UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C. 20460

OFFICE OF THE ADMINISTRATOR
SCIENCE ADVISORY BOARD

May 22, 2014

EPA-SAB-14-006

The Honorable Gina McCarthy
Administrator
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, N.W.
Washington, D.C. 20460

Subject: SAB Advice on Advancing the Application of CompTox Research for EPA Chemical Assessments

Dear Administrator McCarthy:

Risk assessment is central to the mission of the U.S. Environmental Protection Agency (EPA), and the Science Advisory Board (SAB), as well as the National Academy of Sciences, has encouraged the EPA to incorporate 21st century approaches into its risk assessment practices and to modify its single-chemical approach. Tens of thousands of chemicals are currently in commerce and hundreds more are introduced every year, yet only a small fraction have been adequately assessed for potential hazard. To meet this challenge, the EPA established the Computational Toxicology (CompTox) Research Program to explore and develop ways to exploit modern advances in molecular biology, chemistry, exposure science and computer science to more effectively and efficiently assess chemical hazards and along with exposure information, their risks. An effort central to the CompTox program is ToxCast, in which data from a battery of rapid *in vitro* assays have been assembled for a variety of compounds.

The CompTox research program has the potential to provide the agency with a means to evaluate individual chemicals more rapidly. Moreover, it can facilitate the shift from the EPA's 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 (EHHC) to evaluate how the products from the CompTox research program are being used by the 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 for risk assessment and decision-making can be identified and addressed. The EHHC, 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 of EPA risk assessments. In the enclosed report, the SAB provides its analysis and advice regarding the issues that the agency should consider as it moves forward with implementation. This letter highlights the SAB's major recommendations.

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 – or a battery of assays –to predict adverse effects in animals; and the ways in which these patterns of data predict the risk of human disease. The SAB offers recommendations on several applications for ToxCast data:

• Using ToxCast data to develop Adverse Outcome Pathways (AOPs): The agency is currently evaluating whether information derived from ToxCast is predictive of *in vivo* toxicity, using the Endocrine Disruptor Screening Program (EDSP) as the focus of this validation work. This is a reasonable focus and the agency is reporting some success. However, the SAB recommends that the agency look not only at whether the ToxCast dataset for the EDSP validation is predictive of *in vivo* toxicity, but also whether the endpoints captured *in vivo* have associated molecular endpoints in ToxCast. This balance in information derived from the *in vitro* and *in vivo* setting is critical to the validation process, and evaluating information flow in both directions will be crucial to validating the ToxCast enterprise and streamlining the required battery of assays. The agency also is in the process of developing "Adverse Outcome Pathways (AOP)" that will help link results from *in vitro* high-throughput screening assays to human disease. As the agency develops these AOPs for the human population, the SAB recommends that the agency explore partnerships with professional societies and institutions devoted to the development of new information and therapeutic approaches for specific diseases.

• Using ToxCast data in a consistent manner: Following the Deepwater Horizon accident in 2010, a subset of ToxCast assays was used to evaluate the endocrine toxicity of eight different commercial dispersants being considered for use in the Gulf of Mexico. Considering this application as a case study, the SAB finds that the logic underlying the specific choice of assays – even the choice of endocrine toxicity as opposed to other mechanisms of toxicity – as well as the interpretation of the data derived from these assays requires a more structured and transparent approach. EPA should examine their decision to use these ToxCast assays to determine if the information they provided was the most appropriate for the intended purpose (i.e., one that minimized risks to human health and the environment). The SAB recommends that the agency develop a structured approach, such as a Data Use Guide, to obtain and use ToxCast data, especially in emergency situations. A retrospective study of the Deepwater Horizon accident can help inform the development of this structured approach. A Data Use Guide or other structured approach would be useful for other ToxCast applications as well.

• Using ToxCast data for evaluating the toxicity of mixtures: The CompTox research program offers the agency a unique opportunity to develop data on environmentally relevant mixtures. This is an important element of the rationale for ToxCast, but requires development of the practical aspects of studying mixtures using *in vitro* assays and validation of the predictive nature of the assays since a large dataset of *in vivo* data on mixtures is not available. The SAB recommends that the agency begin a formal approach to address these challenges in the field of mixture research using ToxCast and other CompTox technologies.

The CompTox research program is at a stage of maturity where researchers within the EPA – as well as partners working along with the agency – would benefit from input regarding the applicability of program outputs. Therefore, the SAB recommends that the agency use an advisory committee to promote engagement with a multi-disciplinary group of external scientists, including scientists with expertise in social and decision science, epidemiology and basic science, among others disciplines. The goal of this advisory group would be to help ensure that the agency continues to generate tools that are useful not only within the EPA but also to a broad array of extramural stakeholders. This will help the ToxCast program remain relevant to the overall mission of the agency and will improve the ability of the

outside community to provide thoughtful input to the program. In addition, the SAB notes that the CompTox data frequently will be used in conjunction with other hazard and exposure information and recommends that the program seek input on the integration of CompTox products with other data essential to assessing risk.

The SAB commends the CompTox program for efforts to reach out to EPA program offices and stakeholders to communicate the value and utility of the research program. These efforts are laudable and should continue. The program has developed Computational Toxicology Communities of Practice, 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. This development is an especially worthy effort and should be continued.

The SAB appreciates the opportunity to provide these recommendations and we look forward to continued progress in ToxCast and other CompTox research programs.

Sincerely,

/signed/ **/signed/**

David Allen, PhD
Chair, Science Advisory Board

Thomas Zoeller, PhD
Chair, SAB Exposure and Human Health Committee

Enclosure

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1. EXECUTIVE SUMMARY

The EPA's Computational Toxicology Research Program (CompTox) is part of the EPA's broader research program on Chemical Safety for Sustainability. Traditional chemical toxicity testing is expensive, time-consuming and uses a significant number of animals. As a result, a large number of chemicals in commerce have not been evaluated sufficiently – or at all – for safety yet the agency must make hundreds of decisions each year based on limited information. CompTox is researching new, more efficient, ways to evaluate the safety of chemicals, particularly in assessing chemicals for potential risk to human health and the environment. EPA's National Center for Computational Toxicology (NCCT) was established in 2005 to coordinate computational toxicology research on chemical screening and prioritization, informatics and systems modeling. A core program managed by the NCCT is the ToxCast program. ToxCast is building a database of chemical activities by evaluating a large number of chemicals in a very large (700+) battery of high-throughput *in vitro* assays. The agency is publishing an increasing number of research papers describing the results of this program and the ways in which the information can be validated for use in agency decisions.

To assist the EPA in this process, the SAB asked its Exposure and Human Health Committee (EHHC) to evaluate how the products from the CompTox research program are being used by the agency, 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 EHHC, 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 input to EPA risk assessments. This report provides analysis and advice regarding the issues that the agency should consider as it moves forward with implementation. Because the ToxCast program is more advanced than other programs within CompTox, much of the focus of this report is on ToxCast.

Using ToxCast data to develop Adverse Outcome Pathways (AOPs). ToxCast outputs currently are being used principally within a research context. The agency is evaluating whether information derived from ToxCast is predictive of *in vivo* toxicity, using the Endocrine Disruptor Screening Program (EDSP) as the focus of this validation work. This is a reasonable focus and the agency is reporting some success. However, the SAB recommends that the agency look not only at whether the ToxCast dataset is predictive of *in vivo* toxicity, but also whether the endpoints from *in vivo* studies have associated molecular endpoints in ToxCast. This balance in information derived from the *in vitro* and *in vivo* setting is critical to the validation process, and evaluating information flow in both directions will be crucial to validating the ToxCast enterprise and streamlining the required battery of assays. The agency also is in the process of developing "Adverse Outcome Pathways (AOP)" that will help link results from high-throughput *in vitro* assays to human disease. As the agency develops these AOPs for the human population, the SAB recommends that the agency explore partnerships with professional societies and institutions devoted to the development of new information and therapeutic approaches for specific diseases.

Using ToxCast data in a consistent manner. The SAB learned about the Deepwater Horizon accident, where a subset of ToxCast assays were used to evaluate the endocrine toxicity of eight different commercial dispersants. This application illustrates important benefits of the ToxCast program, as well as opportunities to strengthen it for use in future emergencies and other uses. Examination of the Deepwater Horizon case showed that it would have been helpful to have a more structured and

transparent rationale for the choice of specific assays, the choice of endocrine toxicity as opposed to other mechanisms of toxicity, as well as the interpretation of the data derived from these assays, and whether the ToxCast assays provided the right information. Therefore, the SAB recommends that the agency develop a Data Use Guide or other structured approach to the use of ToxCast data in various applications, including the interpretation of the resulting information. The Deepwater Horizon accident should be studied in retrospect to help inform this structured approach.

Using ToxCast data for evaluating the toxicity of mixtures. The CompTox research program offers the agency a unique opportunity to develop data on environmentally relevant mixtures. This is an important element of the rationale for ToxCast, but requires development including the practical aspects of studying mixtures in *in vitro* assays, and in validating the predictive nature of the assays in the absence of a large dataset of *in vivo* data on mixtures. The SAB recommends that the agency begin a formal approach to addressing these challenges in the field of mixture research using ToxCast and other CompTox technologies.

The SAB recommends that performance characteristics of assays that relate to specificity, sensitivity and reliability be made available on the EPA website. The SAB understands that the suitability of *in vitro* data for regulatory decisions depends on the type and level of decisions to be made. This underscores the importance of a principal research goal of the program, that 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 CompTox research program, specifically ToxCast, is at a stage of maturity where researchers within the EPA – as well as partners working along with the agency – would benefit from input regarding the applicability of program outputs. Therefore, the SAB recommends that the agency use an advisory committee to promote engagement with external scientists. This will help the ToxCast program remain relevant to the overall mission of the agency and will improve the ability of the outside community to provide thoughtful input to the program. In addition, the SAB notes that the ToxCast data frequently will be used in conjunction with metabolic and exposure information and recommends that the program seek input on the integration of ToxCast products with other data essential to assessing risk.

2. INTRODUCTION

EPA's Computational Toxicology (CompTox) Research Program has been underway since 2003. EPA established the National Center for Computational Toxicology in 2005. The goal of this Center is to oversee the development of innovative *in vitro* tools and methodologies as alternatives to animal studies for use in chemical risk assessment. The EPA defines computational toxicology as the integration of modern computing and information technology with molecular biology and chemistry to improve risk assessment and prioritization of the data EPA requires to assess chemicals. The three long-term goals for the program are: (a) to provide EPA risk assessors with improved methods and tools to better understand and describe linkages across the source-to-outcome paradigm; (b) to provide EPA Program Offices with advanced hazard characterization tools to prioritize and screen chemicals for toxicological evaluation; and (c) to provide EPA risk assessors and regulators with new models based on the latest science to reduce uncertainties in dose-response assessment, cross-species extrapolation, and quantitative risk assessment. A more extensive account of the history and goals of the program are included in Appendix A of this report.

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. Consistent with the recommendations of the NRC report, the EPA in that same year launched ToxCast™, an initiative central to the CompTox research program, to develop rapid automated chemical toxicity tests. Including ToxCast, the CompTox research program manages 10 research areas (Table 1). These research areas are intended to provide innovative research that integrates advances in molecular biology, chemistry and innovative computer science, and to communicate this information so as to more effectively and efficiently rank chemicals based on risks.

Table 1. CompTox Research Program: Managed Research Areas

ACToR – Aggregated Computational Toxicology Resource
Determining Uncertainty
Distributed Structure-Searchable Toxicity (DSSTox) Public Database Network
ExpoCast
ToxCast
Toxicological Priority Index (ToxPi)
Toxicity Reference Database (ToxRefDB)
Tox 21
Virtual Liver Project (v-Liver)
Virtual Embryo Project (v-Embryo)

In a 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 2011). 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 data and technologies overall 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. Recognizing these potential benefits and impacts of CompTox data and technologies for the EPA, the SAB has requested that its Exposure and Human Health Committee (EHHC) develop advice to assist in advancing the application of CompTox research products for human health risk assessment to meet EPA's programmatic needs.

The members of the SAB Exposure and Human Health Committee (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] Members of the panel engaged in discussions with the Office of Research and Development (ORD) and EPA program offices that currently use or plan to use the CompTox research outputs. Discussions addressed the following questions:

1. How are the outputs of the CompTox research program being used currently by EPA? What are the challenges that EPA has had to overcome when using these outputs?
2. How well do the outputs of the CompTox research program match with EPA's environmental assessment and management goals and/or the needs of key partners?
    a. Are there plans to align the outputs and needs more closely?
    b. What is the timetable and are those plans appropriate?
    c. How can EPA align the outputs of the CompTox research program and EPA assessment/management needs more closely?
3. Are there obstacles (conceptual, legal, policy, institutional, other) preventing EPA from using the outputs from the computational toxicology program in EPA assessments and to support management decisions? How significant are these barriers? How might they be overcome? What would be a realistic timetable for overcoming them? What are the requirements of data from high-throughput screening that would allow them to be informative to hazard characterization and/or risk assessment?
4. How would the outputs of the computational toxicology program be effectively communicated to the public as part of a new EPA assessment approach? What research or other steps should be taken to build an effective approach for communicating this new science?

The EPA representatives described the overall scope, structure and organization of the CompTox research program, and the use of CompTox information within agency programs. The CompTox research program has generated a large number of products including web sites and web-based tools, and publications describing various research activities and outcomes of the research. These were effectively reviewed by the EPA's Board of Scientific Counselors (BOSC) in 2009 (U.S.EPA BOSC, 2010). The goal of the current SAB process was not to duplicate the work of the BOSC but rather to specifically evaluate how CompTox tools are being implemented within the agency to date. As a result of the information presented by EPA representatives to the panel, this report focuses largely on ToxCast.

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

ToxCast products are a central element of CompTox research and have begun to be used in specific situations.

The ToxCast program[2] currently consists of nearly 700 pre-existing individual *in vitro* assays provided by nine companies. A foundational element of ToxCast is a chemical library in which a large number of chemicals are tested simultaneously to create toxicity profiles in these assays. In Phase I, chemicals for which there is a substantial amount of toxicity data (including 309 pesticide active ingredients and commercial chemicals) have been evaluated in the ToxCast battery of *in vitro* assays. Data generated from this exercise have been analyzed 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 are being evaluated similarly from a broad range of sources including industrial and consumer products, food additives, "green" products that reduce or eliminate the use or generation of hazardous substances, nanomaterials and drugs that never made it to the marketplace. 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.

A draft SAB committee report was developed during the summer of 2012 and discussed at a teleconference on September 24, 2012. The draft report was then considered by the chartered SAB on March 7, 2013 and revised based on member's comments. The following report outlines the SAB's recommendations on how to enhance the utility of the products generated by the CompTox research program, and more specifically the ToxCast program outputs.

[2] More information regarding the ToxCast program is available at the following URL: http://www.epa.gov/ncct/toxcast/ and in a fact sheet at: http://www.epa.gov/ncct/download_files/factsheets/Tox_Cast_Fact_Sheet.pdf

3. FINDINGS AND RECOMMENDATIONS

3.1. Applications of CompTox Data to EPA Programs

Study Question 1. How are the outputs of the CompTox program being used currently by EPA? What are the challenges that EPA has had to overcome when using these outputs?

Currently, the outputs of CompTox are being used largely within a research context. It is essential to recognize that this is a highly focused applied research program designed to determine whether, and how, large sets of *in vitro* data may be used to enhance the mission of the EPA in the short and long-term. Therefore, at present, CompTox outputs, and specifically ToxCast, have been used primarily within ORD to compare *in vivo* data with the resulting *in vitro* data gathered from a variety of assays to determine their predictive capacity and reliability for use in various types of decision-making (e.g., screening, prioritization) by agency programs. 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 communication between the different programs within the agency to ensure that the outputs meet the needs of the specific programs. At the SAB 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.

The ToxCast program within CompTox is designed to evaluate hundreds to thousands of chemicals in hundreds of *in vitro* tests; many of these chemicals have known toxicological phenotypes for cancer, reproductive impairment and developmental disorders. The initial goal is to acquire sufficient information on a range of chemicals so that "bioactivity profiles" or "*in vitro* signatures" can be discerned that predict patterns of toxic effects or phenotypes corresponding to those observed in traditional animal toxicity testing. An underlying assumption in the design and execution of this research program is that the pattern of responses observed in the *in vitro* assays will match toxicological profiles in traditional *in vivo* studies, despite the fact that neither (*in vitro* assays or *in vivo* studies) was designed with this intention in mind.

3.1.1. Challenges of Relating ToxCast Outputs to *in vivo* Assay Results

The ToxCast research program is at the stage where enough data are available to compare the *in vitro* profiles against *in vivo* data; therefore, soon researchers will be able to know the extent to which the program will be predictive and how they may be able to use it. A straightforward approach to address the characterization of the ToxCast database is to determine whether the data from *in vitro* assays in ToxCast are predictive of *in vivo* toxicity using a database of toxicity studies conducted with guideline, *in vivo* test systems in addition to data derived from peer-reviewed non-guideline studies. In general, chemicals that acted on estrogen- or androgen-sensitive assays in ToxCast were reported to have a high degree of probability of acting on estrogenic or androgenic endpoints *in vivo.* However, chemicals acting on thyroid-sensitive assays in ToxCast were not identified as acting on thyroid-sensitive endpoints *in vivo*. These findings indicate that the ToxCast data will be reasonably predictive of endpoints of estrogen and androgen toxicity in the EDSP, but will not be predictive of thyroid toxicity. This application of ToxCast data represents a good beginning to test reliability because there are considerable data *in vivo* to compare with the ToxCast data. In addition, the SAB recommends that the agency consider information flow in both directions; that is, from *in vitro* to *in vivo* and from *in vivo* to *in vitro*. In this manner, the questions become whether data from specific assays in ToxCast are predictive of effects observed *in vivo*, and whether molecular and cellular events that lead to effects on endpoints identified in tier 1 of the EDSP are represented by specific assays in ToxCast.

3.1.2. Development of Adverse Outcome Pathways

The structured framework within which ToxCast data may be linked to adverse outcomes (i.e., be predictive of toxicity), both in the EDSP and potentially in other applications including in the human population, is called the Adverse Outcome Pathway (AOP). Building these AOPs represents a very important link from *in vitro* high-throughput assays to human disease, and this effort is just beginning. The SAB recommends that the EPA 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 considering upstream events, the EPA can use CompTox data to evaluate how chemical exposure and disease AOPs may intersect leading to a more complete understanding of chemical action (NRC 2009). Building and characterizing these AOPs would benefit from the expertise of those in the scientific community who conduct research on specific health outcomes, and the SAB recommends that the agency partner with professional societies and/or public institutions to engage with these experts. Examples might include the American Lung Association or the National Heart, Lung and Blood Institute to aid in building AOPs related to lung disease or disorders.

As ToxCast data are evaluated for deployment in different applications, the approach to test reliability becomes less obvious and has not been articulated. The agency is 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 integrated data within the context of AOPs. In principle, weight-of-evidence approaches would be developed to guide the integration of this information into different kinds of practices for hazard identification and perhaps risk assessment. The SAB agrees that, if successful, this effort would be enormously valuable, but the realization of this is distant.

3.1.3. Current Use of ToxCast Outputs

One example where the EPA has employed the ToxCast program to help inform an important decision was after the Deepwater Horizon accident. In this situation, the agency used a subset of ToxCast assays to assess the endocrine toxicity of eight oil dispersants that had the potential to be employed in the Gulf after the oil spill. Having this research program in place provided an important example of how the system can be used now and in the future. Moreover, this situation illustrates a number of important ancillary benefits of this program, including having developed an infrastructure that would allow rapid data generation so that the agency can make better-informed decisions in an emergency situation. Another benefit is that development of the overall CompTox research program 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 as well as the sometimes unanticipated needs of the agency, and helps risk assessors identify early the data gaps that may be filled by ToxCast.

However, the Deepwater Horizon accident also illustrates that in emergency situations ToxCast data may be generated and used very rapidly without the opportunity to fully evaluate the rationale behind the selection of a subset of assay data or the interpretation of those data for agency decisions, and without an obvious results-based validation that the decisions made based on these data were the best. Therefore, the SAB strongly encourages the agency to engage in strategic planning so that the endpoints and assays employed in a specific set of circumstances are maximally useful for that situation. Maximal utility would include a thoughtful analysis of the strengths and weaknesses of the subset of ToxCast data collected for a particular situation. The SAB recommends that the agency develop Data Use Guides to assist the agency in the design of assays and interpretation of ToxCast data generated in an emergency situation. The SAB also recommends that the agency use the Deepwater Horizon incident as a case study for the use of ToxCast data.

The ToxCast program also provides a unique way of evaluating the risk posed by exposures to mixtures of chemicals. This may be a means of enhancing the agency's traditional, more limited, focus on single stressors, endpoints, sources, pathways, and environmental media to a broader focus on the combination of these factors or the potential co-occurrence among these factors. The SAB recommends that the agency consider the use of ToxCast to explore the impact of mixtures.

3.2. Evaluating CompTox Outputs for Decision-making

Study Question 2. How well do the outputs of the CompTox program match with EPA's environmental assessment and management goals and/or the needs of key partners?

a) Are there plans to align the outputs and needs more closely?

b) What is the timetable and are those plans appropriate?

c) How can EPA align the outputs of the CompTox program and EPA assessment/management needs more closely?

This question is related to the first but is focused specifically on the degree to which the outputs from the CompTox research program are tailored to match the needs of the agency and key partners. In general, the administrative structure within ORD is designed to ensure that the work being performed – and the outputs generated – is matched to EPA's assessment and management goals. This includes both planning and implementation of ORD's research programs and periodic reviews by the BOSC, as well as feedback from regional offices and national program directors. Thus, there is every reason to believe that the organizational structure of the CompTox research program within ORD is being constantly evaluated and tailored to meet the agency's needs.

While the structure is in place to ensure that CompTox outputs are relevant to the agency and its partners, there are two fundamental issues that influence the use of these data in decision-making at different levels. First is what might be called "data integrity." That is, the data from each individual assay should be specific, sensitive and reliable. The second is "relevance." That is, the data should be informative at the level of prioritization, screening or green chemistry. These issues are briefly discussed below because these issues impact the degree to which CompTox outputs will become useful within the agency.

3.2.1. Specificity, Sensitivity, Reliability, and Relevance of ToxCast Data

The technical characteristics of the many assays that make up ToxCast – data integrity – were not discussed in detail at the May 30, 2012 meeting because reviewing these characteristics was beyond the scope of the committee's charge. The SAB expects that these technical issues of performance characteristics have been evaluated by the agency and suggests that this information be made available. Performance characteristics for individual assays were not discernible from the data posted to the website, but may influence the weight that a single assay should have within a battery.

The relevance of the data to decision-making is another matter. This will depend on the level of decision (prioritization, screening or green chemistry). There are, however, additional situations where the data may be used in different ways, such as in the case of Deepwater Horizon accident. It will be important to proactively envision the various ways that these data may be employed and develop a structured approach to demonstrate relevance for that application.

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 in various kinds of regulatory decisions. The answer to this question will undoubtedly depend on the level of

decisions to be made. For decisions with significant regulatory consequences, an important – if not essential – goal will be to sufficiently demonstrate the relevance of and obtain widespread acceptance of the data generated from ToxCast by risk assessors, risk managers and the regulated community. The data also will 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 EPA 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 *in vivo* studies associated with the same chemical. This strategy is made possible by the considerable amount of *in vivo* data associated with the Phase I chemicals. Of course, the assays included in ToxCast were pre-existing high-throughput assays developed for the pharmaceutical industry; they were not designed for ToxCast to correlate with endpoints employed in *in vivo* studies. Therefore, the SAB recommends that the agency consider developing the theoretical framework that would support the effectiveness of this strategy. That is, rather than evaluating how well ToxCast data predict the *in vivo* data, develop a structure that predicts how well ToxCast data *should* predict the *in vivo* data. Essentially, this amounts to developing "AOPs" for the *in vivo* studies and matching the *in vitro* assays to those AOPs.

The EPA's second strategy is to develop AOPs for human disease that may be reflected in the ToxCast data. This strategy highlights an important weakness in these two strategies that can be addressed in 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. 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).

The concept is that by evaluating the behavior of known toxicants in the ToxCast battery, patterns of toxicity linking this high-throughput behavior to adverse outcomes and thereby enhancing predictability will become apparent and will serve as validation of the predictive capability of the assay. However, this is a very retrospective approach that implies that strong patterns of activity within a subset of the 700 assays of ToxCast will be readily visible. The fact that investigators outside the agency evaluating ToxCast data are not finding the same predictive capacity of the data (Thomas et al. 2012) may indicate that the agency should take a more prospective approach to evaluating the relevance of these data.

Ultimately, the usability of a given result will be dependent upon the availability of additional data for the chemical in question and information regarding the tests and pathways affected by that chemical. The advantage of ToxCast 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 value of the information obtained from the breadth of assays. For example, the testing may be relevant to some cancers, developmental and reproductive endpoints, and endocrine and metabolic endpoints, but not, for example, eye health or neurological health. Positive results on subsets of assays or patterns of effects in certain biochemical pathways could implicate further testing and/or *in vivo* studies. Of particular importance for public health is the relevance of a negative result in an assay system, which in a screening step could result in a

decision not to proceed with further testing. Regarding more advanced uses of ToxCast outputs beyond hazard identification (e.g., use in dose-response assessment and risk assessment), the following additional concerns were raised by the SAB:

- Have the most predictive assays been identified in the ToxCast assays (which were pre-existing and not designed for this application per se)?

- How well do these ToxCast assays 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 uncertainty factors, 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?

The SAB also notes that the ToxCast research program should develop strategies for studying environmental chemical mixtures - not just the effects of one chemical at a time. The importance of using ToxCast 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 ToxCast 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 acceptable for a given AOP?

3.2.2. Incorporating Exposure Information

The SAB emphasizes the importance of considering the potential for exposure to the chemical when determining the degree of testing required. 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 that might be needed for agents to which 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. However, work is currently underway to address these issues (Gangwal et al. 2012; Wetmore et al. 2012).

As already noted, exposure is a key component of risk assessment and one that needs greater attention 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 may lead to misclassification of exposure. 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. 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.

3.2.3. Structured Approaches for Data Use

A key issue affecting use of ToxCast data is the need for a transparent structured approach to obtain and interpret data for various applications, especially in emergency situations. 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, structure-activity relationship (SAR), read-across approaches, other supporting *in vitro* data), it would be beneficial to establish general principles for the use and interpretation of the output for specific situations in Data Use Guidelines. Guidance for data needs (and sufficiency or appropriateness of data) must come from a clear characterization of EPA programmatic needs – identification of both the intended goals of a risk assessment or a prioritization effort – and examples in which ToxCast information appears to add real value. Key aspects regarding the performance of assays that are used should also be addressed in such a guidance document. Suggested criteria for assessing these assays can be found in Appendix B.

The concept of a Data Use Guideline is not new. For example, the Centers for Disease Control/ National Health and Nutrition Examination Survey (CDC/NHANES[3]) 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 (e.g., specificity, sensitivity) of the biomarker itself. This information is meant to aid in the interpretation of the data by various stakeholders and avoid the over-interpretation of the data.

The SAB also recommends that the agency work toward developing a clear rationale describing the different uses of the data depending upon specific effects. For example, the CompTox program's "ToxPi" (or Toxicological Priority Index) 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. In addition, in cases where only a subset of assays is employed for a particular application, there is no transparency in the logic about the construction of that subset as opposed to other subsets.

3.3. Building Scientific Acceptance of CompTox

Study Question 3. Are there obstacles (conceptual, legal, policy, institutional, other) preventing EPA from using the outputs from the computational toxicology program in EPA assessments and to support management decisions? How significant are these barriers? How might they be overcome? What would be a realistic timetable for overcoming them? What are the requirements of data from high-

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

throughput screening that would allow them to be informative to hazard characterization and/or risk assessment?

There are a number of challenges facing the ToxCast 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. Barriers to the use of ToxCast data include:

- 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 ToxCast assays and thus it will be difficult to determine the reliability of the *in vitro* assay;
- Lack of 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*;
- Dose-response assessment that does not 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;
- The potential for false negative results when considering a chemical for increased usage based upon ToxCast results; and
- Limited exposure information, which is a key part of any screening and prioritization program, as well as necessary for risk assessment.

The ToxCast program is in the development stage, as noted in response to Study Question 1, and so its use is still restricted to a largely research domain. The program has not had sufficient time to demonstrate that it can deliver on its promise. Questions remain 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, and the value of the current design of the system to generate the kind of information needed to be predictive; these 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 ToxCast 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 ToxCast assays to predict human disease. In the absence of such an accepted method, the agency will be limited in associating ToxCast data to data generated from guideline assays and this would be a severe limitation.

One of the ways to improve acceptance of CompTox outputs and overcome some of the barriers to their use is to demonstrate that they provide equivalent (or more accurate) answers relative to the currently accepted methods for characterizing hazard and estimating risk. Moreover, if CompTox 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 other components of the CompTox program, such as ExpoCast, in parallel to ToxCast 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 that, "...*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 EPA pursues this path, the agency will need to address several questions:

- How well do the *in vitro* and *in silico* (computer simulation) 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, gastro-intestinal, 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 that 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 come from, and how will they 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 ToxCast 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. Finally, the interactions between ORD and the various EPA programs (e.g., pesticides, water or toxics) that will use ToxCast data are commendable and should continue in order to understand what would make such data most useful. In addition to providing opportunities for program office scientists to spend time in the ORD laboratories to become familiar with the ToxCast program, extensive remote learning and training modules could be developed to reduce the cost and logistic challenges. This may also serve to engage more EPA scientists outside of the ORD laboratories located in Research Triangle Park, North Carolina.

3.4. 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 in 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 ToxCast 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 ToxCast assays to predict any particular endpoint in isolation and in combination with data from other ToxCast assays, and providing a broader understanding of what is known about a chemical's biological activity based upon ToxCast 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.

Outreach to epidemiologists and clinical investigators needs to be part of the communication 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 ToxCast 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 need 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 ToxCast. The agency can utilize existing relationships via community outreach and translation cores which support gene and cell therapy clinical trials and include 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 ToxCast 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 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 to target for outreach are risk assessors and public health professionals in state and tribal environmental and health agencies. Additional suggestions for research regarding communication and achieving a broader understanding of the potential contributions and limitations of these approaches include: (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. Such a study might identify structural reasons why there might be communication difficulties and how they could be addressed.

3.5. Other Issues

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

1) The SAB recommends that the agency clarify the goals and objectives for ToxCast with respect to chemical screening, prioritization and risk assessment. How will application of ToxCast 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 assessing the utility of particular types of information? Resolution of some of these structural issues could be a useful contribution of the ToxCast 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 ToxCast 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 chemicals will be evaluated? What health effects can be assessed at the current time

and what health effects may be assessed in the future? The identification of critical pathways is an important step toward clarifying key risk challenges – mixtures, interactions with background exposures, existing conditions and susceptibilities – and it provides an attractive possible approach for using ToxCast 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, ToxCast 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 the 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 ToxCast 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 ToxCast 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 ToxCast 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 ToxCast; 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 the EPA handle contradictions between *in vivo* or human studies and 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 ToxCast results. This is an inherent characteristic of science and is not unique to the ToxCast program. What will be EPA's approach to handling comments and perceptions regarding whether the ToxCast 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 ToxCast program and the use of its outputs?

7) Clear and effective communication with all stakeholders, both within and outside the agency, will be essential to the long term use of ToxCast and ExpoCast findings.

8) A community of scientists should be developed to provide feedback on ExpoCast in a parallel fashion to ToxCast.

9) Finally, the SAB recommends that the EPA consider establishing an ongoing external advisory process to help institutionalize a long term program built around the idea of incremental transformation. An independent perspective on the current program and prospects for the future can be provided along with constructive suggestions.

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 Program is part of EPA's broader Chemical Safety research efforts. The CompTox research 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. 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 – a suffix that refers to a broad field of study in biology that includes molecular biology specialties such as genomics, proteomics, and transcriptomics.

NCCT - or the National Center for Computational Toxicology is the largest component of EPA's Computational Toxicology Research Program. It was established in 2005 to coordinate computational toxicology research on chemical screening and prioritization, informatics and systems modeling.

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).

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APPENDIX A. HISTORY OF THE COMPTOX PROGRAM

[The information contained in this appendix was extracted from the *U.S. EPA Office of Research and Development Computational Toxicology Research Program Implementation Plan for Fiscal Years 2009 to 2012,* http://epa.gov/ncct/download_files/basic_information/CTRP2_Implementation_Plan_FY09_12.pdf]

History of the Computational Toxicology Research Program (CTRP) and the National Center for Computational Toxicology (NCCT)

A. Defining the Mission of Computational Toxicology at EPA

Computational toxicology applies mathematical and computer models and molecular biological and chemical approaches to explore both qualitative and quantitative relationships between chemical exposure and adverse health outcomes. Recent technological advances make it possible to develop molecular profiles using high-throughput and high-content methods that identify the impacts of environmental exposures on living organisms.

CTRP Mission Statement: To integrate modern computing and information technologies with molecular biology to provide the agency with decision support tools for high-throughput risk assessment.

With these tools, scientists can produce a more detailed understanding of the hazards and risks of a much larger number of chemicals. The integration of modern computing with molecular biology and chemistry is enabling scientists to better understand a chemical's progression through the environment to the target tissue within an organism and, ultimately, to the key steps that trigger an adverse health effect. Currently, risk estimates most often are based on gross outcomes of disease, such as occurrence of cancer, a neurological disorder, or a visible birth defect. The National Research Council (NRC) in its 2007 report, *Toxicity Testing in the 21st Century: A Vision and a Strategy*, called for a concerted effort to move toxicology from a primarily descriptive science to a more predictive one by utilizing largely human based in vitro studies to understand the biological pathways by which chemically induced diseases occur. The Environmental Protection agency's Computational Toxicology Research Plan is working to aid this transformation by evaluating the key molecular changes occurring in the function of critical human toxicity pathways within cells, tissues, individuals, and populations. The key will be connecting these changes quantitatively and systemically to the types of adverse health effects that have been the traditional basis of EPA risk assessments and to use this understanding to reduce the current uncertainties in the extrapolation of effects across dose, species, and chemicals. The Office of Research and Development CTRP is composed of three main elements. The largest component is the National Center for Computational Toxicology , which was established in 2005 to coordinate research on chemical screening and prioritization, informatics, and systems modeling. The second element consists of related activities in the National Health and Environmental Effects Research Laboratory (NHEERL) and National Exposure Research Laboratory (NERL) of ORD. The third and final component consists of academic centers working on various aspects of computational toxicology and funded by the EPA Science to Achieve Results (STAR) Program.

The rapid and ongoing success of the CTRP is impacting hazard and exposure identification and helping to close data gaps, identify toxicity pathways, suggest modes of action, and make for more efficient utilization of precious resources on the highest priority chemicals. Besides these initial outcomes from the higher throughput approach of the CTRP, informatics and modeling efforts will provide more in-

depth and quantitative molecular understanding of how biological systems respond to environmental chemicals. These knowledge databases and *in silico* tools will reduce or quantify uncertainties relating to biological susceptibility, species differences, and dose response as part of a faster and more intelligent targeted testing paradigm in support of quantitative risk assessments.

B. Timeline of CTRP Development

In fiscal year 2002, Congress ordered a redirection of $4 million from available EPA funds, "…for the research, development and validation of non-animal alternative chemical screening and prioritization methods, such as rapid, non-animal screens and Quantitative Structure Activity Relationships (QSAR), for potential inclusion in EPA's current and future relevant chemical evaluation programs."

To fulfill this directive, EPA embarked on development of a research program that (1) was consistent with the Congressional mandate, (2) complemented and leveraged related ongoing agency sponsored efforts to consider alternative test methods, (3) further advanced the research to support the agency's mission, and (4) would not duplicate the mission and programs in this area conducted by other agencies (see Figure 1 below for a timeline of CTRP development).

Thus the CTRP was initiated to target these goals and, in the process, significantly advance toxicology and risk assessment as currently practiced by the agency and the broader environmental sciences community. In FY2002 to 2003 pilot projects were funded to demonstrate computational toxicology could be adapted to the study of endocrine disruptors. Early successes of these efforts included refinement of estrogen receptor ligand binding data for development of quantitative structure-activity models, evaluation of EPA-developed cell lines for detecting estrogen and androgen activities from various species and the development of an alternative test method for evaluating effects on steroidogenesis in H295R cells (see EDSP Assay Status).

With increasing attention to and expectations for the CTRP in FY2003, ORD developed *A Framework for a Computational Toxicology Research Program*, which was published in FY2004 and provided strategic direction for the program. This document was the product of a cross- ORD design team of scientists and was reviewed by the Science Advisory Board (SAB). ORD hosted a workshop in Research Triangle Park, NC, in late FY2003 to introduce the CTRP framework from which the three objectives for EPA computational toxicology were translated into the three initial long-term goals (LTGs) for the program:

1) risk assessors use improved methods and tools to better understand and describe the linkages of the source-to-outcome paradigm,
2) EPA program offices use advanced hazard characterization tools to prioritize and screen chemicals for toxicological evaluation, and
3) EPA assessors and regulators use new and improved methods and models based on the latest science for enhanced dose-response assessment and quantitative risk assessment.

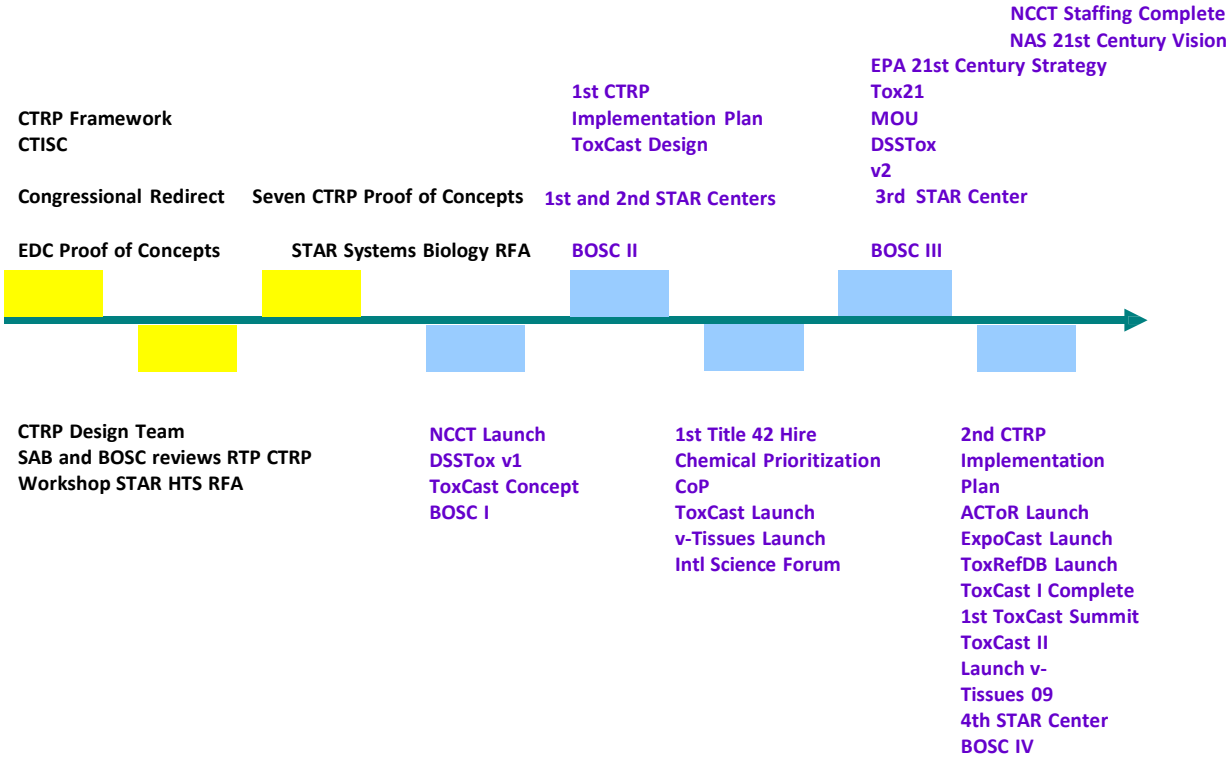
With issuance of the CTRP framework, ORD began the process of implementing a more formalized program. A cross-agency working group, the Computational Toxicology Implementation Steering Committee (CTISC) was formed in FY2004 to oversee the selection and funding of projects across ORD. Seven cross-ORD projects were initiated as result of CTISC action, and these "new start" projects became a critical component of the first generation CTRP implementation plan.

In October 2004, then EPA Science Advisor and Assistant Administrator for ORD, Dr. Paul Gilman, announced the formation of the NCCT, which began official functions in February 2005. The announcement states:

"The Center will advance the science needed to more quickly and efficiently evaluate the potential risk of chemicals to human health and the environment. The Center will coordinate and implement EPA's research on computational toxicology to provide tools to conduct more rapid risk assessments and improve the identification of chemicals for testing that may be of greatest risk."

NCCT quickly became the hub for ORD CTRP research. NCCT formed key partnerships with the other laboratories and centers within ORD, which formed the second critical element. Partnerships with NHEERL, NERL, the National Risk Management Research Laboratory, and the National Center for Environmental Assessment helped in the execution of not only the seven cross-ORD "new start" projects awarded by the CTISC in 2004, but also in several original NCCT-led projects, including the Distributed Structure-Searchable Toxicity Database (DSSTox), Toxicity Forecasting Project (ToxCast^{TM}), Toxicity Reference Database (ToxRefDB), and the virtual tissues projects looking at liver and embryo.

FIGURE 1
ORD Computational Toxicology
Research Program Development



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APPENDIX B. ASSAY PERFORMANCE CRITERIA

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, hormetic and essential);
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.