

FORUM SERIES—PART VIII

Toxicity Testing in the 21st Century: A View from the Chemical Industry

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The rapid onset of the science and experimental tools of molecular biology and genomics has presented the toxicology community with an unprecedented opportunity to reexamine, and potentially significantly restructure, many of the decades-long principles and practices guiding translation of conventional animal toxicology data to scientifically informed assessments of adverse human health risks. Implementation of this energizing and transformational technology into toxicology has of course been recently catalyzed by the report of the U.S. National Research Council (NRC) report, *Toxicity Testing in the 21st Century: A Vision and a Strategy* (National Research Council, Committee on Toxicity Testing and Assessment of Environmental Agents, Board of Environmental Studies and Toxicology, Institute for Laboratory Animal Research, 2007a), and was overviewed in Part I of this Forum series (Andersen and Krewski, 2009). The NRC report outlined the promise that the new technologies could dramatically increase both the number of chemicals comprehensively evaluated as well as broadening and improving the human relevance of toxicity end points assessed. Such outcomes, if successfully achieved, would significantly improve and inform science-based health assessments on a broad range of environmental chemicals.

It has long been recognized that the ability to fully characterize the potential hazard properties of the many thousands of environmental chemicals associated with chemical production by conventional toxicity test methods is pragmatically constrained by laboratory, animal, financial, and expertise resource limitations. And, of course, there are yet many thousands more chemicals originating as by-products of other human endeavors or as natural substances in the environment, all of which cannot be ignored if we are truly interested in understanding the contributions of environmental

chemicals to adverse public health outcomes. The resource limitations associated with conventional toxicity testing are further emphasized when examining the testing resources necessary to develop pharmaceutical or pesticide products. Testing of these agents mandate use of an extensive and regulatory-proscribed core battery of sequential tests that cost millions of dollars per chemical and 4–5 years at a minimum to complete (and consume many thousands of animals).

The chemical industry, facing the challenge of testing many thousands of more chemicals (to which human exposures may already be occurring) relative to the limited numbers of drug and pesticide products, has generally applied a tier-based testing framework for evaluation of chemicals and by-products of chemical production (Becker *et al.*, 2007). The test protocols used are the same or very similar to those used in drug and pesticide evaluations; however, toxicity test implementation is sequenced using triggers to guide decisions on which tests of the total test battery are needed. Triggering decisions are driven by considerations of both observed toxicity in early-stage tests and/or information on potential margins of exposure anticipated or identified from substance use. Although the expressed intent of the NRC vision was to largely replace the need for live animal testing, the 21st century technologies might well be productively applied to further examining the scientific merits of tier-based testing strategies versus conduct of full test batteries.

Enthusiasm engendered by the promise of the transformational 21st century technologies to de-bottleneck resource needs for improving understanding of environmental chemicals hazards and risks has rightly led to calls to establish government-funded programmatic investments to facilitate effective and coordinated implementation of these new technologies into the future mainstream of toxicity testing and human risk evaluation. In an immediate follow-up to the *21st Century* report, the NRC report *Applications of Toxicogenomic Technologies to Predictive Toxicology and Risk Assessment* (National Research Council, Committee on Applications of Toxicogenomic Technologies to Predictive Toxicology, Board of Environmental Studies and Toxicology, Board of Life Studies, Division of Earth and Life Studies, 2007b) recommended funding a large-scale and

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TABLE 1

Differential Implications of False-Positive/Negative Outcomes of High-Throughput 21st Century Toxicity Testing Approaches for Pharmaceutical Compounds in Development Versus Environmental Chemicals Already Presenting Real-World Human Exposures

High-throughput responses	Developmental pharmaceuticals	Environmental chemicals
False positives	Abandon promising compounds Develop alternatives with less desirable properties	Unnecessary bans or deselection pressures on valued compounds Misdirect valued resources from problems of greater concern Deny introduction of productive "green chemistry" alternatives
False negatives	Delay decisions on drugs doomed to fail Human clinical trials backstop false negatives from toxicity testing	Delay action on chemicals of high human health concern Encourage introduction of "green chemistry" alternatives ultimately presenting unacceptable health risks

coordinated program aimed specifically at addressing key implementation issues associated with translation of toxicogenomic technologies into actual practice. The *Applications* report also outlined some of the key challenges as well as opportunities facing effective and efficient implementation of this new technology and included specific commentary on some of the important issues specifically associated with assessment of environmental chemicals.

Although the NRC reports provided important high-level insights of how the new technologies could serve as a foundational mechanism to reframe a modern era of toxicity testing, they did not (and reasonably could not be expected to) define a precise blueprint of how the objectives of the vision would or should be constructed. However, the NRC reports suggest that the road to achieving the objectives of the vision, the rewards of which will be high, will nonetheless be complicated, technically challenging, and likely filled with many potential turns and possible dead ends. That perspective alone mandates that research efforts to integrate these new technologies into the future practice of toxicology and risk assessment must involve an extraordinary level of creative and closely coordinated planning that is highly transparent and participatory to all segments of toxicology and risk scientists, i.e., academia, government, industry, nongovernment organizations, and ultimately the public.

One of the values of this Forum series is to capture a spectrum of diverse experiences and perspectives of toxicologists from differing sectors of our discipline, with the intent that these insights may further stimulate effective and efficient integration of these exciting new technologies into a world of improved toxicity and risk evaluations. The purpose of this Forum piece was to offer the perspective of the chemical industry on this issue, with our particular focus and experience with industrial and environmental chemicals.

PERSPECTIVES TO CONSIDER

Environmental Chemical Assessments have Different False-Positive/Negative Implications than New Chemicals Undergoing Closed Development Programs

The NRC *Applications* report (National Research Council, Committee on Applications of Toxicogenomic Technologies to

Predictive Toxicology, Board of Environmental Studies and Toxicology, Board of Life Studies, Division of Earth and Life Studies, 2007b) acknowledged that evaluation of new compounds in development in highly regulated and contained environments, e.g., pharmaceuticals and pesticides, presented different types of toxicity assessment and interpretative challenges relative to the world of industrial chemicals and other environmental substances. Specifically, the public health implications of either false positives or negatives is distinctly different for compounds within the contained environments of precommercial development processes as compared to chemicals that are already in widespread direct use by the public and/or are widely distributed throughout the environment as by-products of production processes, consumer uses, or pollutants associated with other human endeavors (Table 1).

For compounds in development programs, false-positive responses may mean that compounds with potential valuable health, societal, and/or commercial promise are unnecessarily abandoned. Such outcomes may result in ultimate introduction of substitutes that are not as effective in achieving commercial and/or societal expectations, or alternatively, cause continued use of existing materials possessing less than desirable properties, e.g., emerging "green chemistry" concerns. Conversely, false negatives may delay timely decisions to halt costly development on substances ultimately doomed to failure. In both of these cases, assuming the false negatives do not survive to commercial development, the immediate impact is largely to the institution developing the chemistry and/or associated products and technologies. In the specific case of pharmaceuticals, human clinical evaluations also further backstop development decisions associated with either potential false-negative or false-positive findings from preclinical toxicity evaluations, including those incorporating information from application of the new molecular/cellular technologies. Importantly, such clinical trial backstop options do not exist, for ethical reasons, for assessment of environmental chemicals.

Apart from contained development scenarios, environmental chemicals present an entirely different set of health implications associated with false-positive or false-negative testing outcomes. Because many of these chemicals are already in the real-world environment, false-positive responses can result in

ill-informed regulatory or other public responses, e.g., product bans or deselection pressures, resulting in society being denied continued access to valued agents. In addition, false positives may misdirect valued toxicological and other resources from problems ultimately of much higher public health concern. False negatives are equally a concern in that they may delay or even eliminate actions that in fact should be higher priorities for protecting public health.

The false-positive/negative issues uniquely associated with environmental chemical demand that any transition to a future testing paradigm based predominately on nonwhole-animal testing be implemented with great caution. Such a transition, if it is to be successful, must be strongly focused on identifying true human health risks with a higher degree of confidence than that associated with existing test systems. It will always be necessary to evaluate relevance, reliability, sensitivity, and specificity of advanced high-throughput molecular screening and computational profiling methods prior to regulatory acceptance so that regulatory agencies, the regulated community, and the public have sufficient confidence in the decisions based on such methods. While traditional structures for conducting method validation and demonstrating model predictivity may not be practical, approaches such as those discussed by the National Research Council, Committee on Applications of Toxicogenomic Technologies to Predictive Toxicology, Board of Environmental Studies and Toxicology, Board of Life Studies, Division of Earth and Life Studies (2007b) with respect to validation of toxicogenomic technologies as well as practices embodied in the Organization for Economic Cooperation and Development (OECD) principles and guidance for the validation of quantitative structure activity relationships (OECD, 2007) and evidence-based toxicology (Guzelian *et al.*, 2005; Hoffmann and Hartung, 2006) should be considered.

The emergence of “green chemistry,” as a discipline devoted to promoting development of chemical products and processes that reduce or eliminate the use and generation of hazardous substances, carries with it a need to conduct side-by-side comparisons of hazard (and risk) profiles across a series of molecules with comparable beneficial uses. These comparisons would, theoretically, permit selection of the least hazardous (and risky) molecule(s). Such comparisons, however, will require a sufficient degree of confidence in the hazard profiles of the substances being compared. As noted above, it will not be efficient or cost-effective to conduct extensive and complex *in vivo* longer-term toxicity tests for all possible “green” molecules. So, if green chemistry is to have a substantial impact, there is a pressing need for more efficient tools to provide “data” on toxicity end points of concern and to support science-based decisions to select the “greenest” molecules in terms of potential hazards and risks. The approaches outlined by the *21st Century Report* and now under development by Environmental Protection Agency (EPA), National Institute of Environmental Health Sciences, and National Institutes of

Health (Collins *et al.*, 2008) as well as researchers at the Hamner Institute (Thomas *et al.*, 2007) have great promise in increasing the efficiency of selection. But again, as discussed above, the confidence in green chemistry decisions based on such methods will be dependent on demonstration of the relevance, reliability, and predictivity of the methods used to generate the “equivalent” hazard profile information.

Any New Testing Paradigm must Adequately Account for Complex Biological Events Driving Expression of Toxicity

In an earlier commentary in this Forum series, our pharmaceutical colleagues MacDonald and Robertson (2009) noted the distinct challenge for the mostly *in vitro* testing paradigm proposed in the NRC vision is for it to adequately encompass the broad range of intracellular and intraorgan and interorgan pharmacokinetic and pharmacodynamic phenomena driving expression of whole-animal toxicity responses. These concerns are equally shared by environmental chemicals, and the many examples of such complexity in the toxicology literature are far too numerous to productively enumerate here. Thus, the expectation that any battery of *in vitro* technologies will be able to adequately replicate such biological and toxicological complexity is likely to prove an exceedingly difficult challenge. This is not to say, however, that marrying the new technologies with modified approaches to whole-animal toxicity testing, e.g., using these technologies to eliminate lifetime bioassays or other rodent toxicity studies, might be one of the major “turns in road” associated with implementation of the vision. Exactly where such future forks in road decisions will take toxicology is as yet uncertain but the end objective must be to develop toxicity evaluation programs to better inform decisions on potential adverse human health outcomes than conventional toxicity testing and risk assessment practices now in practice.

Integrating the Expanding Body of Knowledge on Human Exposures to Environmental Agents is a Key Opportunity for Implementation of the Vision

Over the last several decades, the advent of government regulatory and industry product stewardship practices has significantly reduced the concentrations of many chemicals in the environment. For the most part, these efforts have increasingly distanced the doses used in conventional environmental chemical toxicity testing from human exposure-doses actually encountered in the real world. This growing disparity, and its importance to the future of toxicity testing, was noted 10 years ago by the Society of Toxicology Risk Assessment Task (Conolly *et al.*, 1999). The Society of Toxicology Task Force stated: “The relevance of using doses that are many multiples of conceivable human exposures. . . is, at most, quite dubious. [and] . . . the predicted risks may have little or no relationship to risk in the real world.” A distinct advantage of the new technologies is their ability to rapidly and relatively efficiently explore a much wider-range of dose and response

and particularly to explore the shape of the dose-response curve at doses/concentrations much more reflective of real-world human exposures to environmental chemicals.

Examination of a wider, and particularly a lower, range of chemical dose will afford the opportunity to significantly increase the human relevance of many environmental chemical assessments. Interpretation of low-dose effects observed in *in vitro* or other high-throughput systems will be significantly facilitated if accompanied by contextual relationships to both no-effect and effect level doses reported in whole-animal toxicity tests. Although the advent of sophisticated physiologically based pharmacokinetic and other exposure-response models is significantly advancing these objectives, there remains an important opportunity to routinely assess internal dosimetry in animal bioassays of environmental chemicals. All too often existing toxicity studies of environmental chemicals express dose simply as external milligram per kilogram or parts per million when in fact the interpretive value of dose information really lies in what is delivered to the systemic circulation and target tissues. With the relatively recent advances in analytical sciences technologies, there is a distinct opportunity for capturing this vital information with little modification to existing toxicology studies (Saghir *et al.*, 2006). In addition, when animal internal dosimetry findings are integrated with the rapidly expanding data emerging from human biomonitoring programs and further with dose-response information from high-throughput technologies, the collective body of information will be of much great value in informing future health risks. These types of opportunities have recently been illustrated in efforts to develop "Biomonitoring Equivalents" assessments of environmental chemical exposures. Such efforts, which bridge actual human exposures to doses in toxicity test systems and current regulatory exposure standards, will further define the strategic path for efficient selection of environmental chemicals deserving of more detailed health assessments (Hays *et al.*, 2007; Meek *et al.*, 2008). Biomonitoring equivalents can serve to "anchor" exposure concentrations used in high-throughput and high-content screening to concentrations in humans that correspond to meaningful health risk assessment benchmarks such as Reference Doses (Aylward and Hays, 2009). Thus, there is little doubt that application of high-throughput technologies to dose-response evaluations at environmentally relevant exposures will translate to better informing the potential for adverse health outcomes in humans.

The future focus on toxicity evaluations at low environmentally relevant doses must be accompanied, however, by enhanced attention to what represents an "adverse" response, a concern of particular emphasis for environmental chemicals for which large segments of the population are already exposed to. The NRC *Vision* articulated the opportunity for emerging technologies to rapidly interrogate "toxicity pathways" which in fact are normal physiologic and biochemical pathways. We believe that toxicity pathway terminology must be carefully employed within the toxicological community in order to avoid

overly precautionary reactions to environmental chemicals. Past experiences have strongly suggested that if the future outputs from *in vitro* or other high-throughput technologies are simply described as affecting toxicity pathways, an immediate inference is that such chemically induced effects must be adverse. As well appreciated by toxicologists, many such perturbations, dependent on dose, may represent normal and health homeostatic responses to low-level chemical interactions with cellular systems, as has been highlighted in an earlier commentary by Meek and Doull (2009). While some have referred to an "exposure-adverse effect continuum" in reality, it is a "discontinuum." A discontinuum is a more accurate description because as long as low doses cause responses that remain within "homeostatic tolerance limits of the normal state," then no adverse effects would be expected. This would apply to even the most sensitive life stages and to individuals with increased susceptibility. Therefore, there is a pressing need for more research using systems biology constructs of toxicity pathways to obtain knowledge on the "range and tolerance limits" of normal homeostatic responses and the dose-dependent transitions, which shift such systems from the normal state to first an adaptive state, and then at still higher doses, to a state reflective of adverse effect(s).

The New Technologies Should Not be Solely Focused on Predicting Toxicity Outcomes, but also Offer Enormous Opportunity to Re-examine and Experimentally Test Default Assumptions Underpinning Environmental Chemical Risk Assessments

Many of the human health risk assessments of environmental chemicals are primarily based on outputs from animal toxicity studies. A central component of risk assessment is the scientifically challenging effort of extrapolating results from high-dose animal toxicity studies and potential interspecies, strain, or sex differences to human health risks in man. Lacking a necessary understanding of the critical biology underpinning the toxicity responses, risk assessors routinely use default assumptions or "uncertainty" factors to correct for the absent information. These factors can range from fractions or multiples of "10" to the assumption that some chemical responses are linear with no threshold. The application of defaults, and the surrounding scientific and policy debates on their application, is often a root cause of lengthy and frustrating delays in implementing scientifically sound decisions on environmental chemicals of concern (National Research Council, Committee on Improving Risk Analysis Approaches Used by the U.S. EPA, Board of Environmental Studies and Toxicology, Division of Earth and Life Studies, 2008a).

The new 21st Century technologies offer an exciting opportunity to experimentally test the scientific validity of many of the currently used default assumptions. As outlined in both the NRC *Applications* report (National Research Council, Committee on Applications of Toxicogenomic Technologies to Predictive Toxicology, Board of Environmental Studies and

Toxicology, Board of Life Studies, Division of Earth and Life Studies, 2007b) and in a joint SOT-Society of Toxicology and Chemistry Pellston Conference report (Bus *et al.*, 2007), those explorations offer the promise of direct replacement or refinement of the commonly used defaults with human-relevant biological and toxicological information. The toxicology community is already well started on constructing the road of how this science may impact the replacement/refinement of key default assumptions. Some examples include:

1. Naciff *et al.* (2005) have demonstrated the potential value of genomic analyses to explore the shape of the dose-response curve for endocrine disruptors and particularly to more comprehensively test for the presence or absence of low-dose mediated nonmonotonic responses. Similar opportunities exist to more robustly examine the potential for genotoxic agents to exhibit biologically based thresholds, a default significantly impacting risk evaluations of many environmental chemicals (Pottenger *et al.*, 2007). The systems biology approach outlined in the NRC *21st Century Vision* (Andersen and Krewski, 2009; National Research Council, Committee on Toxicity Testing and Assessment of Environmental Agents, Board of Environmental Studies and Toxicology, Institute for Laboratory Animal Research, 2007a) affords an extremely valuable opportunity to refine or replace extrapolation defaults with biologically and experimentally based information.

2. In an earlier commentary in this Forum series, MacDonald and Robertson (2009) noted that the new technologies are powerful tools in building rapid and more comprehensive understanding of chemical modes of action for pharmaceuticals. These same opportunities apply to environmental chemicals and likely present one of the greatest immediate and long-term opportunities to replacing defaults with actual science in environmental chemical risk assessments. The high-throughput technologies clearly have the ability to rapidly inform high dose, cross-species, and other mode of action extrapolation issues current challenging toxicology and risk assessment.

3. A clearer understanding of specific genetic influences on toxicity expression illuminated by genomic technologies has catalyzed development of improved animal models, which more closely mirror human responses to environmental chemicals. Availability of humanized mouse models specifically tailored to chemical modes of action now permits thorough analysis of dose-response, particularly in the low-dose region, of environmental chemicals that could not otherwise be ethically conducted in humans. Such efforts can provide important empirical experimental insights into the impact of genetic polymorphisms as determinants of low-dose responses to environmental chemicals. Availability of paraoxonase 1 humanized mice is one example of how such *in vivo* models can significantly improve understanding of human risks associated with low-dose exposures to neurotoxic organophosphate chemicals (see National Research Council, Committee on Applications of Toxicogenomic Technologies to

Predictive Toxicology, Board of Environmental Studies and Toxicology, Board of Life Studies, Division of Earth and Life Studies, 2007b, for overview).

4. Knowing that humans are continually exposed to large numbers of environmental chemicals, the question of “how to add” the toxicity potential of mixtures, particularly at relevant environmental doses-exposures, is a growing human health focus (Bus *et al.*, 2007; National Research Council, Committee on Applications of Toxicogenomic Technologies to Predictive Toxicology, Board of Environmental Studies and Toxicology, Board of Life Studies, Division of Earth and Life Studies, 2007b, 2008b; Teuschler *et al.*, 2002). The power of the new technologies to rapidly and comprehensively examine the full range of dose-response and associated modes of action will likely provide significant insights into scientifically defensible refinements of default assumptions and risk models applied to mixture risk assessments. An important perspective that must be factored into such approaches, however, is the fact that the largest exposures to “environmental” chemicals comes from the myriad of natural dietary chemicals present in fruits, vegetables, and other foods. Since natural chemicals possess many if not all the toxicity properties of anthropogenic compounds, a key challenge (and opportunity!) will be to determine how the new technologies might better differentiate “healthy” from “adverse” and to clarify how the added stressors of anthropogenic environmental chemical mixtures can be more confidently teased from background influence of natural dietary chemicals (Mattsson, 2007).

Closing Observations

The advent of the tools and technologies associated with *21st Century Vision* offers a “once in a generation” opportunity for toxicologists to initiate a significant reframing of a toxicity testing and risk assessment paradigm that has been only incrementally refined over multiple decades of experience. Although there is enormous enthusiasm to introduce this strategic vision into the mainstream of toxicity evaluations as rapidly as possible, that enthusiasm must be tempered with the understanding that those same many decades of toxicology experience and practice have constructed a huge reservoir of knowledge that must be tapped for effective implementation of the *Vision*. Perhaps most importantly, the new technologies now afford significant opportunities to more richly understand the toxicological significance of the foundational mantra of toxicology, “The dose makes the poison,” and how this applies across the range of life stages and host factors. With this standard as the beacon illuminating the twists and turns of the road ahead, toxicologists can be confident that implementation of the NRC *21st Century Vision* is indeed headed in the right direction.

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