

Developments in Chemical Toxicity Testing

EPA relies on toxicology studies for its regulatory decision making, but traditional toxicology testing has led to slow and expensive chemical-by-chemical analysis. New developments promise to revolutionize toxicity testing, with huge potential impacts on EPA regulatory programs.

I. Introduction

Information and models regarding the potential human toxicity of chemicals are perhaps the most important ingredients in regulatory decisions regarding chemicals management. Such regulatory decisions can have substantial impact on the bottom lines of chemical companies as well as on the well-being of persons exposed to the affected chemicals, so it is no surprise that scientific and political controversies regarding toxicity testing have often been prolonged and intense. However, the basic structure of system for providing toxicity information from animal testing has changed only very slowly—until recently.

With the advent of new sciences such as informatics and genomics and the development of new high-throughput testing techniques, the Environmental Protection Agency (“EPA”) and regulated entities alike are poised for a revolution in toxicity testing. How this scientific and technological revolution will ultimately impact screening, prioritization, risk assessment, and regulation of chemicals, particularly in light of the intensifying debates over the Toxic Substances Control Act (“TSCA”), will depend in part on the resolution of a number of key questions and on the funding and pace of implementation of the new toxicity testing methodologies.

II. Background

Toxicity studies generally involve testing whole, living animals (“*in vivo*” testing). They are based on observing adverse health outcomes in large numbers of laboratory animals that have been exposed to relatively high doses of specific chemical substances. The observed outcomes are then extrapolated to humans using uncertainty factors. This basic approach is decades old. It has been only gradually modified by altering existing tests or adding new tests to account for new concerns and scientific knowledge, despite great advances in these areas, such as new techniques for testing outside a whole, living animal (“*in vitro*” testing). The *in vivo* testing approach has been increasingly criticized as time-consuming, expensive, ethically controversial due to its heavy reliance on test animals, insufficiently reliable, and altogether inadequate in its resulting coverage of chemicals, chemical mixtures, outcomes, and complexities. In addition to *in vivo* testing, EPA uses structure-activity relationship (“SAR”) analyses to estimate the properties of some chemicals based on the properties of other chemicals with similar structure, but the reliability of these analyses is limited by their base data sets and by the complex and somewhat hypothetical nature of the models, particularly for health endpoints.

Moreover, the growing gap between EPA’s information needs for its increasingly complex regulatory mandate, on one hand, and the limited available toxicity data and testing

capacity, on the other, is an important impetus for calls for reform of TSCA,¹ the keystone law regulating industrial chemicals in the United States. Critics of TSCA point to the more than 80,000 chemicals on the TSCA Inventory, for many of which there is limited or no toxicity information.² One legislative proposal to overhaul TSCA, the Kid-Safe Chemicals Act (“KSCA”),³ likely to be reintroduced in 2009, would specify minimum health and safety data that would have to be generated at great expense by companies for all new or existing chemicals.

A number of important developments in toxicology techniques have the potential to change the shape of the chemicals policy debate by increasing the efficiency and reliability of toxicity testing. Research in biology, medicine, data processing, and related fields has rapidly advanced. It provides the groundwork for a dramatic revolution in chemical toxicity testing and modeling. Many of these remarkably rapid developments in toxicity testing are being paralleled by advances in ecological and environmental impact testing and modeling, which are beyond the scope of this update but which implicate many of the same benefits and concerns for stakeholders.

III. Developments

A. The National Research Council’s Vision of a Transformation of Toxicity Testing in the 21st Century

In 2004, EPA, supported by the National Toxicology Program (“NTP”) of the National Institute of Environmental Health Sciences (“NIEHS”), commissioned the National Research Council (“NRC”) to provide a report on the long-term advancement of toxicity testing of environmental contaminants in light of innovations in computational and molecular techniques. EPA specifically directed the NRC to address the often conflicting goals of providing both breadth and depth (including improved assessments of key exposures and outcomes), while using time, money, and laboratory capacity more efficiently and also promoting animal welfare. The NRC issued its report, *Toxicity Testing In The 21st Century: A Vision And A Strategy*, in 2007.⁴

While the NRC report recognizes that decision-making contexts will influence the shape of toxicity testing for various sets of risks, the core changes it suggests would be applicable to the majority of toxicity assessments:

- The NRC vision would generally replace analysis of ultimate disease end-points in whole animals with analysis of upstream perturbations in cellular response pathways, or “toxicity pathways.” Perturbations in these cellular response pathways can cause adverse health effects if they are of sufficient duration and magnitude to overcome homeostatic (i.e., equilibrium-maintaining) processes.

¹ 15 U.S.C. §§ 2601-2692.

² See, e.g., RICHARD DENISON, ENVIRONMENTAL DEFENSE, NOT THAT INNOCENT: A COMPARATIVE ANALYSIS OF CANADIAN, EUROPEAN UNION AND UNITED STATES POLICIES ON INDUSTRIAL CHEMICALS IV-1 to IV-32 (2008), available at http://www.edf.org/documents/6149_NotThatInnocent_Fullreport.pdf.

³ S. 3040, 110th Cong. (2008); H.R. 6100, 110th Cong. (2008).

⁴ NRC, TOXICITY TESTING IN THE 21ST CENTURY: A VISION AND A STRATEGY (2007), available at http://www.nap.edu/catalog.php?record_id=11970.

- The NRC vision therefore initially depends on finding and analyzing a suite of these toxicity pathways using cell biology, functional genomics,⁵ systems biology,⁶ bioinformatics,⁷ and other modern biology techniques.
- Once such pathways have been sufficiently identified, the effects of chemical substances on the toxicity pathways would be efficiently tested via high-throughput, robot-assisted *in vitro* screening assays, based on similar assays developed by the pharmaceutical industry. Ideally, these assays would use human cells from stem cell lines, but could also use lower-order animals (e.g., fish or worms instead of rodents) and some isolated molecular targets.
- Targeted and improved whole-animal testing would continue to provide important information, especially until more reliable prediction of *in vivo* metabolism from *in vitro* systems could be attained.
- Dose-response relationships based on expected adverse outcomes from conditions of *in vivo* exposure would then be defined and modeled using computational systems biology; such modeling would include sensitive subpopulations.
- Finally, population-based studies and human exposure data (including biomonitoring) would add essential insight to the other steps of toxicity testing.

This thoroughly revised mechanism-based approach presents a number of advantages. Most notably, the assays could characterize dose-response relationships far more quickly and cost-efficiently. The increase in efficiency would allow for a greater number of chemicals to be addressed under existing regulatory programs. It would also allow for each chemical assessment to account for complexities such as interaction effects and the susceptibility of sensitive subpopulations due to disease, developmental stage, or genes. Reliability would also be expected to increase: first, the assays could utilize a much broader range of doses, and second, extrapolations to low environmental doses and across species would present fewer problems. However, if use of new methods outpaces the understanding of such methods and their relation to real world outcomes, the anticipated “deluge of data” could lead to excessively conservative risk assessments.⁸

B. EPA’s Policy Implementation of the National Research Council Vision

The innovative vision described above presents extraordinary practical challenges. Questions abound concerning many toxicity pathways, their dose-response behaviors, and their relationships to downstream disease responses. Cellular assays cannot yet account for the complexities of whole-human metabolism. The new approach demands better understanding of the relationship between *in vitro* and expected *in vivo* concentrations (for example, using

⁵ Functional genomics is focuses on understanding the function and regulation of gene sequences and their products.

⁶ Systems biology is an interdisciplinary field focused on integrative, rather than reductive, study of complex interactions among parts of biological systems.

⁷ Bioinformatics applies information technology and advanced computer science to manage and analyze large amounts of biological and molecular data.

⁸ See American Chemistry Council Long-Range Research Initiative, Research Strategy 2009-2015: Modernizing Approaches to Chemical Risk Assessment (2009), available at http://www.americanchemistry.com/s_acc/bin.asp?CID=1389&DID=5074&DOC=FILE.PDF.

physiologically-based pharmacokinetic (“PBPK”) models). Additionally, technical aspects of the assays still need to be addressed to account for the wide diversity of chemical substances not yet tested. The NRC report suggested that implementing its recommended toxicity testing program would require an investment of hundreds of millions of dollars over one to two decades, a level of funding that is well above even the combined resources of EPA and similarly situated science agencies.

Despite the high hurdles, EPA is moving forward implementing NRC’s vision. EPA’s peer-reviewed *Strategic Plan for Evaluating the Toxicity of Chemicals* was released in March, 2009.⁹ EPA’s eight strategic goals build on the recommendations of the NRC report:

- Toxicity pathway identification and *in vitro* assay development;
- Chemical prioritization;
- Development of integrated inventories (“knowledgebases” as opposed to databases) of toxicity pathways, plus complementary effort for environmental exposure pathways;
- Development of virtual tissues, organs, and systems that would work at multiple scales, developed by knowledge-discovery and computer simulations and tested fully;
- Proof of concept efforts via human evaluation and quantitative risk assessment;
- Operational transition, including new guidance to ensure consistency of approaches within and outside EPA over the gradual course of adoption of the new vision;
- Organizational transition, e.g., hiring and training; and
- Outreach to the public and stakeholders, emphasizing transparency and involvement.

The *Strategic Plan* states an expectation that full implementation will be an “iterative and long-term effort . . . likely encompassing more than a decade.”¹⁰

Related efforts to implement the NRC report’s recommendations are also moving forward. As noted in the *Strategic Plan*, EPA’s Office of Research and Development (“ORD”) signed a memorandum of understanding with the NTP and the National Institutes of Health (“NIH”) Chemical Genomics Center (“NCGC”)¹¹ in January, 2008 to coordinate efforts to develop new test methods in support of the NRC vision.¹² The collaboration is known as “Tox21.” It aims to develop predictive models through robotic testing at the NCGC, workshops,

⁹ EPA SCIENCE POLICY COUNCIL, THE U.S. ENVIRONMENTAL PROTECTION AGENCY’S STRATEGIC PLAN FOR EVALUATING THE TOXICITY OF CHEMICALS (2009), available at http://www.epa.gov/osa/spc/toxicitytesting/docs/toxtest_strategy_032309.pdf.

¹⁰ *Id.* at 7.

¹¹ NCGC (2009), <http://www.ncgc.nih.gov/>. “Genomics is the comprehensive study of the genetic information of a cell or organism. This includes the number of genes in an organism, the function of specific genes, the influence of one gene on another, and the activation and suppression of genes.” Archon X PRIZE, *What is Genomics?* (2009), <http://genomics.xprize.org/discover>.

¹² Memorandum of Understanding on High Throughput Screening, Toxicity Pathway Profiling, and Biological Interpretation of Findings (2008), available at <http://www.niehs.nih.gov/news/releases/2008/docs/ntpncgcepmou.pdf>.

focus groups, and public participation.¹³ Moreover, “[i]n preparation for a revolution in toxicity testing,” the NRC and EPA sponsored a scientific symposium in May, 2009 entitled “Toxicity Pathway-Based Risk Assessment: Preparing for Paradigm Change.”¹⁴

C. EPA’s Technical Programs

While NRC’s vision and EPA’s *Strategic Plan* are transformative, EPA has also been incrementally laying the groundwork for many of the recommended technical improvements for years, even prior to the release of NRC report. For example, several of the strategic goals in EPA’s plan expand on ToxCast™,¹⁵ the advanced toxicology program that EPA launched in 2007. The ToxCast program combines high throughput screening techniques, computer modeling, and data compilation in order to enhance EPA’s ability to prioritize chemicals. ToxCast has begun by conducting assays on several hundred well-characterized chemicals (mainly pesticides), creating a database within the existing Aggregated Computational Toxicology Resource (“ACToR”). The database created from the assays is used to identify toxicity pathways and create predictive capacity. ToxCast will then use that information as well as information from outside sources to screen additional chemicals. Thereafter will come further evaluation and validation of the predictive bioactivity signatures developed from the first round of assays, and refinement of ToxCast as a high-throughput tool for screening and prioritizing chemicals. EPA recently held the first ToxCast Data Analysis Summit, where researchers presented analyses based on the Phase 1 ToxCast dataset.¹⁶ EPA is also engaged in two related high-level research programs to develop sophisticated virtual models of the human embryo (v-Embryo™¹⁷) and liver (v-Liver™¹⁸).

On a more basic level, because animal testing data are lacking for so many chemicals and endpoints, EPA already uses computer models in making regulatory decisions as well as in screening for research prioritization. SAR analysis uses computer models to predict the relative toxicity and unknown properties of a chemical from its physical characteristics, based on knowledge of other comparable chemicals. Quantitative Structure-Activity Relationship (“QSAR”) analysis operates similarly but uses statistical regressions. Both have been used for many years in reviewing pre-manufacture notices under TSCA. The proposed pathway-based assays and associated models would go far beyond the (Q)SAR models, however, in their level of complexity and in the intended reliability of final results.

¹³ Christopher Austin, Robert Kavlock & Raymond Tice, *Tox21: Putting a Lens on the Vision of Toxicity Testing in the 21st Century*, ALTTOX (2008), available at <http://www.alttox.org/ttrc/overarching-challenges/way-forward/austin-kavlock-tice/>.

¹⁴ National Academy of Sciences Standing Committee on Risk Analysis Issues and Reviews, Symposium on Toxicity Pathway-Based Risk Assessment: Preparing for Paradigm Change (2009), http://dels.nas.edu/best/risk_analysis/symposium.shtml.

¹⁵ EPA National Center for Computational Toxicology, ToxCast™ Program (2009), <http://www.epa.gov/ncct/toxcast/>.

¹⁶ ToxCast™ Data Analysis Summit, Transforming Toxicity Testing From In Vivo to In Vitro: A Computational Toxicology Challenge, May 14-15, 2009. Agenda and presentations are available at <http://www.epa.gov/NCCT/toxcast/summit.html>.

¹⁷ EPA National Center for Computational Toxicology, The Virtual Embryo Project (v-Embryo™): A Computational Framework for Developmental Toxicity (2009), <http://www.epa.gov/ncct/v-Embryo/>.

¹⁸ EPA National Center for Computational Toxicology, The Virtual Liver Project (v-Liver™): A Computational System for Simulating Chemical-Induced Injury in Hepatic Tissues, (2009), http://www.epa.gov/ncct/virtual_liver/.

D. Developments in Toxicity Testing Outside EPA

EPA's *Strategic Plan* also discusses a few of the related efforts elsewhere in the federal government. In addition to the NTP and the NCGC, the *Strategic Plan* also mentions the National Center for Toxicological Research at the Food and Drug Administration¹⁹ and the Interagency Center for the Evaluation of Alternative Toxicological Methods.²⁰ Notably, the NTP recently developed new criteria for classifying results of its non-cancer hazard studies, using five levels of strength of evidence similar to those used for cancer studies (i.e., clear, some, equivocal, or no evidence, or inadequate study).²¹ The NTP has also been working steadily on developing high-throughput screening assays for use in the new chemical testing system.²²

Foreign government entities are also contributing to the overhaul of toxicity testing. EPA's *Strategic Plan* notes research being undertaken by the European, Japanese, and Korean Centers for the Validation of Alternative Methods and by Organization for Economic Co-Operation and Development ("OECD") workgroups. Moreover, the expected "avalanche of data" from the European Union's Registration, Evaluation, Authorization and Restriction of Chemical Substances ("REACH") regulation has been highlighted as an important driver of the changes in toxicity testing and chemical risk assessment.²³ A formal agreement regarding international cooperation on alternative test methods was recently reached by research institutions in Japan, the European Union, and the United States.²⁴

Industry, academic, and professional groups around the world are also playing prominent roles in shaping the future of EPA's chemical toxicity evaluations. Prominently, the Long-range Research Initiative ("LRI") of the American Chemistry Council and the International Council of Chemical Associations is hosting an ongoing series of workshops on new toxicity testing and risk assessment methods and their regulatory implications.²⁵

¹⁹ FDA's National Center for Toxicological Research Home Page (2009), <http://www.fda.gov/NCTR/>.

²⁰ The NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM): Advancing Public Health and Animal Welfare (2009), <http://iccvam.niehs.nih.gov/>.

²¹ NTP Unveils New Non-Cancer Evaluation Criteria (2009), available at <http://www.niehs.nih.gov/news/events/pastmtg/2009/sot-mtg/docs/sot.ntp.criteria.pdf>.

²² See, e.g., NTP, Request for Information Meeting, High Throughput Screening Approaches for Toxicology, Sep. 11-12, 2008, <http://ntp.niehs.nih.gov/files/HTSAgendaSept081.pdf>.

²³ See, e.g., Peter Preuss, EPA, Presentation at the National Academy of Sciences, *supra* note 14, available at http://dels.snas.edu/best/risk_analysis/Documents/Symposium%20Presentations%20Approved%20to%20Post%26%20Preuss.pdf.

²⁴ Memorandum of Cooperation Between the Japanese Center for the Validation of Alternative Methods, National Toxicology Program, European Centre for the Validation of Alternative Methods, and Health Canada's Environmental Health Science and Research Bureau Regarding International Cooperation on Alternative Test Methods (ICATM), April, 2009, available at http://iccvam.niehs.nih.gov/docs/about_docs/ICATM-MOC.pdf.

²⁵ See Flyer for LRI-sponsored program "Connecting Innovations in Biological, Exposure and Risk Sciences: Better Information for Better Decisions," Charleston, S.C., June 16-17, 2009, http://www.americanchemistry.com/s_acc/bin.asp?CID=1369&DID=8930.

IV. Conclusion

This summary provides only a brief overview of a far-reaching and very rapidly developing area at the intersection of science, technology, and policy. The implications of the ongoing developments in toxicity testing are likewise far-reaching. Industry stakeholders should be prepared for significant changes, not only in the conduct of toxicity testing and risk assessment themselves, but also in the broader debates over chemicals policy in the United States and elsewhere.

The paradigm change in toxicity testing presents a number of opportunities for improved regulatory outcomes. The efficiency gains in terms of both time and money would alleviate animal testing expenses in both the public and the private sector. The focus on pathways, mechanisms, and modes of action and the use of more realistic doses could reduce some instances of excessive conservatism in risk assumptions and produce more accurately targeted regulation.

Moreover, faster and higher volume testing could reduce the pressure for the most drastic proposals for revising TSCA, by eliminating the perceived gap between the information EPA needs to manage chemicals and the information that it currently is able to obtain from companies for new or existing chemicals. On the other hand, the improved testing capability can spur changes to TSCA that would direct EPA to prioritize and evaluate large numbers of chemicals on an ambitious timetable.

Yet there are daunting procedural and substantive hurdles before the potential benefits can be realized. The transition will be long and expensive, and resources are scarce. The ability of EPA and similarly situated agencies to utilize improved toxicity testing programs will also depend on obtaining acceptance from a variety of sectors. Internally, they may have to fight institutional resistance to innovation; this will require training on and promotion of the new techniques. Externally, they must gradually win regulatory and even legislative incorporation and judicial acceptance, which will depend in part on sufficient validation. EPA must carefully decide the extent to which the new paradigm will be used either merely for screening for additional testing, or as the basis for actual risk assessments and regulatory decisions.

Even the smoothest possible transition process, however, would be worthless if the implementation of the new toxicity testing paradigm fails to sufficiently ensure the accuracy of the resulting data. Implementation of the screening and regulatory aspects of the new paradigm cannot be allowed to outpace the production of necessary exposure data. Nor can implementation of the new techniques outpace the understanding and proper interpretation of the data they will produce. For example, exposure of “naked” cellular targets to chemicals may produce perturbations which in the real world would be prevented, modulated, or suppressed by larger metabolic systems, and such larger interactions must be known in order for cellular assays to be at all realistic. EPA must also be careful to validate its new models as much as possible against reality, not simply against its old animal-testing models. Funding, which as always is limited, must therefore be appropriately targeted and paced. Industry stakeholders should closely monitor future developments in the conduct and regulatory use of toxicity testing and contribute their views to decision makers.

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