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## FORUM

# The Vision of Toxicity Testing in the 21st Century: Moving from Discussion to Action

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Over the past year, a series on commentaries have appeared in the *Toxicological Sciences* Forum Series related to the 2007 National Research Council (NRC) publication, *Toxicity Testing in the 21st Century: A Vision and A Strategy*. The first article in the series provided an overview of the vision and was accompanied by an editorial by the three editors of *Toxicological Sciences*. During the past year, eight invited commentaries from the academic, industrial, and regulatory sectors have provided diverse perspectives on the vision, noted challenges to its implementation, and highlighted aspects of toxicity testing that were not addressed in the original NRC report. Here, we offer a summary of the main points raised by the commentators in tabular form, identify a number of common themes, and finish the series by providing our perspective on several key issues in charting the path forward to move from discussion to action.

**Key Words:** toxicity pathway perturbations; *in vitro-in vivo* extrapolations; 2007 NRC report on Toxicity Testing; adversity; risk assessment of environmental agents.

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Three years ago, the U.S. National Research Council (NRC, 2007) published a report entitled *Toxicity Testing in the 21st Century: A Vision and a Strategy*. The purpose of this report was to develop a long-range strategic plan to modernize the way environmental agents are tested for toxicity. In a previous editorial in the *Toxicological Sciences* Forum Series, Andersen and Krewski (2009) outlined the components of the NRC vision; the editors subsequently invited eight commentaries on this editorial (cf., Holsapple *et al.*, 2009). The present article provides a synthesis of the various commentaries and some thoughts on moving from discussion of the report to its implementation in practice.

## OVERVIEW OF COMMENTARIES

The eight commentaries covered a broad range of topics (Table 1). Several overarching themes were present. Two of the commentaries (Bus and Becker, 2009; Meek and Doull, 2009) were extremely cautious, even pessimistic, about any rapid change to the current toxicity testing methods without insuring that the scientific tools were fit for purpose and that the results could be appropriately applied beyond simply hazard identification. The pharmaceutical industry perspective (MacDonald and Robertson, 2009) highlighted some differences in the process of safety and risk assessment for environmental agents and for pharmaceuticals. The pharmaceutical industry has had more experience with mechanistic *in vitro* tests and high throughput screening and brings valuable experience tempered with some caution about setting overly high expectations for the proposed toxicity testing technologies. These three were categorized as “guarded to various degrees” in Table 1. Four others, from Hartung (2009), Hubal (2009), Chapin and Stedman (2009), and Boekelheide and Champion (2010), looked primarily at issues relating to the process by which the NRC vision could be implemented. Hartung (2009) focused on regulatory change needed to facilitate any change in testing, Chapin and Stedman (2009) outlined promises and challenges with human stem cell technologies, and Hubal (2009) noted the coordinate need for improved exposure assessment tools to complement the toxicity testing initiative. Boekelheide and Champion (2010) addressed the larger issue of how the results from a battery of *in vitro* assays and associated interpretive methodologies will be used to define “adversity.” These four commentaries are “guardedly optimistic” in tone. The commentary by Walker and Bucher (2009) provided a “one size is not likely to fit all” warning about toxicity testing needs with engineered nanomaterials. Table 1 highlights key points from each commentary.

**TABLE 1**  
**Summary of Commentaries on *Toxicity Testing in the 21st Century: Bringing the Vision to Life***

Commentary (authors)	Specific comments
<i>Original editorial</i>	
Toxicity testing in the 21st century: bringing the vision to life (Andersen and Krewski, 2009)	<p>Current toxicity testing paradigm cannot meet the challenge of evaluating the large number (some 100,000) environmental agents to which humans are potentially exposed</p> <p>NRC vision is based on understanding toxicity pathways and identification of critical pathway perturbations that can lead to adverse health outcomes in humans</p> <p>Critical pathway perturbations will be identified using suites of high throughput screen (HTS) assays based on human cells and cell lines</p> <p>A concerted effort on the part of the full scientific community will be required to bring the vision to life</p>
<i>Introduction to the forum series</i>	
The vision for toxicity testing in the 21st century: promises and conundrums (Holsapple <i>et al.</i> , 2009)	<p>Commitment to the three R's: replacement, reduction, and refinement</p> <p>Vision integrates state of the art mechanistic modeling and risk assessment approaches</p> <p>Current toxicity testing practices originated 40–50 years ago and are time consuming and expensive</p> <p>Boundaries of “adverse” and “adaptive” effects need to be clearly defined</p>
<i>Commentaries guarded to various degrees: Vision lacks specificity about how new toxicity testing results will be used and how the new results will be interlaced with past experience</i>	
Pragmatic challenges for the vision of toxicity testing in the 21st century in a regulatory context: another Ames test?...or a new edition of “the Red Book”? (Meek and Doull, 2009)	<p>Need to define what constitutes an adverse effect (cell homeostasis does not necessarily reflect an adverse outcome)</p> <p>NRC vision does not address short-term chemical risk management needs of regulatory agencies</p> <p>Need to integrate pragmatic aspects of risk management to meet progressive regulatory requirements</p>
Toxicity testing in the 21st century: a view from the chemical industry (Bus and Becker, 2009)	<p>Examine merits of tiered testing versus the use of a full battery of tests within the context of the NRC vision</p> <p>False positives and false negatives have different implications in the chemical and pharmaceutical industries</p> <p>NRC vision will test current default assumptions</p> <p>HTS allows for a range of relevant dosages to be evaluated</p> <p>Reservoir of current knowledge must be used</p> <p>Must establish homeostatic tolerance limits of chemicals</p>
Toxicity testing in the 21st century: a view from the pharmaceutical industry (MacDonald and Robertson, 2009)	<p><i>In vitro</i> and <i>in vivo</i> testing does not necessary predict adverse health outcomes</p> <p>Proteomics and metabolomics will greatly enhance our understanding of how chemical interactions can affect health risk</p> <p><i>In silico</i> chemical characterization will aid in the risk assessment process</p> <p>Use a hybrid approach including both old and new testing strategies until the NRC vision is validated</p>
<i>Guardedly optimistic commentaries: The vision has clear merits, but what else is needed to ensure that a change of this magnitude will be successful?</i>	
A toxicology for the 21st century—mapping the road ahead (Hartung, 2009)	<p>Focus on test strategies instead of individual tests: Several tests combined will reduce false positive rate</p> <p>Consider specificity and sensitivity of the test when setting thresholds for what constitutes an adverse effect</p> <p>Although the gold standard for validation of new toxicity tests are <i>in vivo</i> test results, a mechanistic standard would be more relevant</p> <p>Evidence that the new toxicity testing methods are superior to existing methods will be needed to motivate regulatory change</p> <p>Globalization of markets may pose an obstacle to the implementation of the NRC vision: International acceptance of the vision will be a prerequisite to its success</p> <p>Quality assurance will be essential to the success of the vision: will need stringent quality standards and documentation for the new types of toxicity tests</p> <p>Need to organize the transition to the new type of testing through communication and workshops</p> <p>Academia, industry, and government need to work together to implement the new vision for toxicity testing</p>

TABLE 1—Continued

Commentary (authors)	Specific comments
Biologically relevant exposure science for the 21st century (Hubal, 2009)	New tools to measure environmental exposures need to be developed to define hazard-exposure relationships within the context of the risk assessment process Range of doses that are relevant to real-world exposures must be used in HTS <i>in vitro</i> assays New NRC committee on exposure science in the 21st century will address outstanding issues in exposure assessment
Endless possibilities: stem cells and the vision for toxicity testing in the 21st century (Chapin and Stedman, 2009)	HTS assays, one of the main elements of NRC vision, require robust, stable abundant cell lines Stem cells may be advantageous because they can differentiate into any cell type and maintain their genotype; there is an unlimited source of these cells iPS stem cells derived from adult cells can be used to test sensitive phenotypes in the population Stem cells can differentiate into 3D spheroids that may emulate <i>in vivo</i> models
Toxicity testing in the 21st century: using the new toxicity testing paradigm to create a taxonomy of adverse effects (Boekelheide and Campion, 2010)	Regulatory health guidelines must clearly distinguish between adaptive and adverse responses Presents a sequential model of adverse effects: A series of “latent failures” (such as electrophilicity of the test chemical, irreversible toxicity pathway perturbations, abrupt dose-response transitions, and mitochondrial dysfunction) can lead to “active failure” of an adverse effect Development of a new paradigm to systematically analyze large reservoir of high throughput screening data called the “Toxicological Factors Analysis and Classification System (TFACS)” Presents TFACS framework for defining adverse effects based on three tiered categories: chemical characterization, toxicity pathways, and dose-response and extrapolation modeling Information mined from databases and analyzed by TFACAS framework will establish a <i>Taxonomy of Adverse Effects</i> <i>Taxonomy of Adverse Effects</i> will require national and international collaboration
<b><i>Commentary on challenges of novel environmental agents: The development of new chemistries will require attention to optimum test methods and may not easily be transferred from in vivo technologies to in vitro HTS assays</i></b>	
A 21st century paradigm for evaluating the health hazards of nanoscale materials? (Walker and Bucher, 2009)	Nanoparticles possess unique characteristics with respect to dose, surface area, and behavior in <i>in vitro</i> systems Some, but not all, classes of nanoparticles could be tested in HTS assays

### COMMENTARIES “GUARDED TO VARIOUS DEGREES”

Meek and Doull (2009) had the most pessimistic view of the ability of the scientific community to bring the new technologies described in the 2007 NRC report to bear in toxicity testing. They argued that the committee should have developed bridging strategies for moving from current practice to the proposed new toxicity testing paradigm. In their view, having such a plan in place early on would increase the likelihood of long-term success and reduce the chance that the proposal would alienate current practitioners. They also voiced a concern that agents tested in these *in vitro* batteries will produce multiple perturbations of pathways, as is now evident from phase I ToxCast assay results (Judson *et al.*, 2010). Without a clearly defined approach to categorize *in vitro* effects as beneficial, adverse, or irrelevant (normal variation), there is the concern that pathway perturbation results will not be credible as a risk assessment tool for the regulatory community. They recommended taking a first step that would relate early perturbations to apical endpoints in frameworks designed to systematically consider key events in modes of action and their subsequent implications for dose-response in risk assessment (cf., Meek, 2008). This intermediate step in implementation of any new testing process would be instrumental in advancing common understanding in both the toxicological research and

the risk assessment communities in potential appropriate application of data on early events in a toxicity pathway. Increasing experience gained in making these comparisons could then guide the transition from current practice to those proposed in the 2007 report. Other clear concerns related to the lack of consideration of other approaches, especially in Canada and Europe, to developing progressive regulatory strategies to address much larger numbers of chemicals within existing testing and risk assessment programs. Such initiatives increase throughput while maintaining the current primacy of animal testing for establishing hazard. Summing up their comments was the concern that the report might simply add a suite of *in vitro* tests but lack the tools needed to interpret the results for risk assessment purposes. This first commentary by Meek and Doull (2009) presented a warning volley regarding the possible downsides of moving toward what was seen as a vaguely delineated mechanistic approach to human health risk assessment, without a better definition of the transitional steps necessary to bring the vision to life.

Bus and Becker (2009) offered several similar cautionary notes from the perspective of the chemical industry. They emphasized that *in vitro* methods are unlikely to capture the broad range of intercellular and interorgan phenomena driving expression of whole animal toxicity results and that transition

to an *in vitro* testing system needs to be focused on identifying true human health risks with a higher degree of confidence than that associated with existing test systems. These new technologies are likely to produce many false positives while seeking to identify true human health risks through use of human cells. On a positive note, it was stressed that new technologies, such as those outlined in the NRC report, can evaluate larger ranges of dose and associated modes of action. In this manner, the tools may provide insights into refinements of current default assumptions and risk models. In their closing comments, they suggest that in order to effectively implement the NRC vision, we must tap the knowledge base from our long history of animal studies.

One point of clarification should be added. This commentary (Bus and Becker, 2009) stated that the express intent of the NRC vision was to replace live animal testing. Although minimization of animal use was seen as desirable, the committee was not focused on reduction in animal use as a main criterion in its deliberations. The vision that emerged, with limited animal use, was chosen because it represents the preferred option for toxicity testing with improved *in vitro* test methods, mode of action information using human cells as pertinent testing systems, and understanding responses over a range of concentrations.

The pharmaceutical perspective provided by MacDonald and Robertson (2009) brings a different viewpoint from an industry that has a primary focus on predicting and avoiding human toxicity from compounds that will be administered to humans at levels eliciting a beneficial response. This toxicity testing constituency has concerns regarding interspecies extrapolation but lesser concerns about extrapolations to low doses. The goal with pharmaceuticals is to predict toxicity at levels where there are biological effects in the majority of the patient population. MacDonald and Robertson (2009) offered comments on the inability of either *in vitro* or animal *in vivo* studies to be completely predictive of subsequent human toxicity and that a hybrid approach of *in vivo* test strategies with *in vitro* mechanistic methods will likely be required until the new vision is implemented. Overall, their comments show some enthusiasm for the concepts underlying the NRC vision but highlighted the need to inject a large dose of reality about prospects to deliver on the promise of new and emerging toxicity testing methodologies in the near term.

#### “GUARDEDLY OPTIMISTIC” COMMENTARIES

Hartung (2009), bringing long experience in developing alternative test strategies, noted that the vision appears to be an idea whose time has come and focused primarily on challenges in regulatory implementation once the technical aspects of the vision are achieved. He listed 10 challenges. Some are very practical. Challenge #3 (threshold setting) asks how the *in vitro* results will produce a value for regulatory action. Challenge #4 (what to validate new test against) emphasizes mechanistic

validation, noting that continued validation against animal studies will never overcome the inherent shortcomings of the present testing strategies. (This challenge resonates well with us. One of the most frequent questions we have been asked is how the NRC vision will be validated. Validation cannot be done against animal test results obtained at high doses that we are seeking to replace; rather, validation can only be achieved through an in-depth understanding of toxicity pathways, identification of critical pathway perturbations, and the demonstration that *in vitro* tests are able to identify those perturbations, with high sensitivity and specificity. Thus, validation of the NRC vision will not be done against an existing “gold standard,” but rather through a detailed mechanistic understanding of toxicity pathways, as envisaged by Hartung (2009).) Challenge #8 asks the broad question: “How to change with step by step developments becoming now available?” Two basic strategies are mentioned: (1) running two parallel approaches forward for comparison purposes in preparation for the transition or (2) take new problems and new opportunities and start the new paradigm with these technologies or endpoints. He cautions that either one has “the trap of just adding new patches without substantial change,” concluding that we will have to approach the transition in an organized fashion. Hartung’s (2009) paper has a figure with the steps necessary to arrive at a new approach to regulatory toxicology that deserves study by all of us who are interested in bringing these new test methods to the mainstream of regulatory risk assessment.

Chapin and Stedman (2009) discuss progress in stem cell biology and the application of toxicity testing tools based on stem cells for implementing the vision. They note two ways that stem cells may be used in toxicity testing: (1) by differentiation into cultures of “different” human cell types whose response to chemicals can be tested and (2) by evaluation of responses in their undifferentiated state (or during differentiation). They discuss the development of 3D-cultures (which they refer to as “tissue doppels”) from multiple cell types, including liver spheroids, and suggest how these systems may be used in toxicity testing in the future. They speculate:

“On the other hand, it may be true that in the final version of this testing scenario, we would not need to know how a toxicity will manifest but would only need to know which tissue doppels *in vitro* are sufficiently affected to pass over the threshold of change into toxicity. Perhaps, we will not need to reconstruct all the steps leading from reduced neuronal steroid sensitivity to increased ovarian steroid output to altered estrous cycle (persistent estrus) to infertility; eventually, it may be that seeing the neuronal change will be enough to flag a compound as potentially toxic and lead to its testing in animals. It is likely that *in vitro* testing would be designed to identify the boundaries of threshold responses; the population health

protection goal would then be to maintain corresponding human exposures below these levels.”

This high-level summary accurately captures the intended directions for toxicity testing in the NRC report.

Boekelheide and Campion (2010) provided the final perspective, discussing challenges of using *in vitro* rather than *in vivo* test results for risk assessment. The collection of new information will require a definition of apical endpoints to change to a biological, chemical, or mechanistically based endpoint based on *in vitro* systems. They propose a framework for defining adverse effects based on three tiered categories: chemical characterization, toxicity pathways, and dose-response and extrapolation modeling with information mined from databases and analyzed by a consistent framework to establish a *Taxonomy of Adverse Effects*. In the discussion, the paper provides an articulate overarching statement of a key goal for Toxicity Testing in the 21st Century:

“We all seek a mode of action-based molecular understanding of how the initiating events arising from the interactions of a toxicant with a living system produce adverse effects. One advantage of this new approach is a deeper and coherent appreciation of the contributing components that ultimately manifest as an adverse effect.”

Hubal (2009) emphasized that successful implementation of the NRC vision, including assay design and development of the necessary tools for interpreting the results of the new assays for purposes of human health risk assessment, is still only a step along the way to achieving comprehensive risk assessments for specific populations. A coordinated effort is also required to modernize exposure science in order to measure environmental exposures and insure that real-world exposures become a key component of risk assessments based on *in vitro* test methodologies. This recommendation reflects concerns of other commentators that toxicity pathway perturbations need to be interpreted both with respect to their relevance to a traditional adverse health response and with respect to their relevance for describing dose-response relationships for human populations.

#### COMMENTARY ON “CHALLENGES OF NOVEL ENVIRONMENTAL AGENTS”

Walker and Bucher (2009) warn that *in vitro* technologies may not be useful for novel environmental agents, such as nanomaterials, and that chemical characterization and the interrelated aspects of dosimetry for nanomaterials would require more attention than would be applied to most chemicals. In addition, physical characteristics of these materials may not be amenable to high throughput evaluations. The NRC committee did foresee challenges with new technologies that could require targeted *in vivo* testing and novel protocol development before *in vitro* test systems could

be developed and validated. High throughput assays are more likely to be useful for working with libraries of chemicals in order to evaluate structural attributes of compounds activating specific pathways. For any individual compound or small numbers of compounds, the need is not high throughput but assays that can be rapidly performed and interpreted to assess perturbations (reflecting possible hazards) and possible risks (i.e., determination of those hazards that are likely under specific human exposure conditions). The goal of the NRC report was to outline both toxicity testing and risk assessment tools that would ensue from results of the assays, not simply the endorsement of high throughput technologies.

#### RECURRING THEMES EXPRESSED IN THE COMMENTARIES

We are grateful to the editors and the authors for the thoughtful commentaries, even though some may have been provocative on our first reading. Despite the broad diversity of comments, there are a few general themes that deserve attention. Before having these final thoughts, it is necessary to recount the path from publication of the report in 2007 until the present. There has been and remains significant interest in the 2007 NRC report and its recommendations for modernizing the manner in which we conduct toxicity tests with environmental agents. Committee members have now provided over 75 presentations on the NRC report since publication (see Supplementary material for details). Our thoughts on the future steps in moving the vision forward have been affected by our service on the committee, by the efforts in report writing, by the continuing dialog with both interested and skeptical audiences, and by the contributions that are the focus of this article. We acknowledge an enormous debt to all our fellow committee members for their contributions to the formulation of the original vision for the future of toxicity testing set out in the 2007 NRC report. At the same time, it is difficult to completely dissociate our postcommittee experiences over the past 3 years in representing the toxicity testing report and simply talk about the intent in the original NRC document. With this caveat noted, we would like to discuss four recurring themes. (1) Because not all responses observed *in vitro* assays will be adverse, how will a determination be made as to which responses warrant attention from a risk assessment perspective? (2) Because *in vivo* responses frequently require multitissue interactions absent from *in vitro* testing assays, how can apical responses in intact mammalian systems be predicted on the basis of *in vitro* data? (3) The ultimate goal of risk characterization is the establishment of a recommended human exposure guideline, traditionally done by extrapolation of animal toxicity data to humans. Why the committee did not propose methodologies for deriving human exposure guidelines based on toxicity pathway perturbations? And, (4) how can such fundamental changes in the way we do toxicity testing

be achieved in a smooth efficient manner? Are such changes even possible?

### *Adversity*

The 2007 NRC report discusses a continuum ranging from subthreshold doses to moderate doses causing at most modest responses with adaptation, through to higher doses with perturbations that are likely to lead to adverse responses if they were present in an *in vivo* situation. At present, such dose dependencies are essentially ignored, with high dose-responses treated as if they will occur throughout a wide dose range. Thresholds are assumed for noncancer responses but subject to application of multiple uncertainty factors, all treated as if they are independent. The NRC report broadly outlined the role of nonlinear computational systems biology modeling of response pathway circuitry and network dynamics that underlie dose-dependent transitions and that will likely guide discussions on how we differentiate adaptive from adverse responses. These computational models are under development for a limited number of pathways, but the technology for toxicity pathway mapping and dose-response modeling is developing rapidly in the biomedical engineering arena. The contribution of the “dose-response and extrapolation modeling” component of the NRC vision, often overshadowed by the “toxicity testing” component, will be key in making decisions about adversity, dose-dependent transitions, and thresholds. The quantitative tools for these assessments will need to develop along with the experimental approaches for understanding pathway circuitry, pathway dynamics, and defining adverse levels of perturbation. The perspective by Boekelheide and Campion (2010) on adversity is particularly pertinent to this point because one of the authors (K.B.) was a member of the committee and takes a careful look at questions of adversity through a postcommittee lens.

### *Predicting In Vivo Results from In Vitro Toxicity Pathway Assay Results*

This question—will the *in vitro* methods predict *in vivo* responses—was not fully articulated in the original NRC report. To address this point, it is important to remember that the ultimate goal of toxicity testing is to prevent the occurrence of adverse health effects in human populations exposed to environmental agents. At present, this involves the identification of (usually high) levels of exposure that will lead to adverse health outcomes in animals, followed by extrapolation to exposures that are not expected to lead to adverse health effects in humans. The process expressed in the NRC report, followed to its logical conclusion, will be to avoid critical pathway perturbations: regulatory risk assessment will seek to restrict human exposures to levels corresponding to those that do not lead to excessive *in vitro* perturbations. Once an appropriate suite of high throughput *in vitro* assays has been developed, environmental agents capable of causing toxicity pathway perturbations would be rapidly identified. In the

future, the emphasis in toxicological risk assessment would shift toward the prediction of exposures that will not cause critical toxicity pathway perturbations and away from the present practice of identifying (high) levels of exposure in animals that lead to adverse health effects as the point of departure for establishing human exposure guidelines. (This change in mindset is not appropriate for pharmaceuticals, where the goal remains prediction of likely human responses to biologically active levels of drugs.)

In many ways, this redirection of thinking about managing the population health risks associated with environmental agents may be the most difficult from a regulatory perspective. Today, chemicals are labeled as toxic based on adverse health outcomes seen at high doses in animals. Once labeled as “toxic,” it becomes more difficult to remember that because of dose-dependent transitions, compounds will usually pose little or no risk to humans at ambient exposure levels. With the new approach to toxicity testing, evaluation of the physical and chemical properties of environmental agents, *in silico* evaluations of structure activity relationships, and pathway activation patterns identified *in vitro* might indicate that various end organ responses *could* occur at sufficiently high exposures. Control below some level consistent with the *in vitro* assay results and extrapolation modeling would then predict regions of exposure that would not demonstrate toxicity in humans. This change in perspective—from risk assessment based on high dose animal testing to risk avoidance based on results of *in vitro* assays—would represent fundamental refocusing of the interpretation of toxicity test results for inferring human health risks.

### *Setting Standards from Results of In Vitro Assays*

With an *in vitro* concentration in hand that has caused an “excessive” perturbation, how would this be transformed into a proposed acceptable tissue concentration and then to an appropriate environmental exposure guideline for humans? These are excellent questions, which the committee wisely avoided. The use of uncertainty factors, especially the practice of treating them as independent, and multiplying them together is controversial. The manner in which these decisions will evolve depends at least in part on the development of the assays and the computational modeling of pathway dynamics. This point was side stepped in the original NRC report and will need to be revisited as the tools and technologies mature.

### *How Can the Change from Current Practices to a New Paradigm Occur?*

This point was raised forcefully by Meek and Doull (2009). Our original perspective suggested taking the first steps by using prototype compounds for which a comprehensive toxicological database exists, including mode of action studies that indicate the toxicity pathway(s) involved with higher dose toxicity. These compounds could be tested in assays that query the known targets of the environmental agent. These prototype assays

would be test beds for the elucidation of toxicity pathways and the development of computational systems biology models for mechanistically motivated dose-response modeling, along with physiologically-based pharmacokinetic modeling models for *in vitro* to *in vivo* extrapolations. By taking advantage of compounds with well-developed *in vivo* databases, comparisons with the results of new test methods based on “21st century approaches” would be facilitated. This process could be repeated for several prototypes (Andersen, 2010) and the results and proposed midcourse refinements used “to organize the transition,” as recommended by Hartung (2009).

### SUMMARY

The NRC vision for the future of toxicity testing represents a paradigm shift in the manner in which the toxic potential of chemical substances will be assessed. The vision focuses on the identification of critical perturbations of toxicity pathways that may lead to adverse health outcomes in humans using modern scientific tools and technologies. A particularly important element of the vision is the use of suites of rapidly performed *in vitro* assays using human cells that will be amenable to scale-up for high throughput screening to process large numbers of chemicals in a matter of days or weeks at a wide range of doses, including those within the human exposure range. The vision has received strong initial endorsement from the scientific (Collins *et al.*, 2008) and regulatory (Cohen *et al.*, 2008) communities, and aspects of the vision have been incorporated into the recent U.S. Environmental Protection Agency *Strategic Plan for Evaluating the Toxicity of Chemicals* (EPA, 2009). The series of commentaries discussed in the present article represent a healthy and necessary discussion within the scientific community about the opportunities and challenges provided by the NRC vision for the future of toxicity testing.

The dialog within the scientific and regulatory communities on the NRC vision is just beginning (Stokstad, 2009). The risk assessment implications of the NRC vision were debated in a parallel series of commentaries appearing in *Risk Analysis* in 2009 in response to an editorial by Krewski *et al.* (2009). A forthcoming special issue of the *Journal of Toxicology and Environmental Health* (2010) includes 14 invited papers that discuss specific aspects of the NRC vision. These papers address issues relating to computational toxicology, physiologically based biokinetic and *in silico* modeling, exposure assessment, current and future practices in toxicity testing, risk assessment, and implementation of the NRC vision.

On one point, all the authors of the eight commentaries and the two of us agree fully. This adventure will require collaborations across various groups in toxicology, in cell biology, and in computational systems biology to bring this vision for the future of toxicity testing to life. The path forward will not be easy. It will require hard work, commitment to improving our

current test methods, and an ability to make midcourse changes as scientific advances in toxicity testing are realized and the interpretive tools needed to evaluate new toxicity test data mature. The larger question is whether the effort is worthwhile. Our opinion on this remains unchanged. Toxicity test methods need to make better use of human biology and mode of action information to adequately assess risks posed to humans at relevant exposure levels. In addition, with the large number of untested or inadequately tested environmental agents that lies before us, the change proposed in the NRC vision for the future of toxicity testing is desperately needed to provide public confidence that compounds in commerce have been adequately tested. We would like to conclude by expressing our sincere thanks to all the authors of the commentaries for their thoughtful reactions to the vision. We look forward to many more productive discussions on progress toward making the NRC vision a reality in the years ahead.

### SUPPLEMENTARY DATA

Supplementary data are available online at <http://toxsci.oxfordjournals.org/>.

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### REFERENCES

- Andersen, M. E. (2010). Calling on Science: Making “Alternatives” the New Gold Standard. *ALTEX* 27, 135–143.
- Andersen, M. E., and Krewski, D. (2009). Toxicity testing in the 21st century: bringing the vision to life. *Toxicol. Sci.* 107, 324–330.

- Boekelheide, K., and Campion, S. N. (2010). Toxicity testing in the 21st century: using the new toxicity testing paradigm to create a taxonomy of adverse effects. *Toxicol. Sci.* **114**, 20–24.
- Bus, J. S., and Becker, R. A. (2009). Toxicity testing in the 21st century: a view from the chemical industry. *Toxicol. Sci.* **112**, 297–302.
- Chapin, R. E., and Stedman, D. B. (2009). Endless possibilities: stem cells and the vision for toxicology testing in the 21st century. *Toxicol. Sci.* **112**, 17–22.
- Cohen, K. M., Crofton, K., Hartung, T., Krewski, D., Locke, P., and Stephens, M. L. (2008). Toxicity testing in the 21st century: better results, less use of animals. *Environ. Forum* **25**, 46–51.
- Collins, F. S., Gray, G. M., and Bucher, J. R. (2008). Transforming environmental health protection. *Science* **319**, 906–907.
- Environmental Protection Agency (EPA). (2009). *The U.S. Environmental Protection Agency's Strategic Plan for Evaluating the Toxicity of Chemicals*. U.S. Environmental Protection Agency, Washington, DC. Available at: <http://www.epa.gov/spc/toxicitytesting/>. Accessed July 1, 2010.
- Hartung, T. (2009). A toxicology for the 21st century—mapping the road ahead. *Toxicol. Sci.* **109**, 18–23.
- Holsapple, M. P., Afshari, C. A., and Lehman-McKeeman, L. D. (2009). Forum series: the “vision” for toxicity testing in the 21st century: promises and conundrums. *Toxicol. Sci.* **107**, 307–308.
- Hubal, E. A. (2009). Biologically relevant exposure science for 21st century toxicity testing. *Toxicol. Sci.* **111**, 226–232.
- Journal of Toxicology and Environmental Health*. (2010). *Part B* **13**, 51–384.
- Judson, R. S., Houck, K. A., Kavlock, R. J., Knudsen, T. B., Martin, M. T., Mortensen, H. M., Reif, D. M., Rotroff, D. M., Shah, I., Richard, A. M., et al. (2010). *In vitro* screening of environmental chemicals for targeted testing prioritization: the ToxCast project. *Environ. Health Perspect.* **118**, 485–492.
- Krewski, D., Andersen, M. E., Mantus, E., and Zeise, L. (2009). Toxicity testing in the 21st century: implications for human health risk assessment. *Risk Anal.* **29**, 474–479.
- MacDonald, J. S., and Robertson, R. T. (2009). Toxicity testing in the 21st century: a view from the pharmaceutical industry. *Toxicol. Sci.* **110**, 40–46.
- Meek, M. E. (2008). Recent developments in frameworks to consider human relevance of hypothesized modes of action for tumours in animals. *Environ. Mol. Mutagen.* **49**, 110–116.
- Meek, B., and Doull, J. (2009). Pragmatic challenges for the vision of toxicity testing in the 21st century in a regulatory context: another Ames test?. or a new edition of “the Red Book”? *Toxicol. Sci.* **108**, 19–21.
- National Research Council (NRC). (2007). *Toxicity Testing in the 21st Century: A Vision and a Strategy*. National Academies Press, Washington, DC.
- Stokstad, E. (2009). Putting chemicals on a path to better risk assessment. *Science* **325**, 694–695.
- Walker, N. J., and Bucher, J. R. (2009). A 21st century paradigm for evaluating the health hazards of nanoscale materials? *Toxicol. Sci.* **110**, 251–254.