



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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OFFICE OF THE ADMINISTRATOR
SCIENCE ADVISORY BOARD

DATE

EPA-SAB-12-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 Science, has often encouraged the EPA to improve its risk assessment practices and to modify its single chemical approach. With tens of thousands of chemicals currently in commerce, and hundreds more introduced every year, only a small fraction of chemicals have been adequately assessed for potential risk. The EPA's Computational Toxicology (CompTox) Research Program was established to explore ways in which advances in molecular biology, chemistry and computer science can more effectively and efficiently assess chemical risks. The goal of the CompTox Research Program is to provide high-throughput decision support tools for assessing chemical exposure, hazard and risk and to address the need for faster, cheaper alternative risk assessment methodologies.

The SAB has previously underscored the importance of this research program and has been interested in the successful application of CompTox data to advance EPA's hazard and risk assessment. The CompTox program 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 a more broad focus on the potential interaction between these factors. Consequently, the SAB asked its Exposure and Human Health Committee (EHHC) to look at how the research products from the CompTox program are currently being used by EPA, if they align with EPA's programmatic needs and if there are limitations or challenges to using CompTox in decision making for risk assessment and risk characterization. The SAB EHHC along with members of the EPA Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel received briefings from EPA representatives regarding the implementation of CompTox research program outputs into EPA risk assessments. Although the EPA has not yet begun to incorporate the information generated by the CompTox research program into various applications, e.g.,

1 screening, prioritizing or risk assessment, the SAB, in their attached report, has provided advice
2 regarding the issues that the Agency should consider as the move forward with implementation.
3 This letter highlights the SAB's major recommendations.

4 The SAB commends the Agency on undertaking the immense effort of developing the CompTox
5 research program. The CompTox program is currently in the development stage so the extent of
6 the use of its outputs is necessarily limited. There are exceptions to this; for example, following
7 the Deepwater Horizon accident, the high-throughput screening (HTS) assays that form the basis
8 of the CompTox program were used for gathering toxicity data on the 8 oil dispersants employed
9 by BP in the Gulf of Mexico. The fact that there was a formal CompTox program in place within
10 EPA at the time of the Deepwater Horizon accident made it easier to employ these assays. Thus,
11 there are ancillary benefits that "spin off" from this program and the Agency is realizing these
12 benefits. Another ancillary benefit is that the alignment of the CompTox program and the
13 program's goals with Agency needs requires a great deal of interaction among the various
14 programs and offices within the Agency and this will have long-lasting benefits.
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16 The challenges the Agency currently faces in regards to the various ways CompTox data may be
17 used are substantial and are well known to the Agency. These include but are not limited to
18 physical characterization of each individual assay, how the data generated predict effects on
19 apical endpoints in validated guideline studies, and the ways in which patterns of data predict
20 human disease. This latter issue is the most difficult and, as the Agency develops the Adverse
21 Outcome Pathways (AOPs) that would link these patterns to human disease, it would be well-
22 served to partner with professional societies and research institutes whose mission is to
23 understand the diseases under investigation. In addition, while the CompTox research program is
24 currently focused on understanding AOPs, a similar effort to incorporating metabolism, and
25 understanding exposure should also be pursued.
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27 Guidance for data needs (and sufficiency or appropriateness) must be matched to guidance for
28 data use and this will be derived from a good characterization of programmatic needs – what are
29 the intended goals of a risk assessment or a prioritization effort – together with the identification
30 of examples of where CompTox information appears to add real value. A clear explanation of the
31 limitations of the models, the reliability of the assay systems, the certainty associated with an
32 AOP and knowledge of the metabolism of the chemical being assessed are only some of the
33 important considerations that should be addressed when developing a risk assessment.
34 Furthermore, methods for incorporating biomonitoring data, exposure pathways, chemical source
35 information and human activity patterns on human exposure also need to be developed.

36 One of the ways to improve acceptance of CompTox and overcome some of the barriers is to
37 demonstrate that it provides equivalent (or more accurate) answers relative to the currently
38 accepted methods for estimating risk. This will require a combination of research to develop
39 reliable methods and experience in using the methods to predict risk. Through incremental
40 change to the current approaches of assessing risk, supplementing and then replacing existing
41 methodologies and demonstrating the value of new technologies through practice will lead to
42 greater confidence in the use of CompTox as a predictive tool.
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1 Outreach, training and communication are all necessary and important components for the
2 effective implementation of CompTox outputs and advancing EPA risk assessment. The
3 approaches for reaching out to the diverse EPA program offices that could use CompTox data
4 and trying to understand what would make such data most useful for them are laudable and
5 should continue. We commend EPA's Computational Toxicology Communities of Practice
6 which is composed of more than 300 people from over 50 public and private sector organizations
7 that are interested in the application of computational toxicology and exposure science to EPA's
8 risk assessments. This is a phenomenal tool for keeping up with technical issues that the EPA is
9 dealing with and addressing as a part of the CompTox program.

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11 The SAB applauds the Agency for their efforts within the CompTox program, and encourages
12 and recommends the continued development of CompTox to lead to a better understanding and
13 expansion of the potential utility of this technology. As the EPA gains more experience and
14 expertise in the use of CompTox outputs in risk assessment, we look forward to future
15 opportunities to provide advice to EPA on this important effort.

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Sincerely,

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1 **Background**

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3 The NRC Committee on Toxicity Testing and Environmental Assessment published in 2007,
4 “Toxicity Testing in the 21st Century: A Vision and a Strategy”. In this report, the Committee
5 developed a very strong rationale for developing a program using modern tools that would
6 provide relevant information about chemical toxicity that could be integrated into current risk
7 assessment practices, the goals of which would be to enhance the efficiency and effectiveness of
8 chemical safety determination. In the same year, the EPA launched the ToxCastTM initiative as
9 part of the computational toxicology (CompTox) research program, guided by the NRC report
10 and taking advantage of existing technologies, to develop ways to predict the toxicity of the
11 thousands of chemicals for which toxicity testing is lacking or absent.

12

13 Recently the SAB noted in its report, Science Advisory Board Comments on the President's
14 Requested FY 2012 Research Budget (EPA-SAB-11-007), “*the SAB is concerned that there is no*
15 *proactive budget initiative to develop ways of employing the results of the CSS program,*
16 *including high throughput data, into hazard or risk assessment.*” The CSS or Chemical Safety
17 for Sustainability program is one of six transdisciplinary research programs within the EPA
18 Office of Research and Development and is responsible for coordinating the activities of the
19 CompTox research program. Since CompTox has the potential to provide the Agency with a
20 means of modifying its traditional focus on single stressors, endpoints, sources, pathways, and
21 environmental media to a broader focus on the potential interaction between these factors, the
22 SAB has requested that its Exposure and Human Health Committee (EHHC) develop advice to
23 assist in advancing the application of CompTox research for human health risk assessment to
24 meet EPA’s programmatic needs. In developing its advice to EPA, the EHHC engaged in
25 discussions with ORD and EPA offices that currently use or plan to use the CompTox research
26 outputs in order to address the following questions:

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- 28 1) Are the outputs of Comptox currently being used by EPA? How well do the outputs align
29 with EPA’s programmatic needs?;
- 30 2) What issues are there in using Comptox in decision making for risk assessment and risk
31 characterization as opposed to chemical screening, prioritization and green chemistry?;

- 1 3) What are the barriers and limitations that prevent EPA from using CompTox outputs and
2 how might they be overcome? and
3 4) How should the use of the CompTox program be effectively communicated to
4 stakeholders? How can the communication be enhanced?
5

6 The members of the EHHC were joined, for this review, by two members of the EPA Federal
7 Insecticide, Fungicide, and Rodenticide Act Scientific Advisory Panel (FIFRA SAP) who had
8 reviewed elements of the ToxCast program in 2011. The committee received overview
9 information from representatives ORD and program offices which laid out the overall
10 philosophy, structure, and organization of the program and included descriptions from risk
11 assessors within the agency who described their use of the information. The ToxCast program
12 currently consists of nearly 700 individual assays provided by 9 companies. A foundational
13 element of ToxCast is a chemical library in which a large number of chemicals are
14 simultaneously tested to create toxicity profiles in these assays. In Phase I, chemicals, including
15 309 pesticidal actives and commercial chemicals for which there is a substantial amount of
16 toxicity data, have been assayed. These chemicals are intended as a “proof of concept”, i.e., they
17 will be used to develop toxicity profiles and demonstrate the ability of the assays to be
18 predictive. In Phase II, about 2,000 chemicals from a broad range of sources including industrial
19 and consumer products, food additives, “green” products, nanomaterials and drugs that never
20 made it to the market are being screened. This information will be used in the development of
21 pathways of toxicity – patterns of effects observed in the CompTox assays that are plausibly and
22 causally related to observations of apical effects in the *in vivo* assays.
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24 **Response to Charge Questions**

- 25 1. *Are the outputs of Comptox currently being used by EPA? How well do the outputs align*
26 *with EPA’s programmatic needs?*

27 The outputs of Comptox are currently being used primarily in a research domain to determine the
28 reliability of the data for use in the different programs of EPA. There are a few examples where
29 the information derived from the Comptox program were used in a limited fashion to inform
30 Agency decisions (see below), but these were special cases. Despite the limited use of Comptox

1 outputs at this time, the committee feels that the program is on track. In fact, the progress made
2 to date within the 5 years of the development of ToxCast is impressive.

3 There are several kinds of challenges facing the Comptox program in preparing outputs for use
4 in agency decisions, and these challenges are specific to the various uses to which these data may
5 be employed, including informing decisions that must be made without other information,
6 prioritizing chemicals for further toxicity analysis, and for risk assessment. In all cases, a
7 common concern is that the data generated from high throughput *in vitro* assays can be applied
8 reliably to these applications. By “reliable”, the intention is that the data will have been shown
9 to be predictive of toxicity such that agency decisions in each of these domains will be
10 supported.

11
12 The reliability of ToxCast data are currently being explored in two ways. First, data from
13 ToxCast are being compared to data from ToxRef – a database of toxicity studies using
14 guideline, *in vivo* test systems. The theory is that by comparing the effects of individual
15 chemicals in both ToxCast and ToxRef, parallels can be ascertained that will provide confidence
16 that decisions based on ToxCast data will be at least predictive of results obtained from *in vivo*
17 guideline studies. A second approach is to develop pathways of toxicity in the human population
18 that would lead to the manifestation of disease. These, “Adverse Outcome Pathways” (AOP)
19 represent a very important link from *in vitro* high through-put assays to human disease, and this
20 effort is just beginning to be developed.

21
22 The CompTox program is also exploring the possibility that ToxCast data can be combined with
23 large databases of experimental data at the level of the genome, epigenome, proteome and
24 metabolome to provide higher resolution data within the context of AOPs. In principle, weight
25 of evidence approaches would be developed to guide the integration of this information into
26 current data and practices for hazard identification and perhaps risk assessment. The anticipated
27 result is expected to shape the future of toxicity testing at EPA in accordance with previous NAS
28 reports, paying large dividends for the Agency and the American public that are well worth the
29 investments currently being made.

30

1 The outputs of the ToxCast program are being tailored to align with the programmatic needs of
2 the Agency both in the long- and short-term. The Agency devoted a significant amount of time
3 in the face-to-face meeting explaining the ways in which this tailoring is occurring. This is a
4 critical and difficult issue and will require constant communication between the different
5 programs within the Agency to sculpt the program in such a way that the outputs are employed
6 by the Agency. The expectation is that the current research questions and research approach will
7 produce a program that will have broad applicability within the Agency.

8
9 The high-throughput screening (HTS) assays that form the basis of the CompTox program have
10 already been employed in a limited manner to provide the Agency with at least some formal
11 toxicity data on the 8 oil dispersants employed by BP in the Gulf of Mexico following the
12 Deepwater Horizon accident. The fact that there was a formal CompTox program in place within
13 EPA at the time of the Deepwater Horizon accident made it easier to employ these assays. This
14 illustrates an important issue; namely, that there are a number of ancillary benefits of this
15 program. One is to have ready access and infrastructure to handle HTS data generated rapidly to
16 inform the Agency in times of crisis. Another is that development of the CompTox program has
17 facilitated a great deal more interaction between the various offices of the Agency. This
18 communication about data needs and data interpretation has and will continue to have the benefit
19 of bringing the intramural research program into alignment with the routine, and sometimes
20 unpredicted, needs of the Agency as well as to help risk assessors identify early the data gaps
21 that may be filled by the kind of information that CompTox can produce. Additionally, the
22 CompTox program provides an alternative means of evaluating mixtures. The CompTox
23 program provides the Agency with a means of shifting its traditional focus on single stressors,
24 endpoints, sources, pathways, and environmental media to a more broad focus on the potential
25 interaction between these factors.

26
27 A central question at this time is whether the *in vitro* high throughput assays will ever produce
28 data that will be suitable for decision-making such that, eventually, these data could replace *in*
29 *vivo* testing in regulatory decisions. But the answer to this question will undoubtedly depend on
30 the level of decisions to be made. Thus, an important – if not essential – goal will be to obtain
31 widespread support for the characteristics of the data generated from ToxCast that would reflect

1 toxicity in a *predictive* manner. This will also need to be consistent with statutory requirements
2 for the evidence the Agency uses to take some regulatory action. Thus, characterizing the data
3 generated from ToxCast assays in terms of the specificity, sensitivity and reliability of the
4 individual assays as well as their ability to predict toxicity either alone or in combination with
5 other findings, is currently the principle goal of the research domain of the program, and the
6 Agency appears to be making rapid progress toward these specific goals.

7
8 2. *What issues are there in using Comptox in decision making for risk assessment and risk*
9 *characterization as opposed to chemical screening, prioritization and green chemistry?*

10
11 It is expected that the data derived from CompTox assays should lend themselves readily to
12 hazard identification and especially green chemistry. These data may stand alone within a green
13 chemistry paradigm and provide insight guiding the development of chemical products that have
14 a much greater likelihood of being free from toxic properties. Moreover, these data may be
15 combined with information from structure-activity relationship (SAR) and any *in vivo* data that
16 might be available to identify hazard and help guide a weight-of-evidence analysis of hazard.
17 However, there are several precautions that need to be taken when applying the data for hazard
18 evaluation. First, the strengths and limitations of each assay must be recognized, including the
19 potential for false negative and false positive results. Given that pathways of toxicity are poorly
20 understood, current *in vitro* assays cannot be seen as comprehensive in their scope. According to
21 Judson et al. 2010¹, CompTox models developed to screen for chronic, developmental and
22 reproductive toxicity endpoints display high specificity (few false positives) but only moderate
23 sensitivity (multiple false negatives). [Sensitivity relates to the assay's ability to identify positive
24 results. Specificity relates to the ability of the assay to identify negative results.] Therefore, the
25 rate of false negatives is expected to be high at this stage of the program. While some
26 information is better than none, there is the concern that too much confidence will be placed
27 upon the lack of activity in the available assays. If there is a high degree of reliance on data from
28 these assays, it may inappropriately give the appearance that a chemical with no activity is safer
29 than other, alternative chemicals for which more information is available. EPA should include

¹ JUDSON, R., D. J. DIX, K. A. HOUCK, M. T. MARTIN, T. B. KNUDSEN, AND R. J. KAVLOCK. 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.

1 the exposure potential of the chemical when determining the degree of testing required such that
2 even if initial screens of a chemical find little reason for concern, *in vivo* confirmation may still
3 be desirable if its exposure potential is high. Conversely, low exposures may eliminate the need
4 for extensive toxicity testing which might be needed for agents whose exposure is greater. This
5 emphasizes the need for good exposure information which at this point appears to be a limitation
6 of CompTox modeling and Toxic Substances Control Act (TSCA) databases. Second, there is
7 uncertainty of the significance of a positive result in any particular assay within ToxCast. A
8 major effort is apparently under way to link patterns of activities within the battery of ToxCast
9 assays to AOPs. The current utility is limited since many of the screening tests are still under
10 development and going through validation exercises on an individual level and still needing to be
11 understood within the broader context of AOPs and apical endpoints. The concept is that by
12 evaluating the behavior of known toxicants in the ToxCast battery, patterns of toxicity linking
13 this HTS behavior to adverse outcome – and thereby is predictive – will become apparent.
14 Ultimately, the usability of a given result will be dependent upon the context of additional data
15 known about the chemical in question and about the tests and pathways affected by that
16 chemical.

17
18 In other words, for chemical screening and prioritization, the testing should be sensitive (i.e.,
19 detects an effect when there is one) and specific (i.e., does not detect an effect when there is not
20 one). Of particular importance for public health is the accuracy of a negative result – which in a
21 screening step would effectively stop further testing. The advantage of CompTox is that
22 thousands of tests can be conducted – these need to be inclusive of as many potential health
23 effects as possible. The limitations of the breadth of the assays should be transparent. For
24 example, the testing may be accurate for cancer, developmental and reproductive endpoints,
25 endocrine/metabolism endpoints, liver and kidney effects, but not for, say, eye health or
26 neurological health.

27
28 Regarding more advanced uses of Comptox output (e.g., use in dose response assessment and
29 risk assessment) the following additional concerns should be considered to understand chemical
30 hazards: 1) have the most sensitive endpoints been identified for risk assessment; 2) how well do
31 these endpoints relate to apical endpoints such as carcinogenesis, endocrine disruption, organ

1 toxicity, neurotoxicity, immunotoxicity, etc.; 3) would the same uncertainty factors apply to *in*
2 *vitro* screening data as are now used when starting with *in vivo* data (e.g., interspecies,
3 intraspecies, acute to subchronic to chronic study duration, database quality and completeness);
4 4) how would the *in vitro* dose response relate to *in vivo* when considering route of entry,
5 metabolic activation and detoxification systems that may not be present *in vitro*, and other
6 toxicokinetic factors that govern the external dose associated with a particular concentration at
7 the target cell or receptor; and 5) related to #3 above, how well do the *in vitro* test methods
8 capture intra-human variability in terms of susceptible sub-populations and life stages including
9 genetic polymorphisms and disease states.

10
11 For risk assessment that more accurately represents environmental conditions, CompTox needs
12 to also develop strategies for studying environmental chemical mixtures - not just the effects and
13 exposures of one chemical at a time hundreds individually one at a time. The importance of
14 using CompTox to characterize the risk of environmental chemical mixtures cannot be
15 overstated. Moving in this direction requires establishing a scientifically defensible foundation -
16 issues such as defining appropriate AOPs, development of testing methods that address a wide
17 array of AOPs, and evaluation of the accuracy, sensitivity and specificity of the tests, etc. While
18 this may ultimately be a long range goal, the path to studying and estimating risk from mixtures
19 should be outlined. Examples of critical questions include the following: 1) how would relevant
20 mixtures be identified; 2) how can sufficiently similar mixtures be used; and 3) how much risk is
21 allowable for a given AOP. These are difficult questions that the Agency is certainly aware of
22 and are working toward defining.

23
24 As already noted, exposure is a key component of risk assessment and it is a specific issue that
25 needs to be addressed. A general approach based solely on chemical properties that evaluate
26 transport from large sources and partitioning based on fugacity concepts to distribute chemicals
27 from their source to a population will NOT provide a full exposure evaluation and will lead to
28 misclassification of exposure. This is analogous to saying that nothing needs to be known about
29 metabolism of chemicals when determining toxicity- just the overall chemical structure and what
30 functional groups are present to compare among compounds. Exposure occurs where people
31 contact chemical agents and often that is a result of being close to the emission source and the

1 agent is not in equilibrium or at steady state with the environment. For example, an agent
2 produced in relatively small amount compared to a High Production Volume Chemical (HPVC)
3 and that has a fugacity that would limit its transport in the environment but is used in personal
4 products can have a higher exposure than a HPVC agent emitted from point sources away from
5 populations. This would not be predicted based on an exposure model that does not include
6 information on its use and potential contact with people. Thus, if these two agents were equally
7 hazardous, the low production compound would present greater risk and it is unclear if the
8 current assays used in the CompTox program would predict that. This would be true for all of
9 the EPA applications listed, i.e., chemical screening, prioritization, risk assessment or green
10 chemistry.

11
12 Perhaps the greatest issue with the emergence of large volumes of CompTox data is the manner
13 in which these data can be used for various applications. Guidance for data needs (and
14 sufficiency or appropriateness) must come from a good characterization of programmatic needs –
15 what are the intended goals of a risk assessment or a prioritization effort – together with the
16 identification of examples of where CompTox information appears to add real value. While the
17 data are meant to be used within a weight of evidence context that requires integration across all
18 the available data (e.g., *in vivo* toxicology data, SAR, read-across approaches, other supporting
19 *in vitro* data), it may be beneficial to devise general principles for the use and interpretation of
20 the output for any one endpoint in a Data Use Guidance (DUG) document. Such information
21 about the endpoint can include:

- 22 1) name of the assay;
- 23 2) positive control and other agents known to characterize the assay;
- 24 3) dynamic range of the assay;
- 25 4) where the endpoint fits within one or more AOPs;
- 26 5) related CompTox endpoints (i.e., likely to be within the same AOP, but may also
27 include endpoints indicative of similar biological activity but in an independent test
28 system);
- 29 6) interpretative value of the endpoint if altered in isolation;
- 30 7) interpretative value if altered in conjunction with other “aggregated” endpoints;

- 1 8) rate of false positive and negative results if it is to be used for predictive purposes
- 2 (e.g., to forecast *in vivo* endocrine activity);
- 3 9) shape of the dose response curve (e.g., monotonic, non-monotonic, threshold, linear,
- 4 etc.);
- 5 10) potential for the endpoint to be used as a biomarker in toxicity testing or in
- 6 epidemiology studies;
- 7 11) provide an indication of whether the endpoint is also affected by disease processes
- 8 that might potentially lead to a chemical/disease interaction; and
- 9 12) limitations and uncertainties of the endpoint.

10 Perhaps a simple flow chart would help going from least evidence for a meaningful effect
11 (e.g., perturbation only at high dose) → greatest evidence for meaningful effect (e.g., upstream
12 and downstream endpoints affected in a defined AOP with effects occurring on upstream
13 endpoints at low dose and anchored by similar effects from a known toxicant). Perhaps the DUG
14 can also suggest different uses of the data depending upon where on the continuum the evidence
15 for a meaningful effect lies for a particular chemical. The “ToxPi” pie chart of endocrine related
16 effects for a chemical appears to be a useful way to illustrate the types of biological activities a
17 chemical has but the meaning and importance of individual slices relative to other slices is not
18 apparent. The DUG can also have a section on aggregated endpoints that describes the
19 implications of a “slice” of the pie for a particular biological effect and how one determines
20 potency for a slice rather than a particular endpoint.

21
22 The concept of a DUG is not new. For example, the CDC/NHANES biomonitoring data release
23 provides important information for each endpoint including the normative range in the
24 population, any relevant workplace or environmental standards (e.g., OSHA BEIs), and
25 limitations of the biomarker itself (e.g., specificity, sensitivity). This is meant to aid in the
26 interpretation of the data by various stakeholders and avoid the over-interpretation of the data.
27 As previously mentioned, the Deepwater Horizon accident revealed a critical programmatic need
28 – the need for rapid response to emergency or other sudden demands for information and
29 recommendations. Here again, there is a need for developing resources, procedures, and
30 guidance for such responses.

31

1 Finally, for CompTox data to be of sufficient quality for use in risk assessment, it must
2 correspond to validated endpoints or well defined AOPs. Importantly, the batteries of CompTox
3 assays were not formally designed to inform these endpoints, for which *in vivo* assays were
4 developed, in some cases, decades ago. Further, the validated guideline assays were not
5 specifically designed to predict current public health trends. The current strategy is to use the
6 data generated by Phase I of the CompTox program to provide information that would lead to
7 confidence about the relationship between patterns of responses in the battery of tests and the
8 way these chemicals act in guideline studies. This empirical analysis will be difficult in part
9 because chemicals may have more than one mode of action and while two “estrogenic”
10 chemicals may overlap in the patterns of responses observed in the battery of tests, they will
11 likely have large regions of non-overlap. In the absence of prior knowledge of these
12 characteristics, it will be difficult to find the common pattern that predicts the responses
13 observed in guideline studies. However, although difficult, it is not insurmountable and over
14 time, through experience with the rapidly increasing database of information that is being
15 generated, the Agency will develop this knowledge. Just as important will be developing the
16 relationship between the CompTox outputs and the etiology of human disease based on
17 epidemiological data. These are difficult issues, but the Agency has an extraordinary beginning
18 to address these successfully.

19
20 3. *What are the barriers and limitations that prevent EPA from using CompTox outputs and*
21 *how might they be overcome?*

22
23 It is worth repeating several points that likely serve as barriers to the use of CompTox data: 1) If
24 an endpoint is not well anchored in an AOP or read-across approach, then perturbation of that
25 endpoint may be difficult to apply to screening or risk assessment; 2) dose-response assessment
26 must take into account *in vitro* to *in vivo* extrapolation, application of uncertainty factors, special
27 consideration of vulnerable sub-groups; 3) as noted earlier, there is a likelihood for false negative
28 results at this stage of testing which requires caution when considering a chemical for increased
29 usage based upon CompTox results; and 4) exposure information is often limited but is a key
30 part of any screening and prioritization program, as well as in risk assessment; 5) risk
31 management requires an understanding of how to reduce or eliminate exposure not just the

1 toxicity of an agent unless a complete substitution for a newly proposed chemical and/or removal
2 of the chemical already being used from the environment is possible.

3
4 As the CompTox program is still in its infancy and as already noted above in response to Charge
5 Question #1, its use is still very limited. Therefore, a significant barrier is that the Agency has
6 not had enough time in the program to prove that it will work the way it is being described.
7 Questions about the reliability of each individual assay, the power of “pattern recognition” as a
8 predictor of toxicity, the value of the current design of the system to generate the kind of
9 information needed to be predictive, all are legitimate questions that require time and experience
10 to answer. Considering the importance of these goals and the complexity of the issues involved,
11 there will be unavoidable “blind alleys” that the Agency will discover. However, the number of
12 these may be limited by being more proactive about building “Adverse Outcome Pathways” and
13 “Pathways of Toxicity”. In this regard, the fact that there are currently no internationally
14 accepted methods of performing a “weight of evidence” analysis in the scientific literature
15 should not be overlooked. While this is not the purview of the Comptox program per se, the
16 ability of the Agency to employ peer-reviewed science in the Tox21 program would be enhanced
17 by developing a method of analysis that is generally accepted. The absence of this would mean
18 that the Agency will be limited in associating CompTox data to data generated from guideline
19 assays and this would be a severe limitation.

20
21 One of the ways to improve acceptance of CompTox and overcome some of the barriers is to
22 demonstrate that it provides equivalent (or more accurate) answers relative to the currently
23 accepted methods for estimating risk. Moreover, if it does so with lower resource (e.g., cost and
24 time) requirements, thereby allowing for the characterization of the large number of agents that
25 EPA must make decisions about, then it will quickly become the methodology of choice. There
26 is also a need to commit similar resources to develop ExpoCast in parallel to CompTox to
27 support the all of the programmatic needs of EPA. This will require not only acceptance by the
28 exposure group of the National Exposure Research Laboratory (NERL) but also a recognition
29 that exposure is a key component of risk assessment, risk characterization and risk management
30 by others within the Agency and that the volume of an emission is not equivalent to exposure.

31

1 According to the EPA’s March 2009 strategy document², the Agency appears to be following the
2 recommendation of the NRC 2007 committee which said: “...*in vitro* tests would be developed
3 not to predict the results of current [animal] apical toxicity tests but rather as [human] cell-based
4 assays that are informative about mechanistic responses of human tissues to toxic chemicals. The
5 NRC committee is aware of the implementation challenges that the new toxicity-testing
6 paradigm would face”. With this in mind, EPA is currently conducting research to identify
7 AOPs which can serve as predictors of toxicity; the need to relate these AOPs to currently
8 understood toxicity endpoints is critical. Once appropriate AOPs are established, EPA will be
9 positioned to transition to the methodologies recommended by the NRC. However, as EPA
10 pursues this path, there are several issues that will need to be addressed. They include: 1) how
11 well do the *in vitro* and *in silico* tests translate to human systems? ; 2) how predictive of human
12 pathways are the identified AOPs? Data on this is important to share and make public; 3) how do
13 the testing methods account for differences between *in vitro/in vivo* animal testing and human
14 metabolism? For instance, how are chemicals that are cleared through multiple pathways (renal,
15 GI, etc) treated in the analysis? How do these testing methods account for chemicals that are
16 strongly bound to plasma proteins, lipids, etc? As described in Rotroff et al 2010³, there are
17 multiple methods to estimate PK behavior and since the results may differ based on which ones
18 are used and the underlying assumptions, how will decisions be made regarding which ones to
19 use, their accuracy and certainty? ; 4) are the proposed tests useful for chemicals that are stored
20 in humans (e.g., adipose tissue depot)?; 5) how are human exposure characterization and
21 biomonitoring data used in the prioritization and testing of chemicals? Although the tests are
22 designed to identify chemical hazards, if exposure is low or non-existent then how should the
23 chemical be prioritized?; and 6) incorporating human exposure data should be a high priority
24 since it is such an important component of risk assessment - a description of where these data
25 will come from, how they will be used (upper bounds, central tendency, etc) and if EPA will

² EPA/100/K-09/001 I, The U.S. Environmental Protection Agency’s Strategic Plan for Evaluating the Toxicity of Chemicals
March 2009, http://www.epa.gov/osa/spc/toxicitytesting/docs/toxtest_strategy_032309.pdf

³ 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. Incorporating human dosimetry and exposure into high-throughput *in vitro* toxicity screening. *Toxicol Sci.*
2010 Oct; 117(2):348-58. Epub 2010 Jul 16.

1 generate its own exposure data since the data likely only exist for a small fraction of the
2 chemicals to be tested; 7) how is the existing data from the scientific literature incorporated into
3 these AOPs and how is it curated to remain current.

4
5 The scientific acceptance of these approaches in a weight of evidence for decision-making will
6 depend on the accuracy, sensitivity, and specificity of the computational toxicity testing and true
7 human health effects. A transparent strategy for quantifying the endpoints that risk assessments
8 will be based on should be outlined. How would these considerations differ, or be the same, for
9 such EPA applications as chemical screening, prioritization, risk assessment, and green
10 chemistry? While relevance to humans is always important, ranking these applications in order
11 from highest to lowest in terms of the scrutiny with regard to human relevance may be as
12 follows: risk assessment, prioritization, screening chemicals and green chemistry.

13
14 Finally, the approaches for reaching out to the diverse EPA groups that could use CompTox data
15 and trying to understand what would make such data most useful for them are commendable and
16 should continue. Perhaps, in addition to providing individual scientists with opportunities to
17 spend time in the ORD labs to become familiar with the CompTox program, extensive remote
18 learning and training modules could be developed to reduce the cost and logistic challenges. This
19 may also serve to engage more key EPA scientists outside of the Research Triangle Park, North
20 Carolina area.

21
22
23 4. *How should the use of the CompTox program be effectively communicated to*
24 *stakeholders? How can the communication be enhanced?*

25
26 The Agency appears to be doing a very thorough job of communicating to stakeholders. This
27 communication is focused on two areas. First is to convey the importance of the approach and
28 the value of the strategy to stakeholders including the public. Many in the regulated community
29 have worked at developing computational toxicology models of various kinds, often quite
30 specific to their regulated products; they are, obviously, convinced of the strength of the
31 approach or they would not be perusing it. If stakeholders are brought along in a collaborative

1 fashion, they may be more likely to accept it. Second is to provide the data to the general public.
2 The website is relatively easy to navigate, but it would be useful to provide some information
3 about extracting relevant data. Beyond making the data available, possibly the most important
4 element of Comptox communication is to be transparent with respect to the limitations and
5 uncertainties in any particular endpoint in isolation and to provide a broader understanding about
6 what is known about a chemical's biological activity based upon CompTox data in association
7 with SAR, *in vivo* testing, etc. The stakeholders may need to have some summary statistics
8 about the results – perhaps along the lines of AOPs, with a transparent, easily accessible (e.g., on
9 a website) location for the details of the testing– even down to the actual raw data. Uninitiated
10 evaluators of large datasets are often impressed by the sheer scale of the amount of data and may
11 lose focus on the quality of that data. As ExpoCast develops, the web site should incorporate
12 estimates of exposure to chemicals and mixtures potentially stratified by age, gender, regions of
13 the country, population density (rural, suburban, urban), ethnicity etc.

14
15 Communication with epidemiologists and clinical investigators needs to be part of the process. It
16 may be difficult for some health scientists to decipher and to fully understand the potential
17 relevance of computational toxicology to human exposure and health effects. There needs to be
18 data generated by EPA (and collaborators) to demonstrate that the tests utilized are relevant to
19 human health effects, and to explain how they are relevant. In addition, it is critical to clarify in
20 what situations they may fall short and be inadequate. For instance, there is a higher level of
21 uncertainty for specific AOPs, outcomes and/or for specific classes of chemicals. Combining the
22 outputs with data on metabolism of the chemical in humans is essential. Finally, incorporating
23 biomonitoring data, exposure pathways, chemical source information and human activity
24 patterns on human exposure needs to be included.

25
26 The EPA should continue to partner with existing academic health science centers to get the
27 word out. The Agency can utilize existing relationships via community outreach and translation
28 cores. This would allow for the application of high-throughput analysis and predictive modeling
29 of CompTox data sets. The application of non-parametric models would allow for examination
30 of complex interactions between social, natural and built environments with regard to effects on

1 susceptible populations. The EPA may also benefit from more collaboration with international
2 agencies regarding data sources, access and technology transfer.

3 We commend EPA's Computational Toxicology Communities of Practice which is composed of
4 more than 300 people from over 50 public and private sector organizations that are interested in
5 the application of computational toxicology and exposure science to EPA's risk assessments.

6 This is a phenomenal tool for keeping up with technical issues that the EPA is dealing with and
7 addressing as a part of the CompTox program. As AOPs are developed, it would also be useful
8 for the Agency to develop partnerships with relevant professional societies or institutions. For
9 example, a group within the Agency developing an AOP on asthma would benefit from
10 developing a partnership with the American Lung Association or the NHLBI to access
11 physicians and researchers at the cutting edge of developing new knowledge in this field.

12 Some additional suggestions for further research regarding communication and achieving a
13 broader understanding of the potential contributions and limitations of these approaches are as
14 follows: One is an evaluation of the pesticide stakeholder dialog process by an independent
15 expert (group) in communication and stakeholder participation to see what can be learned from
16 that experience. The second is to pursue a mental model study that compares expert and public
17 understandings of how CompTox findings could be informative. The second study, in particular,
18 might identify structural reasons why there might be communication difficulties and how they
19 could be addressed.

20
21

22 **Other Issues**

23 Beyond the charge questions, below are some additional issues that have been identified that
24 EPA should consider as they develop the CompTox research program and as they move towards
25 implementation.

26 1) Are there clear goals for a screening or a prioritization effort? Is the use of this new
27 information making an improvement? Risk assessments are used in a variety of settings for a
28 variety of purposes; demands on the information base will necessarily be different; but are
29 there context-specific criteria for when particular types of information are informative

1 enough to be useful? Getting some of these structural issues resolved could be a useful
2 contribution of the CompTox program even before it is actively producing actionable
3 information. The Deepwater Horizon provides an example of a programmatic need –
4 provision of information in emergency or other fast-moving settings – for which guidance is
5 lacking. Clarification of screening and prioritization objectives is another example where
6 guidance is needed.

7 2) There is a need to delineate better what CompTox results might be able to contribute and
8 what they might not, both types of contribution and the extent to which they might
9 contribute. The delineation should refer to time: what contributions might be feasible over
10 the next few years, what will take longer. How much of the space of chemicals will be
11 covered, soluble? not too volatile? what sort of health effects? (e.g., if cancers are low
12 priority now, but should that continue indefinitely?) The identification of critical pathways is
13 an important step toward clarifying a number of key risk challenges – mixtures, interactions
14 with background exposures, existing conditions and susceptibilities – and it provides an
15 attractive possible link to CompTox findings, but are there risks that may be obscured or
16 ignored in this approach?

17 3) How well developed are EPA’s capabilities for synthesizing and using fragmentary and
18 incomplete information. For the near term at least, CompTox results will be quite limited
19 and their best use likely will be in combination with limited information from other sources.
20 The point of risk assessment, of course, was to be an approach to dealing with limited
21 information, but current practice tends to be both chemical-specific and to focus on particular
22 types of information. How far along is EPA in developing cross-chemical and multi-attribute
23 capabilities for risk interpretation? There is a future vision for CompTox that the data might
24 ultimately deliver a complete identification of critical pathways and a measure of the
25 response along them, but realizing such a vision is still remote. For some period of time,
26 perhaps indefinitely, the information will be fragmentary and new methods will be needed
27 for its interpretation. The primary challenges thus are transitional – how to build analytic
28 structures that can incorporate new kinds of information in incremental steps.

29 4) Typically analytic capabilities are considered, but it is important to also think about
30 institutional capabilities for developing, organizing, and using the information. Are data
31 resources constantly updated and expanded and are there ongoing improvements in

- 1 accessibility and analytic flexibility? Is there an institutional culture that identifies
2 opportunities for the use of new information and is vigilant to detect warning signals
3 concerning new issues and new difficulties? Is there good communication between groups
4 that might use the same or similar information and methods? Can the institutions develop
5 and support incremental changes? Can they enroll stakeholders and other governmental and
6 non-governmental organizations as supporters of such change?
- 7 5) Critical data for steps in the transition will only partly come from CompTox; those data must
8 be synthesized with other, more familiar types of information. Therefore, those data needs
9 and requirements for data quality must be addressed as well.
- 10 6) How will EPA handle the inevitable occurrence when future data from other researchers
11 employing *in vivo* or human studies contradicts the ToxCast data? As the science moves
12 forward, there may/will be results generated from *in vivo* and/or epidemiologic studies that
13 contradict or are not consistent with the CompTox results. This is of course an inherent
14 characteristic of science and occurs in instances apart from the CompTox program.
15 However, as inconsistencies occur how will EPA respond? What will be EPA's approach to
16 handling the comments and perceptions that are sure to arise regarding whether the
17 CompTox data were too conservative or missed the hazard for this chemical? What would
18 the implications be for the CompTox program and the use of its outputs? The public is
19 bombarded with studies that show a risk for chemical X, and then other studies later show no
20 risk, and then another wave of additional studies again showing a risk. The EPA needs to be
21 prepared for the shifting playing field since future data from *in vivo* and human studies will
22 not always be consistent with the CompTox results. The inconsistency that evolves over time
23 as new data are generated is not something EPA has control over (it is a core characteristic of
24 science), but EPA needs to develop a plan to address this as it will definitely occur and its
25 occurrence will accelerate as they roll out more of the results from their testing and begin to
26 use it for prioritization and risk assessment.
- 27 7) It is clear that effective communication will be essential to the long term use of CompTox
28 and ExpoCast findings.
- 29 8) Developing a community of scientists to provide feedback on ExpoCast in a parallel fashion
30 to ToxCast has developed.

31

1 Finally, an ongoing external advisory process would help in the institutionalizing of a long term
2 program built around the idea of incremental transformation. A perspective on the current scene
3 and prospects for the future can be obtained and constructive suggestions to EPA made. The
4 potential for longer term engagement by an external advisory committee should be considered.

5