, Professor and Jacob I. and Irene B. Fabrikant Chair in Health, Risk and Society Associate Dean for Public Health Practice, Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD

**Dr. Edward T. Carney**, Departmental Senior Science Leader and Director of Predictive Toxicology Center, Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI

**Dr. Terry Daniel**, Professor of Psychology and Natural Resources, Department of Psychology, School of Natural Resources, University of Arizona, Tucson, AZ

**Dr. George Daston**, Victor Mills Society Research Fellow, Global Product Stewardship, The Procter & Gamble Company, Mason, OH

**Dr. Costel Denson**, Managing Member, Costech Technologies, LLC, Newark, DE

**Dr. Otto C. Doering III**, Professor, Department of Agricultural Economics, Purdue University, W. Lafayette, IN

**Dr. Michael Dourson**, President, Toxicology Excellence for Risk Assessment, Cincinnati, OH

**Dr. Joel Ducoste**, Professor, Department of Civil, Construction, and Environmental Engineering, College of Engineering, North Carolina State University, Raleigh, NC

**Dr. David A. Dzombak**, Walter J. Blenko, Sr. University Professor of Environmental Engineering, Department of Civil and Environmental Engineering, College of Engineering, Carnegie Mellon University, Pittsburgh, PA

**Dr. T. Taylor Eighmy**, Vice Chancellor for Research and Engagement, University of Tennessee, Knoxville, TN

**Dr. Elaine Faustman**, Professor and Director, Environmental and Occupational Health Sciences, University of Washington, Seattle, WA

**Dr. R. William Field**, Professor, Department of Occupational and Environmental Health, and Department of Epidemiology, College of Public Health, University of Iowa, Iowa City, IA

**Dr. H. Christopher Frey**, Distinguished University Professor, Department of Civil, Construction and Environmental Engineering, College of Engineering, North Carolina State University, Raleigh, NC

**Dr. John P. Giesy**, Professor and Canada Research Chair, Veterinary Biomedical Sciences and Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

**Dr. Cynthia M. Harris**, Director and Professor, Institute of Public Health, Florida A&M University, Tallahassee, FL

**Dr. Robert Johnston**, Director of the George Perkins Marsh Institute and Professor, Economics, Clark University, Worcester, MA

**Dr. Kimberly L. Jones**, Professor and Chair, Department of Civil Engineering, Howard University, Washington, DC

**Dr. Bernd Kahn**, Professor Emeritus and Associate Director, Environmental Radiation Center, Georgia Institute of Technology, Atlanta, GA

**Catherine Karr**, Associate Professor - Pediatrics and Environmental and Occupational Health Sciences and Director - NW Pediatric Environmental Health Specialty Unit, University of Washington, Seattle, WA

**Dr. Madhu Khanna**, Professor, Department of Agricultural and Consumer Economics, University of Illinois at Urbana-Champaign, Urbana, IL

**Dr. Nancy K. Kim**, Senior Executive, Health Research, Inc., Albany, NY

**Dr. Francine Laden**, Mark and Catherine Winkler Associate Professor of Environmental Epidemiology, Harvard School of Public Health, and Channing Division of Network Medicine,

Brigham and Women's Hospital and Harvard Medical School, Boston, MA

**Dr. Cecil Lue-Hing**, President, Cecil Lue-Hing & Assoc. Inc., Burr Ridge, IL

**Dr. Elizabeth Matsui**, Associate Professor, Pediatrics, School of Medicine, Johns Hopkins University, Baltimore, MD

**Dr. Surabi Menon**, Director of Research, ClimateWorks Foundation, San Francisco, CA

**Dr. James R. Mihelcic**, Professor, Civil and Environmental Engineering, University of South Florida, Tampa, FL

**Dr. Christine Moe**, Eugene J. Gangarosa Professor, Hubert Department of Global Health, Rollins School of Public Health, Emory University, Atlanta, GA

**Dr. Horace Moo-Young**, Dean and Professor, College of Engineering, Computer Science, and Technology, California State University, Los Angeles, CA

**Dr. Eileen Murphy**, Director of Research and Grants, Ernest Mario School of Pharmacy, Rutgers University, Piscataway, NJ

**Dr. James Opaluch**, Professor and Chair, Department of Environmental and Natural Resource Economics, College of the Environment and Life Sciences, University of Rhode Island, Kingston, RI

**Dr. Duncan Patten**, Director, Montana Water Center, and Research Professor, Hydroecology Research Program, Department of Land Resources and Environmental Sciences, Montana State University, Bozeman, MT

**Dr. Martin Philbert**, Dean and Professor, Environmental Health Sciences, School of Public Health, University of Michigan, Ann Arbor, MI

**Dr. Stephen Polasky**, Fesler-Lampert Professor of Ecological/Environmental Economics, Department of Applied Economics, University of Minnesota, St. Paul, MN

**Dr. C. Arden Pope, III**, Professor, Department of Economics, Brigham Young University, Provo, UT

**Dr. Stephen M. Roberts**, Professor, Center for Environmental and Human Toxicology, University of Florida, Gainesville, FL

**Dr. Amanda Rodewald**, Professor of Wildlife Ecology, School of Environment and Natural Resources, The Ohio State University, Columbus, OH

**Dr. James Sanders**, Director and Professor, Skidaway Institute of Oceanography, Savannah, GA

**Dr. William Schlesinger**, President, Cary Institute of Ecosystem Studies, Millbrook, NY

**Dr. Gina Solomon**, Deputy Secretary for Science and Health, Office of the Secretary, California Environmental Protection Agency, Sacramento, CA

**Dr. Daniel O. Stram**, Professor, Department of Preventive Medicine, Division of Biostatistics, University of Southern California, Los Angeles, CA

**Dr. Peter S. Thorne**, Director, Environmental Health Sciences Research Center and Professor and Head, Department of Occupational and Environmental Health, College of Public Health, University of Iowa, Iowa City, IA

**Dr. Paige Tolbert**, Professor and Chair, Department of Environmental Health, Rollins School of Public Health, Emory University, Atlanta, GA

**Dr. Jeanne Van Briesen**, Professor, Department of Civil and Environmental Engineering, Carnegie Mellon University, Pittsburgh, PA

**Dr. John Vena**, University of Georgia Foundation Professor in Public Health and Head, Department of Epidemiology and Biostatistics, Georgia Cancer Coalition Distinguished Scholar, College of Public Health , University of Georgia, Athens, GA

**Dr. R. Thomas Zoeller**, Professor, Department of Biology, University of Massachusetts, Amherst, MA

**SCIENCE ADVISORY BOARD STAFF**

**Dr. Angela Nugent**, Designated Federal Officer, U.S. Environmental Protection Agency, Science Advisory Board (1400R), 1200 Pennsylvania Avenue, NW, Washington, DC

# Table of Contents

# GLOSSARY

**AOP** or Adverse Outcome Pathways provide a scientific approach for linking mechanistic information to responses considered relevant to risk assessment and management. AOPs refers to pathways that would: (1) proceed from an initiating molecular event in which a chemical interacts with a biological target (e.g., DNA binding, protein oxidation, or receptor/ligand interaction); (2) continue on through a sequential series of biological activities (e.g. gene activation, or altered cellular chemistry or tissue development); and (3) ultimately culminate in an adverse outcome of relevance to human or ecological risk assessors (e.g., mortality, disrupted reproduction, cancer, or extinction).

**Apical Effects or Endpoints** - empirically verifiable outcomes of exposure, such as developmental anomalies, breeding behaviors, impaired reproduction, physical changes and alterations in the size and histopathology of organs, or death.

**CompTox** or the EPA's Computational Toxicology research is part of EPA's broader Chemical Safety research efforts. CompTox is researching new, more efficient, ways to assess chemical safety for potential risk to human health and the environment that can replace traditional chemical toxicity testing that is expensive, time consuming and uses a significant number of animals.

**CSS** or Chemical Safety for Sustainability is one of six transdisciplinary research programs in EPA's Office of Research and Development which focuses on the use of systems-approaches to advance the understanding of the links between exposures to chemicals and toxicity pathways that lead to the development of disease.

**ExpoCast**- A collaborative effort across the exposure and risk assessment community to provide the exposure science required for interpretation of high-throughput *in vitro* toxicity data. The overall goal of this program is to develop novel approaches and tools for evaluating and classifying chemicals, based on potential for biologically-relevant human exposure, to inform prioritization and toxicity testing.

**Omics** - refers to a broad field of study in biology, ending in the suffix "-omics" such as genomics, proteomics, transcriptomics.

**Tox21**- A collaborative effort of the EPA, National Institutes of Environmental Health Sciences/National Toxicology Program, National Institutes of Health and the Food and Drug Administration that is pooling federal resources and expertise in the use of robotics technology to screen thousands of chemicals for potential toxicity, use screening data to predict the potential toxicity of chemicals and develop a cost-effective approach for prioritizing the thousands of chemicals that need toxicity testing.

**ToxCast**- A multi-year effort launched by EPA in 2007 to develop a cost-effective approach for prioritizing the thousands of chemicals that need toxicity testing. ToxCast uses advanced science

tools to help understand how human body processes are impacted by exposure to chemicals and to determine which exposures are most likely to lead to adverse health effects.

**ToxPi** or Toxicological Priority Index is a tool which profiles the interactions of chemicals with biological processes in ways the public and decision makers can easily understand. ToxPi is a flexible prioritization support software tool that incorporates ToxCast bioactivity profiles, inferred toxicity pathways, dose estimates and chemical structural descriptors to provide a visual representation of the relative contribution of each data domain to an overall priority score.

**ToxRef -** a comprehensive relational database of mammalian toxicity studies developed by EPA's Office of Research and Development in collaboration with EPA's Office of Pesticide Programs (OPP).

# 1. INTRODUCTION

In 2007, the National Research Council (NRC) Committee on Toxicity Testing and Environmental Assessment published a study, *Toxicity Testing in the 21st Century: A Vision and a Strategy* (NRC 2007). In this report, the NRC Committee recommended developing a program that would incorporate modern tools to provide information about chemical toxicity for use in risk assessments. The overall goal of such a program would be to enhance the efficiency and effectiveness of chemical safety determinations. Guided by the NRC report, the EPA in that same year launched ToxCast™, an initiative to employ rapid automated chemical toxicity tests as part of the Computational Toxicology (CompTox) Research Program. The CompTox program conducts innovative research that integrates advances in molecular biology, chemistry and innovative computer science to more effectively and efficiently rank chemicals based on risks. The aim of the CompTox program is to take advantage of existing technologies to develop ways to predict the toxicity of the thousands of chemicals for which toxicity testing is lacking or absent.

In a recent report on the FY2012 EPA research budget, the EPA Science Advisory Board (SAB) noted its concern that, "…*there is no proactive budget initiative to develop ways of employing the results of the [Chemical Safety for Sustainability] CSS program, including high throughput data, into hazard or risk assessment*" (U.S. EPA SAB 2012). The CSS program, one of six transdisciplinary research programs within the EPA's Office of Research and Development, is responsible for coordinating the activities of the CompTox research program. In addition to allowing more rapid evaluation of a large number of individual chemicals, CompTox has the potential to provide the agency with a means of modifying its traditional focus on single stressors, endpoints, sources, pathways, and environmental media to a broader focus on evaluation of these factors in combination to evaluate the potential effects of the co-occurrence of multiple chemicals. For these reasons, the SAB has requested that its Exposure and Human Health Committee (EHHC) develop advice to assist in advancing the application of CompTox research for human health risk assessment to meet EPA's programmatic needs. In developing its advice to EPA, the EHHC engaged in discussions with ORD and EPA program offices, which currently use or plan to use the CompTox research outputs, in order to address the following questions:

1) Are the outputs of CompTox currently being used by EPA? How well do the outputs align with EPA's programmatic needs?

2) What issues are there in using CompTox in decision making for risk assessment and risk characterization as opposed to chemical screening, prioritization and green chemistry?

3) What are the barriers and limitations that prevent the EPA from using CompTox outputs and how might they be overcome? and

4) How should the use of the CompTox program be effectively communicated to stakeholders? How can the communication be enhanced?

The members of the EHHC were joined for this review by two members of the EPA Federal Insecticide, Fungicide, and Rodenticide Act Scientific Advisory Panel (FIFRA SAP) who had reviewed elements of the ToxCast program in 2011[1]. The committee was briefed by representatives from ORD and program offices ((e.g., pesticides, water or toxics)) regarding the overall scope, structure and organization of the program, and the use of ToxCast information within the programs represented. The EHHC met with representatives of the EPA in a face-to-face meeting on May 30-31, 2012, and discussed the study questions. A draft committee report was discussed at a teleconference on September 24, 2012, and the chartered SAB considered the draft report on (DATE). The following report outlines the SAB's impressions of the work undertaken by the CompTox research program and recommendations on how to enhance the utility of the program outputs.

[1] Report available at the following URL: http://www.epa.gov/scipoly/sap/meetings/2011/may/052411minutes.pdf

# 2. SAB STUDY FINDINGS AND RECOMMENDATIONS

## 2.1. Applications of CompTox to EPA Programs

*Study Question 1. Are the outputs of CompTox currently being used by EPA? How well do the outputs align with EPA's programmatic needs?*

The ToxCast program, an effort coordinated by the CompTox program, currently consists of nearly 700 individual assays provided by nine companies. A foundational element of ToxCast is a chemical library in which a large number of chemicals are simultaneously tested to create toxicity profiles in these assays. In Phase I, chemicals for which there is a substantial amount of toxicity data have been assayed, including 309 pesticide active ingredients and commercial chemicals. These chemicals will be assayed to provide a "proof of concept"; i.e., the results will be used to develop toxicity profiles and evaluate the ability of the assays to predict toxicity. In Phase II, about 2,000 chemicals from a broad range of sources including industrial and consumer products, food additives, "green" products (those that reduce or eliminate the use or generation of hazardous substances), nanomaterials and drugs that never made it to the marketplace are being screened. This information will be used to identify pathways of toxicity – patterns of responses observed in the CompTox assays that are plausibly and causally related to observations of apical effects or empirically verifiable outcomes of exposure (e.g., developmental anomalies) in the *in vivo* assays.

At present, the primary use of CompTox outputs has been to determine the reliability of the data for use in various types of decision-making by EPA programs. One example of where information derived from the CompTox research program has been used to inform agency decisions has been provided (see below). Despite the limited use of Comptox outputs to date, the SAB finds that the program is valuable and has made impressive progress in the five years since the inception of ToxCast.

The reliability of ToxCast data is currently being explored in two ways. First, data from ToxCast is being compared to data from ToxRef, a database of toxicity studies conducted with guideline, *in vivo* test systems. By comparing the effects of individual chemicals in both ToxCast and ToxRef, the EPA hopes to identify parallels that will provide confidence that decisions based on ToxCast data will be predictive of results for endpoints assessed using *in vivo* guideline studies. A second approach is to develop pathways of toxicity in humans that would lead to the clinical manifestation of disease.

These adverse outcome pathways" (AOPs) represent a very important link from *in vitro* high-throughput assays to human disease, and this effort is just beginning. EPA should explore AOPs not only based upon how a chemical can perturb biological systems but also from the perspective of how aging and disease processes have underlying AOPs which may be sensitive to chemical effect. By evaluating upstream events, EPA has the ability to use CompTox data for evaluating how chemical and disease AOPs may intersect leading to a more complete understanding of chemical action (NAS 2009).

EPA researchers in the CompTox program are also exploring the possibility that ToxCast data can be combined with large databases of experimental data at the level of the genome, epigenome, proteome and metabolome to provide higher resolution data within the context of AOPs. In principle, weight-of-evidence approaches would be developed to guide the integration of this information into current data and practices for hazard identification and perhaps risk assessment. If successful, this effort is expected to shape the future of toxicity testing at EPA in accordance with the recommendations of previous NAS reports, paying large dividends for the agency, researchers and the American public that are well worth the investments currently being made.

The outputs of the ToxCast program are being developed to align with the needs of EPA programs both in the long- and short-term. The EPA faces significant challenges to understanding how information derived from ToxCast can be employed to inform the various decisions required of the agency. These efforts will require constant communication between the different programs within the agency in order to ensure that the outputs meet the needs of the specific programs. At the SAB committee meeting in May 2012, EPA representatives from ORD and program office (e.g., pesticides, water or toxics) devoted considerable time to describing how this coordination is occurring. EPA's expectation is that the current research questions and research approach, as outlined above, will produce a program that will have broad applicability within the agency.

EPA used the high-throughput screening (HTS) assays that form the basis of the CompTox program in a trial approach to supplement the EPA's response to the Deepwater Horizon accident by calculating toxicity data (endocrine activity screens) on the eight oil dispersants employed by BP in the Gulf of Mexico. Having a formal CompTox program in place within EPA at the time of the Deepwater Horizon accident made it easier to employ these assays. This illustrates a number of important ancillary benefits of this program. One benefit is to have an infrastructure that would allow

rapid data generation so that the agency can make better-informed decisions in a disaster situation. Pairing this data with ExpoCast information (i.e., use novel approaches and tools for evaluating and classifying chemicals, based on potential for biologically-relevant human exposure) to evaluate potential exposure in response to emergencies could provide a more holistic assessment of the associated risk. Another benefit is that development of the CompTox program has required a great deal of interaction between various EPA offices. This interaction will foster greater communication about data needs and data interpretation. This interaction also helps to ensure that the intramural research program aligns with the routine and sometimes unanticipated needs of the agency as well as to help risk assessors identify early the data gaps that may be filled by the kind of information produced by CompTox. Additionally, the CompTox program provides an alternative means of evaluating multiple factors that might influence the risk posed by chemicals through the use of automated assays. The CompTox program provides the agency with a means of shifting its traditional focus on single stressors, endpoints, sources, pathways, and environmental media to a broader focus on the evaluation of these factors in combination or the potential co-occurrence among these factors.

However, the Deepwater Horizon accident also illustrates that in emergency situations CompTox data may be generated and used very rapidly without the opportunity to fully screen a chemical's toxic properties. Strategic planning is needed in advance of such events so the endpoints and assays available are predictive of adverse effects and relevant to the scenario at hand and to ensure some consistency across programs and applications. The limitations of such screening exercises must be described so as not to imply that the data predict risk (by themselves they do not constitute a risk assessment) or that the data present a complete toxicological description of effects the chemical can cause. With such caveats in mind and transparently stated, CompTox can be seen as an aid to risk management. The Data Use Guide (DUG) proposed later in this document will assist in the design and interpretation of CompTox screens for different scenarios.

## 2.2. Evaluating CompTox Outputs for Decision-making

*Study Question 2. What issues are there in using CompTox in decision making for risk assessment and risk characterization as opposed to chemical screening, prioritization and green chemistry?*

### 2.2.1. Specificity and Sensitivity of CompTox Data

A central question at this time is whether the *in vitro* high-throughput assays will produce data that will be suitable for decision-making such that, eventually, these data could replace *in vivo* testing for regulatory decisions. The answer to this question will undoubtedly depend on the level of decisions to be made. Thus, an important – if not essential – goal will be to sufficiently demonstrate and obtain widespread support for the data generated from ToxCast by risk assessors. This will also need to be consistent with statutory requirements for the evidence the EPA uses to take regulatory action. Thus, a principal goal of the research domain of the program is to characterize the data generated from ToxCast assays in terms of the specificity, sensitivity and reliability of the individual assays, as well as their ability to predict toxicity either alone or in combination with other findings. The agency appears to be making good progress toward these goals.

The agency has adopted two general strategies for testing the value of ToxCast data for agency use. The first strategy is to identify the patterns of responses for each chemical in the battery of ToxCast assays and correlate these with the biological activities observed in guideline, *in vivo* studies associated with the same chemical. This strategy is made possible by considerable amount of *in vivo* data associated with the Phase I chemicals. Of course, the assays included in ToxCast were pre-existing HTS assays developed for the pharmaceutical industry; they were not designed for ToxCast to correlate with endpoints in guideline *in vivo* studies. Therefore, it would be useful if the agency considered developing the theoretical framework that would support the effectiveness of this strategy. Essentially, this amounts to developing "AOPs" for the *in vivo* guideline studies.

The second strategy is to develop AOPs for human disease that may be reflected in the ToxCast data. This is an important and valuable strategy and highlights an important weakness in these two strategies that can be addressed in the commission of building these AOPs. Specifically, the ToxCast assays were not designed by the agency to inform *in vivo* endpoints, and the guideline *in vivo* endpoints were not designed overtly to inform human disease. Thus, to build a credible system, the EPA needs to focus on making the case that there is a relationship between what is observed in the ToxCast assays, what is observed in the guideline studies, and what is observed (or expected) in the human population.

The data derived from CompTox assays should lend themselves readily to hazard identification and especially green chemistry. These data may provide insight for the development of chemical products that have a greater likelihood of being free from toxic properties. Moreover, CompTox data may be

combined with information from structure-activity relationship (SAR) evaluation and any *in vivo* data that might be available, to facilitate hazard identification and help guide a weight-of-evidence analysis of hazard. However, there are several cautions that need to be considered when applying the data for hazard evaluation. First, the strengths and limitations of each assay must be recognized, including the potential for false negative and false positive results. Given that pathways of toxicity are poorly understood, current *in vitro* assays cannot be seen as comprehensive in their scope. For example, according to Judson et al. (2010), CompTox models developed to screen for chronic, developmental and reproductive toxicity endpoints display high specificity (few false positives) but only moderate sensitivity (multiple false negatives). [Sensitivity relates to the assay's ability to identify positive results. Specificity relates to the ability of the assay to identify negative results.] Therefore, the rate of false negatives is expected to be high at this stage of the program. While some information is better than none, there is concern that too much confidence will be placed upon the lack of activity in the available assays. If there is a high degree of reliance on data from these assays, it may inappropriately give the appearance that a chemical with no activity is safer than other, alternative chemicals that in fact have more information available.

### 2.2.2. Incorporating Exposure Information

EPA also should consider the potential for exposure to the chemical when determining the degree of testing required such that even if initial screens of a chemical find little reason for concern, *in vivo* confirmation may still be desirable if the chemical's exposure potential is high. Conversely, low exposures may diminish the need for extensive toxicity testing than might be needed for agents whose exposure is greater. These considerations underscore the need for good exposure/biomonitoring information which at this point appears to be a limitation of CompTox modeling. There is also uncertainty about the significance of a positive result in any particular assay within ToxCast. A major effort is underway to link patterns of responses within the battery of ToxCast assays to AOPs. At present, the ability to link patterns of responses and AOPs is limited since many of the screening assays are still under development and going through validation exercises on an individual level. Additionally, there is also a need to better understand the relationships between AOPs and apical endpoints. The concept is that by evaluating the behavior of known toxicants in the ToxCast battery, patterns of toxicity linking this HTS behavior to adverse outcomes and thereby enhancing predictability will become apparent and will serve as validation of the predictive capability of the assay.

Ultimately, the usability of a given result will be dependent upon additional data that is available for the chemical in question and about the tests and pathways affected by that chemical. The advantage of CompTox is that thousands of tests can be conducted – these need to be inclusive of as many potential health effects as possible. EPA should make clear the limitations of the information that can be obtained from the breadth of assays. For example, the testing may be accurate for cancer, developmental and reproductive endpoints, endocrine and metabolic endpoints, liver and kidney effects, but not, for example, eye health or neurological health. Positive results on subsets of tests or tests along certain pathways could suggest further testing and/or *in vivo* studies. Of particular importance for public health is the accuracy of a negative result in an assay system, which in a screening step could result in a decision to not proceed with further testing. In other words, for chemical screening and prioritization, the testing should be sensitive (i.e., detect an effect when there is one) and specific (i.e., does not detect an effect when there is not one).

Regarding more advanced uses of CompTox outputs beyond hazard identification (e.g., use in dose-response assessment and risk assessment) the following additional concerns should be considered:

- Have the most sensitive endpoints been identified in the CompTox assays;
- How well do these CompTox endpoints relate to apical endpoints such as carcinogenesis, endocrine disruption, organ toxicity, neurotoxicity and immunotoxicity;
- How would the uncertainty factors used when starting with *in vivo* data (e.g., interspecies, intraspecies, acute to subchronic to chronic study duration, database quality and completeness) be applied and/or modified for *in vitro* screening data;
- How would the *in vitro* dose-response relate to *in vivo* behaviour when considering route of entry, metabolic activation and detoxification systems that may not be present *in vitro*;
- How would the *in vitro* dose-response relate to *in vivo* behaviour when considering other toxicokinetic factors governing the external dose associated with a particular concentration at the target cell or receptor that may not be taken into account *in vitro*? These factors include metabolic activation and detoxification, as well as, absorption through relevant route(s) of entry, distribution, and excretion; and

- Related to the third bullet above, how well do the *in vitro* test methods capture intra-human variability in terms of susceptible sub-populations and life stages including genetic polymorphisms and disease states?

To move towards the development of risk assessments that more accurately reflect environmental conditions, EPA's CompTox research program also needs to develop strategies for studying environmental chemical mixtures - not just the effects of one chemical at a time. The importance of using CompTox to characterize the hazard, and ultimately the risk, of environmental chemical mixtures cannot be overstated. Moving in this direction requires establishing a scientifically defensible foundation -for example, by defining appropriate AOPs, developing testing methods that address a wide array of AOPs, and evaluating the accuracy, sensitivity and specificity of the tests. While assessment of mixtures may ultimately be a long range goal, the path to studying and estimating risk from mixtures should be outlined. Examples of critical questions include the following: (1) How will relevant mixtures be identified? (2) Can methods be developed to predict the hazard and/or risk of mixtures of chemicals from CompTox data on individual chemicals which affect the same AOP(s); (3) How will risk be quantified for different types of endpoints based on effects on relevant AOPs? and (4) How much risk is allowable for a given AOP?

As already noted, exposure is a key component of risk assessment and one that needs greater focus within the CompTox program. A general approach, based solely on chemical properties, that evaluates transport from large sources and partitioning based on fugacity concepts to predict the distribution of chemicals from their sources to a population will NOT provide a full exposure evaluation and will lead to misclassification of exposure. Such an approach is analogous to saying that nothing needs to be known about metabolism of chemicals when determining toxicity and that the only information needed is the overall chemical structure and what functional groups are present to compare with known compounds. Exposure may occur when people come into contact with chemical agents and often results from being close to the source where the agent is released into the environment. For example, an agent produced in relatively small quantities but used in personal products can result in a higher exposure than a high production volume chemical emitted from point sources located away from populations. The higher exposure potential of a low production chemical would not be predicted based on an exposure model that does not include information on its use and potential contact with people. Thus, if these two agents were equally hazardous, the low production

compound would present greater risk, but it is unclear if the current assays used in the CompTox program would account for this situation. This issue is relevant to all of the EPA applications listed, i.e., chemical screening, prioritization, risk assessment and green chemistry.

### 2.2.3. Data Use Guidelines

A key issue affecting use of CompTox data is the need for a guide to explain the appropriate use of data in various applications. Guidance for data needs (and sufficiency or appropriateness of data) must come from a good characterization of EPA programmatic needs – identification of both the intended goals of a risk assessment or a prioritization effort – and examples in which CompTox information appears to add real value. While the data are meant to be used within a weight-of-evidence context that requires integration across all of the available data (e.g., *in vivo* toxicology data, SAR, read-across approaches, other supporting *in vitro* data), it may be beneficial to establish general principles for the use and interpretation of the output for any one endpoint or health effect in a Data Use Guidance (DUG) document. Key aspects to address in such a guidance document include:

1. Name of the assay;
2. Description of assay design;
3. Name of company that developed the assay;
4. Information on any proprietary constraints of the assay;
5. Positive control and other agents used to characterize the assay;
6. Dynamic range of the assay;
7. Where the endpoint fits within one or more AOPs;
8. Related CompTox endpoints (i.e., endpoints likely to be within the same AOP or that are indicative of similar biological activity but in an independent test system);
9. Interpretative value of the endpoint if altered in isolation;
10. Interpretative value if altered in conjunction with other "aggregated" endpoints;
11. Rate of false positive and negative results if it is to be used for predictive purposes (e.g., to forecast *in vivo* endocrine activity);
12. Shape of the dose-response curve (e.g., monotonic, non-monotonic, threshold, linear);

13. Potential for the endpoint to be used as a biomarker in toxicity testing or in epidemiology studies;

14. Whether the endpoint is also affected by disease processes that might potentially lead to a chemical/disease interaction;

15. Limitations and uncertainties of the endpoint; and

16. Cross reference with other assays that assess the same endpoint(s) and comparison of reliability of the assay in comparison.

It may also be helpful to develop a simple flow chart describing a continuum extending from the least amount of evidence for a meaningful effect (e.g., perturbation only at high dose) to the greatest amount of evidence for meaningful effect (e.g., upstream and downstream endpoints affected in a defined AOP with effects occurring on upstream endpoints at low dose and anchored by similar effects from a known toxicant). The DUG also could provide guidance concerning the different uses of the data depending upon where on the continuum the evidence for a meaningful effect lies for a particular chemical. The "ToxPi" pie chart of endocrine-related effects for a chemical appears to be a useful way to illustrate the types of biological activities of a chemical, but not the meaning and importance of individual slices relative to other slices. The DUG could also include a section on aggregated endpoints that describes the implications of a "slice" of the pie for a particular biological effect and how one determines potency for a slice.

The concept of a DUG is not new. For example, the Centers for Disease Control/ National Health and Nutrition Examination Survey (CDC/NHANES[2]) biomonitoring data release provides important information for each exposure type including the normative range in the population, any relevant workplace or environmental standards (e.g., Occupational Health and Safety Administration (OSHA) Biological Exposure Indices), and limitations of the biomarker itself (e.g., specificity, sensitivity). This information is meant to aid in the interpretation of the data by various stakeholders and avoid the over-interpretation of the data. As previously mentioned, the Deepwater Horizon accident revealed a critical programmatic need – the need for rapid toxicological information in response to emergencies

[2] Information can be accessed at the following URL: http://www.cdc.gov/nchs/nhanes.htm

or other sudden demands for information and recommendations. There is also a need for developing guidance, procedures and resources for the use of Comptox outputs in such events.

### 2.2.4. Relating CompTox Outputs to *in vivo* Assay Results

Finally, for CompTox data to be of sufficient quality for use in risk assessment, it must correspond to validated *in vivo* endpoints or well-defined AOPs. Importantly, the batteries of CompTox assays were not specifically designed to inform these endpoints, in contrast to the *in vivo* assays which in some cases were developed decades ago. Further, the validated *in vivo* guideline assays used in assessing toxicity of chemicals were not designed to predict the full range of endpoints that are currently considered to be of public health importance. Ideally, the results of CompTox assays also should be predictive of additional *in vivo* endpoints of more recent interest – for example, diseases in adulthood resulting from developmental exposures. New methodologies, utilizing new cell and tissue types for DNAse1 Hypersensitive Site correlation analysis, have been reported in the scientific literature (e.g., see Maurano et al. 2012). This research has shown that many common disorders are linked with early gestational exposures or environmental insults. Incorporation of this methodology into ToxCast and CompTox will enhance the ability to identify AOPs relevant to a variety of health outcomes. Developing a CompTox research focus on aging and disease processes common in the U.S. population will allow the exploration of AOPs that are not concerned with how a chemical is perturbing "normal" and healthy systems but how chemicals may act in disease pathways to produce health risks in the population. Just as there is an "omics" explosion in describing chemical effects, there is also an explosion in our understanding disease mechanisms/biomarkers and this information should be integrated to give CompTox maximum relevance to human risk.

The data generated by Phase I of the CompTox program should build confidence about the relationship between patterns of responses in the battery of tests and the effects of the chemicals being assessed using *in vivo* guideline studies. This empirical analysis, however,will be difficult in part because of: (1) the inherent uncertainties about how the *in vitro* dose response relates to *in vivo* when considering route of entry, metabolic activation and detoxification systems that may not be present *in vitro*; (2) the inherent uncertainties about how the *in vitro* dose response relates to *in vivo* when considering other toxicokinetic factors that govern the external dose associated with a particular concentration at the target cell or receptor; (3) the possibility that a chemical may have more than one mode of action; and (4) the possibility that while two "estrogenic" chemicals may overlap in the

patterns of responses observed in the battery of tests, they will likely have large regions of non-overlap. In the absence of prior knowledge of these characteristics, it will be difficult to find the common pattern that predicts the responses observed in current guideline *in vivo* studies. Hopefully, the difficulties in achieving this goal are not insurmountable and over time, through experience with the rapidly increasing database of information that is being generated, the agency will achieve its objective of developing this knowledge.

Just as important, and probably even more challenging, will be to understand the relationship between CompTox outputs and the etiology of human disease based on epidemiological data. The CompTox program should work with epidemiologists within the EPA and extramurally to design epidemiologic studies that incorporate new and improved biomarkers of exposure, subclinical effects and disease. The CompTox program is well on its way to addressing these difficult issues.

## 2.3. Building Scientific Acceptance of CompTox

*Study Question 3. What are the barriers and limitations that prevent EPA from using CompTox outputs and how might they be overcome?*

There are a number of challenges facing the CompTox program with respect to preparing outputs for use in agency decisions and by the broader scientific community. These challenges are specific to the potential types of applications for these data, including informing decisions when other information is not available, prioritizing chemicals for further toxicity analysis and as the basis for risk assessment. In all cases, a common concern is whether the data generated from high-throughput *in vitro* assays can be applied reliably, i.e., that the data will have been shown to be sufficiently predictive of toxicity *in vivo* and ultimately in humans, relative to traditional approaches such that agency decisions can be scientifically defensible within an acceptable level of uncertainty.

It is worth repeating several points that likely serve as barriers to the use of CompTox data:

(1) If an *in vivo* endpoint is not well anchored in an AOP or read-across approach, then perturbation of that endpoint may be difficult to detect in CompTox assays and thus difficult to apply in screening or risk assessment;

(2) There must be an understanding of the impact of the route of entry, metabolic activation and detoxification systems and other toxicokinetic factors that may not be present *in vitro*;

(3) Dose-response assessment must take into account *in vitro* to *in vivo* extrapolation including metabolism and other toxicokinetic factors, application of uncertainty factors and special consideration of vulnerable sub-groups;

(4) There is a likelihood for false negative results at this stage of testing which requires caution when considering a chemical for increased usage based upon CompTox results; and

(5) Exposure information is often limited but is a key part of any screening and prioritization program, as well as necessary for risk assessment.

The CompTox program is in the development stage, as noted in response to Study Question 1, and so its use is still very limited. The program has not had sufficient time to demonstrate that it can deliver on its promise. Questions about the reliability of individual assays, the availability of assays predictive of the full range of relevant endpoints, the power of "pattern recognition" as a predictor of toxicity, the value of the current design of the system to generate the kind of information needed to be predictive, all are legitimate questions that require time and experience to answer. Considering the importance of these goals and the complexity of the issues involved, there will be unavoidable "blind alleys". However, the number of these "blind alleys" may be minimized by being more proactive about building AOPs and pathways of toxicity. In this regard, there are currently no internationally accepted methods in the scientific literature for performing a weight-of-evidence analysis for such pathways. While this task is not the purview of the CompTox program *per se*, the ability of the EPA to employ peer-reviewed science in the Tox21 initiative, which combines the efforts of several federal agencies (i.e., EPA, NIEHS and FDA) to screen thousands of chemicals for potential toxicity, would be enhanced by developing an accepted method of analysis for determining the ability of CompTox assays to predict human disease. In the absence of such an accepted method, the agency will be limited in associating CompTox data to data generated from guideline assays and this would be a severe limitation.

One of the ways to improve acceptance of CompTox and overcome some of the barriers to its use is to demonstrate that it provides equivalent (or more accurate) answers relative to the currently accepted methods for characterizing hazard and estimating risk. Moreover, if it does so with fewer resources (e.g., cost and time), thereby allowing for the characterization of the large number of agents that the EPA must make decisions about, then it will quickly become the methodology of choice.

There also is a need to commit similar resources to develop ExpoCast in parallel to CompTox to more fully support the needs of EPA programs. This will require not only acceptance by scientists at EPA's National Exposure Research Laboratory (NERL) but also a recognition by others within the agency that exposure is a key component of risk assessment, risk characterization and risk management and that the volume of an emission is not equivalent to exposure and the dose humans receive.

According to the EPA's 2009 strategy document on evaluating chemical toxicity (U.S. EPA 2009), the agency appears to be following the recommendation of the NRC 2007 committee, "….*in vitro* tests would be developed not to predict the results of current [animal] apical toxicity tests but rather as [human] cell-based assays that are informative about mechanistic responses of human tissues to toxic chemicals. The NRC committee is aware of the implementation challenges that the new toxicity-testing paradigm would face." With this in mind, the EPA is currently conducting research to identify AOPs that can serve as predictors of toxicity; the need to relate these AOPs to currently understood toxicity endpoints is critical. Once appropriate AOPs are established, the EPA will be positioned to transition to the methodologies recommended by the NRC. However, as the agency pursues this path, EPA will need to address several questions:

- How well do the *in vitro* and *in silico* tests translate to human systems?;
- How predictive of human pathways are the identified AOPs? Data on this is important to share and make public;
- How do the testing methods account for differences between *in vitro/in vivo* animal testing and human toxicokinetics, particularly metabolism but also absorption, distribution, and excretion? (for instance, how are chemicals that are cleared through multiple pathways (renal, GI, etc) treated in the analysis; how do these testing methods account for chemicals that are actively reabsorbed by renal organic anion transporters or those that are strongly bound to plasma proteins, lipids, etc. And how would the testing methods determine the toxicity of chemicals which are initially metabolized in one organ and further metabolized to the ultimate toxic metabolite in another organ?;
- Given that there are multiple methods to estimate pharmacokinetic behavior (as described in Rotroff et al. 2010) and since the results may differ based on which methods are employed, how will decisions be made regarding which ones to use, their accuracy and certainty?;

- Are the proposed tests useful for chemicals that are stored in humans (e.g., adipose tissue depot or other sites)?
- How are human exposure characterization and biomonitoring data used in the prioritization and testing of chemicals (although the tests are designed to identify chemical hazards, if exposure is low or non-existent then how should the chemical be prioritized)?
- Where will human exposure data, which should be a high priority since it is such an important component of risk assessment, come from, how they will it be used (upper bounds, central tendency, etc.); and,
- How are existing data from the scientific literature incorporated into these AOPs and how will the AOPs remain current?

The scientific acceptance of CompTox approaches in a weight of evidence for decision-making will depend on the accuracy, sensitivity and specificity of the computational toxicity testing for predicting actual and potential human health effects. To assist in gaining acceptance, a transparent strategy should be developed for quantifying the endpoints upon which risk assessments will be based. The agency also should indicate what issues should be considered for EPA applications such as chemical screening, prioritization, risk assessment and green chemistry. While relevance to humans is always important, these applications may be ranked in order from highest to lowest in terms of the scrutiny with regard to human relevance as follows: risk assessment, prioritization, screening chemicals and green chemistry.

Finally, the interactions between ORD and the various EPA programs (e.g., pesticides, water or toxics) that will use CompTox data are commendable and should continue in order to understand what would make such data most useful. Perhaps, in addition to providing opportunities for program office scientists to spend time in the ORD laboratories to become familiar with the CompTox program, extensive remote learning and training modules could be developed to reduce the cost and logistic challenges. This may also serve to engage more key EPA scientists outside of the ORD laboratories located in Research Triangle Park, North Carolina area.

## 2.4. Communicating CompTox Approaches and Outputs

*Study Question 4. How should the use of the CompTox program be effectively communicated to stakeholders? How can the communication be enhanced?*

The EPA appears to be doing a very thorough job of communicating to stakeholders about the CompTox program. The agency has created multiple web-based learning tools and models such as webinars and dashboards for the interested public to learn more about the program and to access the data it is generating. The EPA has actively sought input from stakeholders as it developed the CompTox program and also has disseminated information to the scientific community through publications and presentations at scientific conferences. The EPA established the Computational Toxicology Communities of Practice, composed of more than 300 people from over 50 public and private sector organizations that are interested in the application of computational toxicology and exposure science to risk assessments. The SAB commends this effort; it is a powerful tool for keeping up with technical issues that the EPA is confronting and addressing as a part of the CompTox program.

EPA's communication effort has focused on two areas. First is conveying the importance of the approach and the value of the strategy to stakeholders including the public. Many in the regulated community have worked at developing computational toxicology models of various kinds, often quite specific to their products; they are, obviously, convinced of the strength of the approach or they would not be pursuing it. If stakeholders are included in the process of development, validation and application of these methods in a collaborative fashion, they may be more likely to accept the results and provide constructive feedback. Second is providing the data to all stakeholders, including the general public. The CompTox website (http://www.epa.gov/ncct/) is relatively easy to navigate, but it would be useful to provide some information about strategies for extracting relevant data.

As EPA moves forward with the development of the CompTox program, communication can be enhanced by being transparent about the limitations and uncertainties in the use of CompTox assays to predict any particular endpoint in isolation and in combination with data from other CompTox assays, and providing a broader understanding about what is known about a chemical's biological activity based upon CompTox data in conjunction with Structure Activity Relationships (SAR), *in vivo* testing, etc. It may also be useful to provide stakeholders with some summary statistics about the results, perhaps along the lines of AOPs, with a transparent, easily accessible (e.g., on a website) location for the details of the testing and the raw data. However, it should be kept in mind that uninitiated evaluators of large datasets are often daunted by the sheer volume of data and may not consider the quality and limitations of those data. As ExpoCast develops, the website

(http://www.epa.gov/ncct/expocast/) should incorporate estimates of exposure to chemicals and mixtures (especially upper bounds if possible) potentially stratified by age, gender, regions of the country, population density (rural, suburban, and urban), ethnicity and so forth.

Communication with epidemiologists and clinical investigators needs to be part of the process. It may be difficult for some health scientists to discern the potential relevance of computational toxicology to human exposure and health effects. Data generated and provided by EPA (and collaborators) can be used to demonstrate that the tests utilized are relevant to human health effects. In addition, the agency should clarify in what situations the data may fall short and be inadequate. For instance, there is a higher level of uncertainty for specific AOPs, outcomes and/or for specific classes of chemicals. Thus, it is essential to combine CompTox outputs with data on toxicokinetics, particularly metabolism, of the chemical in humans. Finally, biomonitoring data, exposure pathways, chemical source information and human activity patterns related to human exposure needs to be included in the assessment of chemical risk.

The EPA should continue to partner with existing academic health science centers to disseminate information on CompTox. The agency can utilize existing relationships via community outreach and translation cores which support gene and cell therapy clinical trials and includes laboratories for basic research, testing, scale-up, and clinical work. This would allow for the analysis of high-throughput data and development of predictive modeling using CompTox data sets. As AOPs are developed, it would also be useful for the agency to develop partnerships with relevant professional societies or institutions. For example, a group within the EPA developing an AOP on asthma would benefit from a partnership with the American Lung Association or the National Heart, Lung, and Blood Institute to access physicians and researchers at the cutting edge in this field. The EPA also may benefit from more collaboration with international agencies regarding data sources, data access and technology transfer. Another important group of stakeholders are risk assessors and public health professionals in state and tribal environmental and health agencies. Outreach to state and tribal public health scientists would be helpful in informing them about the use of CompTox data in hazard identification and risk assessment.

Some additional suggestions for research regarding communication and achieving a broader understanding of the potential contributions and limitations of these approaches include the following: (1) an evaluation of the pesticide stakeholder dialog process

(http://www.epa.gov/oppfead1/cb/ppdc/#about) by an independent expert (group) in communication and stakeholder participation to see what can be learned from that experience; and (2) pursuing a mental model study to compare expert and public understandings of how CompTox findings could be informative (this might identify structural reasons why there might be communication difficulties and how they could be addressed).

## 2.5. Other Issues

In addition to addressing the study questions, the SAB also identified several additional issues that should be considered by the agency as it continues to develop the CompTox research program and apply the program outputs.

1) The agency should clarify the goals and objectives for CompTox with respect to chemical screening, prioritization and risk assessment. How will application of CompTox information improve current EPA practice? Because risk assessments are conducted for a variety of purposes, demands on the information base will necessarily differ among situations, but are there context-specific criteria for when particular types of information are informative enough to be useful? Resolution of some of these structural issues could be a useful contribution of the CompTox program even before it is producing actionable information. The Deepwater Horizon provides an example of a programmatic need – provision of information in emergency or other fast-moving settings – for which guidance is lacking.

2) There is a need to better delineate what CompTox can and cannot contribute, both now and in the future. Which contributions might be feasible over the next few years versus which ones will take longer to develop? What is the extent of the universe of chemicals that will be evaluated (e.g., soluble? not too volatile?)? What sort of health effects can be assessed at the current time and in the future? The identification of critical pathways is an important step toward clarifying a number of key risk challenges – mixtures, interactions with background exposures, existing conditions and susceptibilities – and it provides an attractive possible approach for using CompTox data to assess risk, but are there risks that may be obscured or ignored when an approach based on critical pathways is used?

3) How well developed are EPA's capabilities for synthesizing and using fragmentary and incomplete information? For the near term, CompTox results will be quite limited and their best use

likely will be in combination with limited information from other sources. Current EPA practice tends to be chemical-specific and to focus on particular types of information. How far along is EPA in developing multi-chemical and multi-factor risk assessments? A future vision for CompTox is that the data will deliver a complete identification of critical pathways and a measure of the response along them, but realizing such a vision is still remote. For some period of time, perhaps indefinitely, the information provided by CompTox will be fragmentary and new methods will be needed for its interpretation. The primary challenges thus are transitional – how to build analytic structures that can incorporate new kinds of information in incremental steps.

4) Analytic capabilities are a major consideration for the CompTox program, but it is also important to think about institutional capabilities for developing, organizing, and using the information. Are data resources constantly updated and expanded and are there ongoing improvements in accessibility and analytic flexibility? How can staff and scientist training and development in use of new CompoTox data be accomplished? Is there an institutional culture that identifies opportunities for the use of new information and is vigilant to detect warning signs concerning new issues and new difficulties? Is there good communication between groups that might use the same or similar information and methods? Can the institutions develop and support incremental changes? Can they engage stakeholders and other governmental and non-governmental organizations as supporters of such change?

5) Critical data for steps in the transition from current risk assessment practices will only partly come from CompTox; those data must be synthesized with other, more familiar, types of information. Data needs and requirements for data quality must be addressed as well.

6) How will EPA handle the inevitable occurrence when future data from *in vivo* or human studies contradict the ToxCast data? As the science moves forward, there may/will be results generated from *in vivo* and/or epidemiologic studies that contradict or are not consistent with the CompTox results. This is an inherent characteristic of science and is not unique to the CompTox program. However, as inconsistencies occur how will EPA respond? What will be EPA's approach to handling the comments and perceptions that are sure to arise questioning whether the CompTox data either overestimated the risk of a chemical or did not identify the hazard(s) of a chemical? What would the implications be for the CompTox program and the use of its outputs? The public is bombarded with studies that show a risk for chemical X, and then other studies later show no risk,

and then another wave of additional studies again showing a risk. The EPA needs to be prepared for the shifting playing field since future data from *in vivo* and human studies will not always be consistent with the CompTox results. The inconsistency that evolves over time as new data are generated is inevitable in scientific research, but EPA needs to develop a plan to address this as it will definitely occur and its occurrence will accelerate as more of the results from CompTox testing become available and begin to be used for prioritization and risk assessment.

7) It is clear that effective communication with all stakeholders, both within and outside the agency, will be essential to the long term use of CompTox and ExpoCast findings.

8) There is a need to develop a community of scientists to provide feedback on ExpoCast in a parallel fashion to ToxCast is needed.

9) Finally, the SAB recommends that the EPA consider establishing an ongoing external advisory process to institutionalize a long term program built around the idea of incremental transformation. This process should be free of members with financial ties to the program. An independent perspective on the current program and prospects for the future can be provided along with constructive suggestions. The potential for longer term engagement by an external advisory committee should be considered.

# REFERENCES

Judson, R., D. J. Dix, K. A. Houck, M. T. Martin, T. B. Knudsen, and R. J. Kavlock. 2010. Predictive Signatures from ToxCast Data for Chronic, Developmental and Reproductive Toxicity Endpoints. Presented at Society of Toxicology Annual Meeting, Salt Lake City, UT, March 07 - 11, 2010.

Maurano et al., 2012. Supplementary Materials for Systematic Localization of Common Disease-Associated Variation in Regulatory DNA. *Science* 337: 1190-1195 (DOI: 10.1126/science.1222794) Published Online September 5 2012.

National Research Council. 2007. *Toxicity Testing in the 21st Century: A Vision and a Strategy*. Washington, DC: The National Academies Press. ISBN-10: 0-309-15173-2, ISBN-13: 978-0-309-15173-3

National Research Council. 2009. Committee on Improving Risk Analysis Approaches Used by the U.S. EPA, "Front Matter." *Science and Decisions: Advancing Risk Assessment*. Washington, DC: The National Academies Press, ISBN-10: 0-309-12046-2, ISBN-13: 978-0-309-12047-0

Rotroff DM, Wetmore BA, Dix DJ, Ferguson SS, Clewell HJ, Houck KA, Lecluyse EL, Andersen ME, Judson RS, Smith CM, Sochaski MA, Kavlock RJ, Boellmann F, Martin MT, Reif DM, Wambaugh JF, Thomas RS. 2010. Incorporating human dosimetry and exposure into high-throughput in vitro toxicity screening. *Toxicol Sci.* 117(2):348-58. Epub 2010 Jul 16.

U.S. Environmental Protection Agency Office of the Science Advisor. 2009. The U.S. Environmental Protection Agency's Strategic Plan for Evaluating the Toxicity of Chemicals, March 2009. EPA/100/K-09/001 I, 2009.http://www.epa.gov/osa/spc/toxicitytesting/docs/toxtest_strategy_032309.pdf

U.S. Environmental Protection Agency Science Advisory Board. 2011.*Science Advisory Board Comments on the President's Requested FY 2012 Research Budget*. EPA-SAB-11-007. 2011.