EPA 100/K-09/001
February 2009

The U.S. Environmental Protection Agency's Strategic Plan for Evaluating the Toxicity of Chemicals

Office of the Science Advisor
Science Policy Council
U.S. Environmental Protection Agency
Washington, DC 20460

Recycled/Recyclable
Printed with vegetable-based ink on paper that contains a minimum of 50% post-consumer fiber and is processed chlorine free.

DISCLAIMER

Mention of trade names or commercial products does not constitute endorsement or recommendation for use. Notwithstanding any use of mandatory language such as "must" and "require" in this document with regard to or to reflect scientific practices, this document does not and should not be construed to create any legal rights or requirements.

AUTHORS AND CONTRIBUTORS

Future of Toxicity Testing Workgroup Co-Chairs

Michael Firestone, Office of Children's Health Protection and Environmental Education, U.S. EPA
Robert Kavlock, Office of Research and Development, U.S. EPA
Hal Zenick, Office of Research and Development, U.S. EPA

Science Policy Council Staff

Melissa Kramer, Office of the Science Advisor, U.S. EPA

Future of Toxicity Testing Workgroup Representatives

Marcia Bailey, Region 10, U.S. EPA
Arden Calvert, Office of the Chief Financial Officer, U.S. EPA
Laurel Celeste, Office of General Counsel, U.S. EPA
Vicki Dellarco, Office of Prevention, Pesticides, and Toxic Substances, U.S. EPA
Scott Jenkins, Office of Air and Radiation, U.S. EPA
Gregory Miller, Office of Policy, Economics, and Innovation, U.S. EPA
Nicole Paquette, Office of Environmental Information, U.S. EPA
Santhini Ramasamy, Office of Water, U.S. EPA
William Sette, Office of Solid Waste and Emergency Response, U.S. EPA

Other Contributors

Katherine Anitole, Office of Prevention, Pesticides, and Toxic Substances, U.S. EPA
Hugh Barton, Office of Research and Development, U.S. EPA
Norman Birchfield, Office of the Science Advisor, U.S. EPA
Michael Brody, Office of the Chief Financial Officer, U.S. EPA
Rory Conolly, Office of Research and Development, U.S. EPA
David Dix, Office of Research and Development, U.S. EPA
Stephen Edwards, Office of Research and Development, U.S. EPA
Andrew Geller, Office of Research and Development, U.S. EPA
Karen Hamernik, Office of Prevention, Pesticides, and Toxic Substances, U.S. EPA
Jean Holmes, Office of Prevention, Pesticides, and Toxic Substances, U.S. EPA
Richard Judson, Office of Research and Development, U.S. EPA
Thomas Knudsen, Office of Research and Development, U.S. EPA
Julian Preston, Office of Research and Development, U.S. EPA
Kathleen Raffaele, Office of the Science Advisor, U.S. EPA
Ram Ramabhadran, Office of Research and Development, U.S. EPA
James Samet, Office of Research and Development, U.S. EPA
Patricia Schmieder, Office of Research and Development, U.S. EPA
Banalata Sen, Office of Prevention, Pesticides, and Toxic Substances, U.S. EPA
Imran Shah, Office of Research and Development, U.S. EPA
Linda Sheldon, Office of Research and Development, U.S. EPA

John Vandenberg, Office of Research and Development, U.S. EPA
Maurice Zeeman, Office of Prevention, Pesticides, and Toxic Substances, U.S. EPA

External Peer Reviewers

John R. Bucher, Ph.D., Associate Director, National Toxicology Program, National Institute of Environmental Health Sciences
George Daston, Ph.D., Research Fellow, P&G
Daniel Krewski, Ph.D., MHA, Professor and Director, McLaughlin Centre for Population Health Risk Assessment, University of Ottawa
Martin Stephens, Ph.D., Vice President for Animal Research Issues, The Humane Society of the United States

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACRONYMS

ACToR	Aggregated Computational Toxicology Resource
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FTTW	Future of Toxicity Testing Workgroup
HTS	High Throughput Screening
IRIS	Integrated Risk Information System
NRC	National Research Council of the National Academies
OPPTS	Office of Prevention, Pesticides, and Toxic Substances
ORD	Office of Research and Development
QSAR	Quantitative Structure-Activity Relationship
SAR	Structure-Activity Relationships

1. INTRODUCTION

EPA bases its regulatory decisions on a wide range of tools and information that represent the best available science. In some situations, where very limited or no animal toxicity data exist, EPA may use tools such as structure-activity relationships (SAR) and quantitative structure-activity relationship (QSAR) modeling, together with information on exposure to make decisions about priority setting and the need for further evaluation (e.g., for new chemicals in the toxics program, high production volume chemicals, and pesticide inerts). To establish regulatory standards, EPA relies heavily on toxicity testing to evaluate clinical or pathological effects in experimental animal models. As such, toxicity testing and related research is currently a multi-billion dollar activity that engages thousands of research scientists, risk assessors, and risk managers throughout the world. To that end, the historical path taken in toxicity testing of environmental agents has generally been either to make incremental modifications to existing tests or to add additional tests to cover endpoints not previously considered (e.g., developmental neurotoxicity). This approach has led over time to a continual increase in the number of tests, cost of testing, use of laboratory animals, and time to develop and review the resulting data. Moreover, the application of current toxicity testing and risk assessment approaches to meet existing, and evolving, regulatory needs has encountered challenges in obtaining data on the tens of thousands of chemicals to which people are potentially exposed and in accommodating increasingly complex issues (e.g., lifestage susceptibility, mixtures, varying exposure scenarios, cumulative risk, understanding mechanisms of toxicity and their implications in assessing dose-response, and characterization of uncertainty)[1].

While the challenges of such information gaps are great, the explosion of new scientific tools in computational, informational, and molecular sciences offers great promise to address these challenges and greatly strengthen toxicity testing and risk assessment approaches. Proven benefits have been demonstrated in allied fields such as medicine and pharmaceuticals. Although untapped, the potential application to toxicity testing and risk assessment has also been recognized by EPA as witnessed by the issuance of a series of papers that provided guidance on the use of genomic data.[2] To better anticipate the potential contribution of new technologies and scientific advances to issues associated with toxicity testing and risk assessment, EPA commissioned the National Research Council (NRC) in 2004 to review existing strategies (NRC, 2006) and develop a long range vision for toxicity testing and risk assessment (NRC, 2007). In the subsequent release of *Toxicity Testing in the 21st Century: a Vision and a Strategy*, a landmark transformation in toxicity testing and risk assessment is envisioned that focuses on "toxicity pathways."[3] This approach is based on the rapidly evolving scientific understanding of how genes, proteins, and small molecules interact to form molecular pathways that maintain cell function. The goal is to determine how exposure to environmental agents can perturb these pathways causing a cascade of subsequent key events

[1] These limitations have been described more fully in *A Review of the Reference Dose and Reference Concentration Processes*: http://www.epa.gov/ncea/iris/RFD_FINAL[1].pdf

[2] Interim Policy on Genomics (2002): http://www.epa.gov/osa/spc/genomics.htm; Genomics White Paper (2004): http://www.epa.gov/osa/pdfs/EPA-Genomics-White-Paper.pdf; Interim Guidance for Microarray-Based Assays (2007): http://www.epa.gov/osa/spc/pdfs/epa_interim_guidance_for_microarray-based_assays-external-review_draft.pdf.

[3] **Toxicity pathways** are cellular response pathways that, when sufficiently perturbed, are expected to result in adverse health effects**.**

leading to adverse health effects. This sequence of events is illustrated in Figure 1 wherein the introduction of an environmental stressor may trigger such a cascade. Successful application of these new scientific tools and approaches will inform and produce more credible decision making with an increased efficiency in design and costs and a reduction in animal usage.

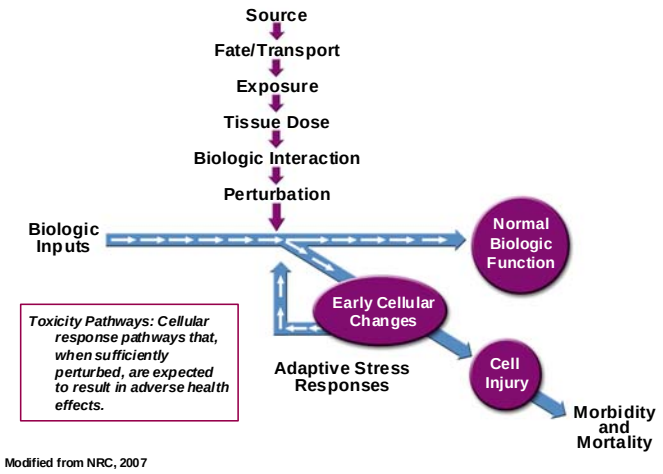

Figure 1. Toxicity Pathways. Toxicity pathways describe the processes by which perturbations of normal biological processes due to exposure to a stressor (e.g., chemical) produce changes sufficient to lead to cell injury and subsequent events (modified from NRC, 2007).

Other agencies have also recognized the need for this transformative shift, including the National Toxicology Program in their Roadmap for the Future and the Food and Drug Administration in their Critical Path Program. In anticipating the emergence, and potential, of this new scientific paradigm, EPA's Office of Research and Development (ORD) and some of the Agency's regulatory programs have also begun to redirect resources in intramural and extramural research programs to "jump start" the process of transformation. For example, ORD created the National Center for Computational Toxicology[4] in 2006. Likewise, ORD National Laboratories and Centers have also begun to incorporate these new scientific tools to better support the research being conducted under several of its multiyear research plans. Several ongoing projects address the use of *in vitro* assays in risk assessment and toxicity testing (e.g., Guyton, *et al*., 2008), and assessments under the Integrated Risk Information System (IRIS)[5] program are describing and evaluating published genomic data. EPA's Office of Prevention, Pesticides, and Toxic Substances (OPPTS) is also actively involved in the development and transition of computational toxicology tools into regulatory practice. OPPTS has developed a multi-year strategic plan to advance computational toxicology tools in its risk assessment and management paradigm. Current activities include assisting ORD by providing the necessary databases to support the development of models for efficiently and credibly predicting toxic potency and levels of exposure, beta testing the new computer models, training staff, and initiating plans for successful international coordination and stakeholder involvement. Furthermore, recognizing the need to partner to achieve the vision and goals laid out by the NRC, EPA recently signed a Memorandum of Understanding for research cooperation with the National Toxicology Program and the National Institutes of Health Chemical Genomics Center as a substantive step forward in building collaborations across sister federal agencies.[6] EPA is also working actively at the international level with programs such as the Organization for Economic Cooperation and Development (OECD) through the Molecular

[4] **Computational toxicology** is the application of mathematical and computer models and molecular biological approaches to improve the Agency's prioritization of data requirements and risk assessments (from *A Framework for a Computational Toxicology Research Program*, EPA 600/R-03/065).
[5] http://cfpub.epa.gov/ncea/iris/index.cfm
[6] http://www.epa.gov/comptox/articles/comptox_mou.html

Screening Initiative, the Integrated Approaches for Testing and Assessment Workgroup, Test Guideline Committees, and the QSAR Expert Group to ensure global harmonization of any new approach that originates from the research program. A more complete listing of these collaborations may be found in the appendix.

In response to the release of the NRC reports, EPA has established an intragency workgroup, the Future of Toxicity Testing Workgroup (FTTW), under the auspices of the Science Policy Council. The FTTW includes representatives from across the Agency, including the Regions and all major Program Offices. It has produced this current document, which will serve as a blueprint for ensuring a leadership role for EPA in pursuing the directions and recommendations presented in the 2007 NRC report. This document presents a strategy that is consistent with the NRC's directions and recommendations. It presents the Agency's vision of how to incorporate a new scientific paradigm and new tools into toxicity testing and risk assessment practices with ever-decreasing reliance on traditional apical approaches. The overall goal of this strategy is to provide the tools and approaches to move from a near exclusive use of animal tests for predicting human health effects to a process that relies more heavily on *in vitro* assays, especially those using human cell lines. The topics to be covered include (1) the applications and impacts/benefits for various types of regulatory activities (Section 2), (2) the research to be conducted to facilitate the screening and prioritization of environmental agents (Section 3), (3) the implementation of a toxicity pathway-based approach to risk assessment (Section 4), and (4) the critical companion component, namely, the institutional transition that must occur before the changes can be fully implemented (Section 5).

As described in Section 6, the workgroup recognizes that the full implementation of the vision set out in this strategy will require a significant investment of resources over a long period of time. The workgroup has identified a range of partners in this effort, and some planning on the relative role of these partners has begun, although the specific areas of work to be conducted/funded by EPA versus other partners needs further assessment. Decisions on the relative roles will have a significant impact on EPA resources required to implement the vision.

Since the NRC charge and report centered on advancing toxicity testing for assessing human health effects of environmental agents, this strategic plan is presented primarily within that context. However, under environmental legislative mandates (e.g., the Toxic Substances Control Act; the Federal Insecticide, Fungicide, and Rodenticide Act; and the Clean Water Act), most EPA programs must regulate compounds to ensure both environmental and human health risks are properly managed. Since statutory language and/or resulting policy typically require single regulatory decisions for a chemical(s) that encompass environmental and human health risks at the same time, accelerated and cost effective approaches for both areas are critical to realize programmatic benefits. As in the human health arena, development and application of approaches described in this strategy apply to ecotoxicology and risk assessment as well. Notable progress is being made within EPA Laboratories and Centers on the development and use of toxicity pathway models and the creation of prioritization schemes, toxicology knowledgebases, and systems biology models in the field of environmental science. The bringing together of relevant disciplines to share data and integrate models is critical to fully achieve increased efficiency in toxicity testing and a reduction in animal usage for both human health and environmental risk assessment. Consequently, the Agency will be implementing this strategy in a manner that addresses both human health and ecological risk assessment. Future versions of the

strategy will summarize progress made in advancing integrated testing and assessment capability and revisit remaining challenges.

2. REGULATORY APPLICATIONS AND IMPACTS

The research arising from implementation of this strategy will change the nature of the methods, models, and data that will inform the major components of the risk assessment process (i.e., hazard identification, dose response, exposure assessment, and risk characterization). Without attempting to be all-inclusive, Table 1 presents some of the major cross-office applications and impacts of these new scientific approaches, with more in-depth discussion of the planned work described in Sections 3-5. The three components of this strategic plan, namely, chemical screening and prioritization, toxicity pathway-based risk assessment, and institutional transition, are not independent elements but rather highly interactive and integrative efforts that will maximize the value and application of the research generated.

2.1. Chemical Screening and Prioritization

An ongoing need of several regulatory offices is to have tools to assist in chemical screening and prioritization, e.g., high production volume chemicals, air toxics, the drinking water Contaminant Candidate Lists, and Superfund chemicals. These programs consider anticipated exposure and hazard to select chemicals to evaluate in longer-term, whole-animal laboratory studies. An early use for data developed under the new paradigm will be as an efficient and cost effective screen for several types of chemical toxicity. Thus, risk assessors could use *in silico* (computer-based) technologies and structure/molecular/bioactivity profiling from diagnostic high-throughput/*in vitro* assays, along with predicted exposure/dose information, to predict chemicals most likely to cause hazards of concern for humans. This approach will also enable risk assessors to determine the specific effects, *in vivo* data, and exposures that would be most useful to assess, quantify, and manage. As the technology develops, EPA will be able to screen previously untested chemicals using libraries of chemical, molecular, biological, and toxicological data and models to identify the types of adverse effects that they are most likely to produce in standard animal bioassays. More importantly, EPA will be able to gain better insight into whether such effects would likely be manifest in humans under various exposure scenarios. As noted earlier, these needs are common to a number of federal agencies; discussions are underway to develop more common paradigms among federal agencies to facilitate data sharing.

2.2. Toxicity Pathway-Based Risk Assessment

The current approach to risk assessment includes uncertainties associated with (1) the human relevance of laboratory animal studies (species extrapolation), (2) the use of high doses in animals to estimate risk associated with lower environmental/ambient exposures (dose extrapolation), and (3) predicting the risk to susceptible populations. In recent years, the consideration of such issues has been better informed by the incorporation of information on potential modes of action through which toxicity may be expressed. The approach outlined earlier in Figure 1 focuses on perturbations in baseline biological processes that may lead down toxicity pathways to adverse health outcome(s). Combining this information with distributional data on population characteristics of exposure and dose (magnitude, frequency, and duration) provides a scientifically based approach for reducing the uncertainties associated with current risk assessments. By relying on a quantitative understanding of perturbations in toxicity pathways that lead to adverse health effects, the new approach to toxicity testing and risk assessment envisioned in this document will greatly increase EPA's capacity to assess individual

chemicals and their mixtures. The new approach will also increase EPA's confidence that the Agency's assessments adequately protect human health. Realization and acceptance of this new approach will likely encounter numerous challenges, but the effort is expected to ultimately lead to better protection of human health.

Table 1. Strategic Plan: Applications and Impacts

	Toxicity Pathway Identification and Chemical Screening & Prioritization	**Toxicity Pathway-Based Risk Assessment**	**Institutional Transition**
Issue	Need to screen 10,000's of chemicals for wide range of endpoints in a manner that considers toxicity pathways and the potential for human exposure.	For many chemicals, the current approach relies on expensive animal testing that takes time to conduct and review. Limitations in the design of *in vivo* studies often prevent complete evaluation of all endpoints and hazard/risk scenarios of concern. Limited understanding of biological mechanisms most often leads to uncertainty in assessing cumulative risk or extrapolating *in vitro* to *in vivo* or across doses, lifestages, species, or genetic diversity.	Implementing the new approach will require significant institutional investment in operational and organizational transition and in public outreach.
Drivers	Need to limit cost and animal usage, improve timeliness, and decrease uncertainty in testing decisions.	New scientific understanding and tools in molecular, computational, and information sciences consistent with applications in allied areas such as medicine and pharmaceuticals represent a path forward.	EPA lacks appropriate expertise and sufficient funding to fully and most efficiently utilize the new toxicity testing technologies when making regulatory decisions.
New Approach	Identification of toxicity pathways for key toxicological endpoints. Combine *in silico* and bioprofiles from HTS[7] along with QSAR approaches linked to animal study data.	Reliance on increased understanding of how perturbations of biological processes at environmentally relevant concentrations trigger events (i.e., toxicity pathway(s)) that may lead to adverse health outcomes. Develop linked exposure/dose models to inform dosing levels for toxicity testing and inform risks.	Fully adopting the new paradigm should be supported by mechanistically based proof-of-concept and verification studies. Further, such adoption will require additional training of existing staff and hiring new staff conversant in state-of-the-science knowledge in fields such as toxicology, biochemistry, bioinformatics, etc.
Impact	Offices would be better able to direct efforts and resources to chemicals with greatest potential risk. Significant increase in efficiency with marked reduction in cost for toxicity testing.	More scientifically relevant data on which to base EPA's regulatory decisions and/or impact analyses that rely on these risk assessments.	A well informed public will have greater confidence as EPA greatly expands the number of chemicals assessed for possible risks and improves existing strategies for hazard and risk assessment!

[7] **High-Throughput Screening (HTS)** refers to robotic technologies developed by the pharmaceutical industry for drug development that enable the ability to evaluate the effects of hundreds to thousands of chemicals per day on molecular, biochemical or cellular processes.

2.3. Institutional Transition

Implementing major changes in toxicity testing of environmental contaminants and incorporating new types of toxicity data into risk assessment will require significant institutional change involving:

- Operational transition – how EPA will transition to the use of new types of data and models for toxicity testing and risk assessment;
- Organizational transition – how EPA will deploy resources necessary to implement the new toxicity testing paradigm such as hiring of scientists with particular scientific expertise and training of existing scientific staff and risk managers;
- Outreach – efforts by EPA to share information with the public and improve risk communication.

The process of moving from research to regulatory acceptance for implementing new science related to toxicity testing will be an iterative and long-term effort (likely encompassing more than a decade). Essential to this iterative process will be the demonstration that the predictive nature of these new approaches is superior to that of our current practices for toxicity testing and risk assessment. It will be critical to begin activities geared toward regulatory acceptance early in the process of implementing this strategic plan.

3. TOXICITY PATHWAY IDENTIFICATION AND CHEMICAL SCREENING AND PRIORITIZATION

The advancements in biotechnology brought about by the sequencing of the human genome and the investment in high throughput screening tools to mine large chemical libraries for potential drugs have for the first time allowed a broad scale, unbiased examination of the molecular and cellular targets of chemicals. At this time, the examination of the relationships between the molecular and cellular targets of chemicals and the traditional endpoints of toxicity is at an early stage of development. Even upon characterization of these types of relationships, significant phenotypic data will be required to critically establish the role of toxicity pathways in evaluating hazards and risks. The great potential is that identification of a toxicity pathway and development of an *in vitro* bioassay for studying its chemical interactions will enable evaluation of the effects of thousands of chemicals in that pathway. Broadening this approach to the many toxicity pathways present in living systems allows a new avenue for identifying those chemicals that pose the greatest potential hazard. Knowledge of the toxicity pathways triggered by any one chemical will also allow targeting of specific *in vivo* tests to more fully characterize the potential hazard and risk. The identification of toxicity pathways for key target tissues, organs, and lifestages, and their linkage across levels of biological organization and exposure pathways and intensities are core elements of this strategy.

As indicated in Figure 2, chemicals may interact with a single pathway (the blue chemical) or multiple pathways (the yellow chemical). Also, multiple pathways can lead to the same expression of toxicity in the target organ as signaling pathways converge on common elements. It is important to note that multiple mechanisms of action[8] for any particular adverse response likely exist, and that many environmental pollutants are likely to have multiple mechanisms of action. Two critical components of the toxicity pathway concept are (1) extending knowledge of molecular perturbations and cell signaling pathways to understand linkages between levels of biological organization and (2) extending knowledge of *in vitro* and *in vivo* markers relevant to adaptive changes and/or adverse outcomes (see Section 5). As the research moves forward, it will be important to capture quantitative relationships between the molecular events and the higher order changes. Demonstration of plausible connectivity along the mechanism of action from initiating event to adverse outcome will serve as the rationale for using data from subcellular or cell-based *in vitro* assays for not only chemical prioritization but also predictive risk assessment. As toxicity pathways are identified, relevant *in vitro* assays can

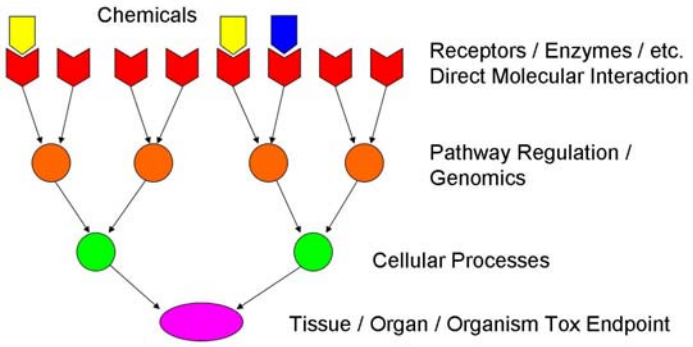

Figure 2. Toxicity Pathways Target Multiple Levels of Biological Organization.

[8] **Mode of action** is defined as a sequence of key events and processes, starting with interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in an adverse health effect. Mechanism of action implies a more detailed understanding and description of events, often at the molecular level, than is meant by mode of action.

be utilized and their results compared to *in vivo* studies as appropriate given the need to predict effects in humans or other species. While comparing responses to those in animal bioassays will be an early milestone of this strategy, the ultimate goal is the prediction of human risk. Therefore, efforts will shift towards that goal as experience with the approach increases. An added benefit to the toxicity pathway approach is that mixtures or their components could be evaluated in this manner, and as knowledge grows, it will be possible to predict where interaction with multiple toxicity pathways might be expected to lead to non-additive outcomes. This later activity will be an important outcome of the research highlighted in Section 4.2 (Strategic Goal 4) that is focused on the development of virtual tissue models. As noted below, virtual tissue models will also provide a basis for predicting emergent properties of tissues by integrating knowledge of molecular and cellular behaviors obtained from reductionist *in vitro* approaches.

In 2007, EPA launched ToxCast™ [9] in order to develop a cost-effective approach for prioritizing the toxicity testing of large numbers of chemicals in a short period of time. Using data from a broad range of state-of-the-art HTS bioassays developed in the pharmaceutical industry, ToxCast™ is building computational models to forecast the potential human toxicity of chemicals. Results from the HTS bioassays are being analyzed for signatures of bioactivity that correlate with known toxicities. These hazard predictions will provide EPA regulatory programs with science-based information helpful in prioritizing chemicals for more detailed toxicological evaluations, and lead to more efficient use of animal testing.

The research described here focuses on two major strategic goals:

1) Identification of toxicity pathways and deployment of *in vitro* assays to characterize the ability of chemicals to perturb those pathways in different biological contexts, and
2) Implementation of ToxCast™, with an initial focus on providing input for chemical prioritization, shifting over time to providing input for dose-response modeling.

A key feature of ToxCast™ is the phased nature of implementation (see Strategic Goal 2, Section 3.2), from proof of concept, to forward validation, and finally to reduction to practice. The number of chemicals will grow from the hundreds to the thousands, and the number of assays will change as experience and biology dictate. As the number of chemicals and breadth of toxicity pathways covered increase, ToxCast™ will improve as a unique resource to build chemo-informatic-based predictions of chemicals' potential human toxicity. Such advancements should help promote improved QSAR models and data upon which to build virtual tissue models.

Exposure science also plays a large role in this strategy. More simple and reliable screening models are needed that predict exposures to chemicals so that information from the full source-to-outcome continuum is brought into consideration in the evaluation of chemicals – a critically important step for new chemicals that have not yet been released into the environment. Examples of such simple methods and models for new chemicals can be found at EPA's Sustainable Futures Initiative[10]. Additional such models should further evaluate exposure based on the life cycle of intended product use and the physical-chemical properties of the chemicals. This

[9] http://www.epa.gov/ncct/toxcast/
[10] http://www.epa.gov/oppt/sf/

research should include the expansion of computational chemistry methods to further predict exposures as well as methods to predict release into the environment during product life cycle. Several additional screening-level models are currently under development in Canada and Europe. Research in this area should be coordinated with these groups to facilitate an international approach for chemical screening. EPA should promote easy public access to all of these additional models through the Internet.

3.1. Strategic Goal 1: Toxicity Pathway Identification and Assay Development

The most systematic and extensive approach currently underway for screening and prioritization is EPA's ToxCast™. Fully implementing the proposed strategy for more efficient toxicity testing will utilize a combination of the more exploratory ToxCast™ chemical signature approach (see Strategic Goal 2), and the more hypothesis-driven approaches to elucidating toxicity pathways. Developing systems-based models will require comprehensive identification of the biological processes that can result in toxicity when they are perturbed by chemical exposures. Therefore, toxicity pathway identification and development of appropriate *in vitro* assays to characterize the dose-response and time course of perturbations to those pathways will be needed. Measurement of chemical form and concentration from *in vitro* assays will also be important in hypothesis-driven research that seeks to establish linkages between perturbations of toxicity pathways and adverse effects, as well as for establishing structure-activity relationships. These research goals will utilize a range of methods (e.g., transcriptomic, proteomic, metabolomic, cellular, and biochemical analyses) to identify toxicity pathways using *in vivo* and *in vitro* systems. The *in vitro* assays and toxicity pathways already included in the ToxCast™ project will be a part of this research, but additional assays providing greater coverage of relevant toxicity pathways will need to be developed. For example, developmental neurotoxicity key responses are known to include cell proliferation, apoptosis, differentiation (into different cell types and creating different functionality/architecture of a cell), neurite outgrowth, synaptogenesis, and myelination (Coeke et al., 2007; Lien et al., 2007), but the underlying molecular pathways are not yet completely identified. Through the informed use of newer "systems-based" approaches (Edwards & Preston, 2008), the flow of molecular regulatory information underlying the control of these cellular events can be characterized, classified, and modeled. To facilitate use in risk assessment, these studies will be coupled with mechanism of action-based studies, including animal and human components as described in Strategic Goal 4.

Current priorities for research include developing *in vitro* assays for the key targets of chemicals in the environment for which limited knowledge is available (e.g., developmental neurotoxicity, immunotoxicity, reproductive toxicity) as well as for relatively well-characterized toxicity pathways such as stress response signaling. Studies representative of the full range of human variability will be necessary to characterize processes that may occur more readily in sensitive populations (e.g., asthmatics) or at certain lifestages (e.g., prenatal development). Additional emphasis needs to be placed on toxicities demonstrated to occur in humans. For example, clinical trials or post-marketing surveillance for pharmaceuticals, as well as molecular and genetic epidemiology studies, afford the opportunity to examine effects of chemicals already introduced into the environment that may not currently be well assessed by *in vivo* animal toxicity studies. Some of these pathways may be important for environmental chemicals with respect to human variability or exposure to complex mixtures.

3.2. Strategic Goal 2: Chemical Prioritization

This strategy extends approaches that are currently under development for EPA's ToxCast™ program to include greater coverage of toxicity pathways and chemicals. The goal of the ToxCast™ program is to provide a comprehensive assessment of toxicity pathways for a relatively low cost per chemical (current estimates are in range of $20-25,000). ToxCast™ (see Figure 3) was designed to collect data from a wide range of *in vitro* assays, mostly mechanistic in nature, to prioritize which chemicals to test further and which *in vivo* studies were likely most important. This screening and prioritization approach provides a near-term benefit during an extended transition to the more comprehensive proposed vision. As more comprehensive descriptions of processes involved in toxicological responses become available, different assays may be identified to replace those in the initial ToxCast™ effort, and the relationship to *in vivo* studies will shift from prioritization to providing input for dose-response modeling.

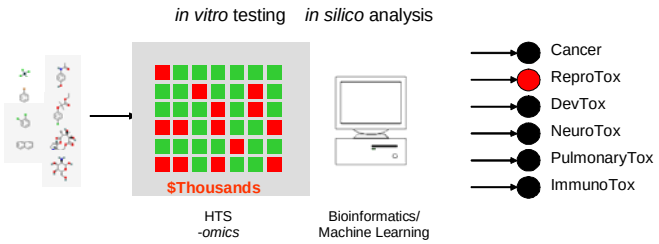

Figure 3. ToxCast™ is using a variety of HTS assays to develop bioactivity signatures that are predictive of effects in traditional toxicity testing approaches.

ToxCast™ is being developed in a phased manner. During FY08-09, substantial progress will be made on the first two phases of the ToxCast™ program (Dix et al., 2007; Kavlock et al., 2008). Phase I is a proof of concept involving 320 chemicals that have robust *in vivo* animal toxicity information. These chemicals have been profiled using over 400 high and medium throughput *in vitro* assays. From these *in vitro* bioactivity profiles, classifiers or signatures predictive of chemicals' *in vivo* toxicity are being derived. Phase II will involve validation of the predictive bioactivity and expansion of the diversity of chemicals tested. Phase III is the most relevant to this strategic plan, as it would begin to apply the knowledge gained in Phases I and II to the tens of thousands of chemicals of concern to EPA regulatory offices. An adaptation of the approach to evaluate the hazardous properties of nanomaterials is also anticipated.

4. TOXICITY PATHWAY-BASED RISK ASSESSMENT

The goals of the proposed new strategy for toxicity testing include collecting mechanistic data, largely *in vitro*, for the purpose of predicting human risk from exposure to chemicals. Prediction of *in vivo* effects in humans requires a combination of measurements and computer modeling to link *in vitro* responses to tissue dosimetry to alterations in the structure and function of tissues and organs. A substantial challenge will be to address the range of human variability arising from differences in age, life stage, genetics, disease susceptibility, epigenetics, diet, disease status, and other factors that potentially influence or interact with toxicity pathways.

The initial process for predicting human risk under this new approach could be summarized as (1) characterizing or predicting potential human exposures; (2) estimating the resulting chemical dosimetry (magnitude, frequency, and duration) for target pathways, tissues or organs; (3) measuring toxicity pathway response at doses consistent with human exposures; (4) predicting the *in vivo* human response resulting from pathway perturbations; (5) quantifying the range of human variability and susceptibility; and (6) validating predictions utilizing *in vivo* systems (e.g., laboratory animals, human data). In the current state of mechanistic toxicology (top row of Figure 4), chemicals are administered to the test animals (usually at high doses), a variety of

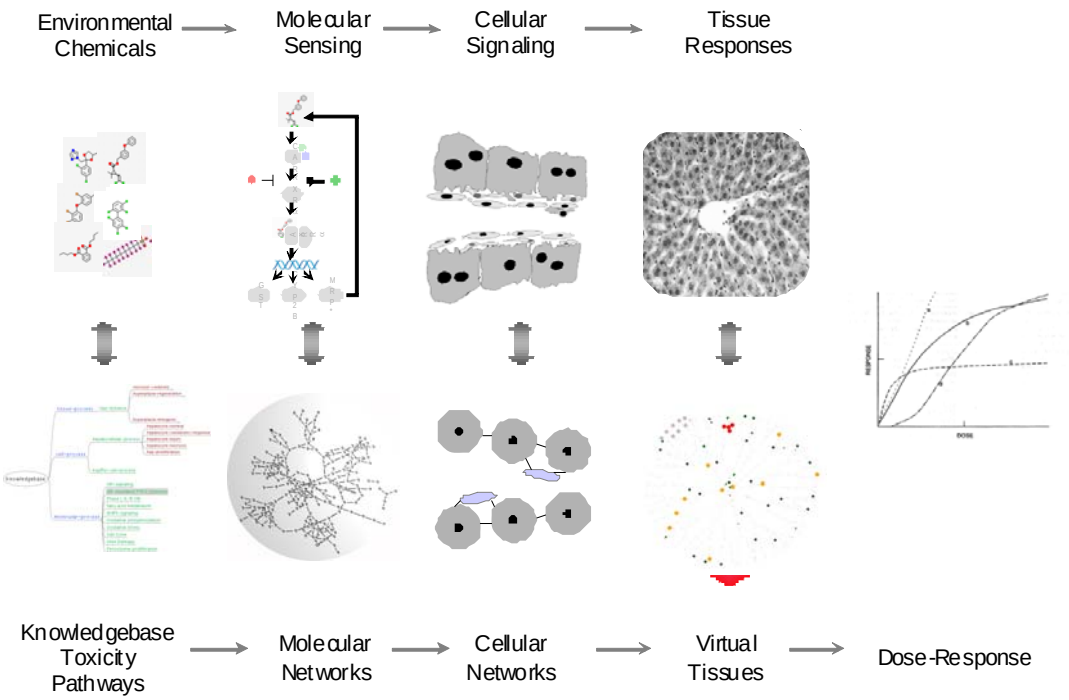

Figure 4. Toxicity Pathways to Dose-Response. The vertical arrows at each step in the process reflect the iterative nature of experimentation and modeling needed to gain full understanding of both the toxicity pathway

biochemical approaches are used to detect alterations in molecular pathways, the data are mined to describe the ensuing cellular alterations (e.g., oxidative stress damage, mitochondrial dysfunction), and tissue changes are confirmed at the level of morphology or function. The

bottom row of the figure depicts the vision for future ways of assessing risk, which includes determining the key toxicity pathways, defining approaches for examining perturbations in molecular networks, and translating the results to responses at the cell, and ultimately tissue and organ level, using computational models of the relevant systems. The expectation is that assessments in the future will utilize data from *in vitro* studies, and the need for *in vivo* animal testing will be substantially reduced. However, until the state of science of this new approach has reached a level of confidence for use in regulatory decision making, the traditional approach to toxicity testing will continue into the foreseeable future. With time, we expect that it will be progressively augmented and ideally replaced by computational models that integrate the information generated from non-animal sources into predictive models of response based upon the underlying biology. The vertical arrows at each step in the process reflect the iterative nature of experimentation and modeling needed to gain full understanding of both the toxicity pathway determination and the relationship to unperturbed biology. One anticipated outcome of the development of virtual tissues will be an increased understanding of the role of metabolism and of intra- and inter-cellular signaling pathways. This understanding will lead to the development of improved *in vitro* systems that, for example, might include combined cell-based systems to provide metabolic competency or to better reflect the intercellular responses in heterogeneous tissues.

As the transition progresses, it is important that increased emphasis will be placed on examination of exposure concentrations that are expected to occur in the environment. The key difference in future toxicity evaluations will be the transition to a focus on ways in which molecular pathways (as detected by *in vitro* models) are perturbed by chemical exposure throughout the range of exposures from environmental to the higher dose levels commonly used in contemporary toxicity studies. Dosimetry measurements coupled with computational modeling will be critical for predicting *in vivo* exposure levels of concern and for determining relevant *in vitro* concentrations. Some responses of targeted toxicity pathways can be evaluated in simpler cell culture models, whereas, in other cases, multiple *in vitro* assays may be necessary for the integration of multiple pathways that produce *in vivo* responses. These situations would require biologically based models for the responses as well as for chemical dosimetry in order to predict the integrated *in vivo* response.

Implementing this new paradigm requires organization of existing scientific information; computational methods for exposure, chemical dosimetry, and perturbations of biological processes; and evaluation of the methods for risk assessment applications. The research program to implement this element of the strategy is defined by three goals: development of toxicity pathway and exposure knowledgebases; development of virtual tissues, organs, and systems; and evaluation of human relevance.

4.1. Strategic Goal 3: Toxicity Pathway Knowledgebases

The underlying basis of the 2007 NRC report is that there are a finite number of toxicity pathways (i.e., in the hundreds) that could be queried using *in vitro* assays to obtain insights into the ability of chemicals to perturb those pathways. It refers to several stress pathways (e.g., oxidative stress response) and notes the general listing of signaling pathways in a previous NRC report (2006). However, an inventory of toxicity pathways and their involvement in a variety of toxicological responses needs to be created. Likewise, from exposure science there needs to be a

The knowledgebase would serve a variety of functions throughout the research and development effort associated with implementing this new approach to toxicity testing and will become a standard tool in the risk assessments of the future. ACToR (Figure 5), the Aggregated Computational Toxicology Resource under development in ORD, is an example of the needed approach of bringing together diverse types of information into a system where interrelationships of individual database elements (e.g., traditional toxicology, chemical structure information, high throughput screening data, molecular pathway analysis, chemical data repositories, peer reviewed published literature, and internal Agency databases) can be explored and utilized (Judson et al., 2008). Key steps in development of these knowledgebases include: (1) creating electronic repositories of existing toxicity information; (2) developing semantics for describing toxicity pathways; (3) automating pathway inference tools to aid in discovering mechanistic links between genomic information and molecular and cellular observations; and (4) creating a toolbox with a user-friendly interface to organize, access, and analyze toxicity pathway assay results.

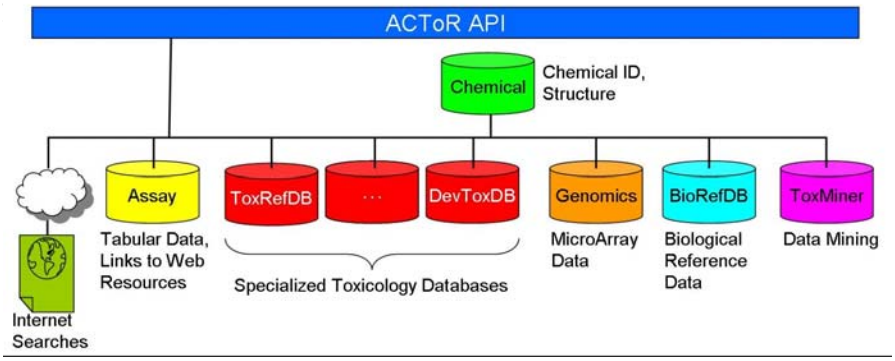

Figure 5. Knowledgebase Development. ACToR brings together a diverse set of currently unlinked resources available from internal and external sources into a system with a user friendly interface to readily mine and analyze toxicity data.

4.2. Strategic Goal 4: Virtual Tissues, Organs, and Systems: Linking Exposure, Dosimetry, and Response

Computational techniques relevant to this strategy fall into two general branches: knowledge-discovery (data-collection, mining, and analysis) represented in Strategic Goal 3, and dynamic computer simulation (mathematical modeling at various levels of detail) described in this section. The central premise of the latter approach is that critical effects of environmental agents on molecular-, cellular-, tissue-, and organ-level pathways can be captured by computational models that focus on the flow of molecular regulatory information (Knudsen & Kavlock, 2008). This information flow is influenced by genetic and environmental signals, with the net outcome being the emergent properties associated with baseline or abnormal collective cell behavior. Thus, computational systems modeling will be used to predict organ injury due to chemical exposure by simulating: (1) the dynamics and characteristics of exposure and dose, (2) the

dynamics of perturbed molecular pathways, (3) their linkage with processes leading to alterations of cell state, and (4) the integration of the molecular and cellular responses into a physiological tissue model. By placing a strong emphasis on understanding the biology of the system and the key regulatory components, these virtual tissue models represent a significant opportunity to better understand the linkage between chemically induced alterations in toxicity pathways and effects at the organ level. This research represents an ambitious effort, conceivable for the first time due to the current technological advances. Virtual tissue and organ system models will initially include liver, cardiopulmonary function, selected immune system tissues, multi-organ endocrine axes, and developing embryonic tissues. Development of these virtual tissue and organ systems will require newly generated data to both fill data gaps identified within the iterative process and test the predictive nature of these virtual systems. Comparative studies should include pathways fundamentally reliant upon cell signaling (e.g., cell proliferation, apoptosis, cell adhesion), intermediary metabolism (e.g., glycolysis, oxygen utilization, fatty acid biosynthesis), differentiation-specific functions (e.g., extracellular matrix remodeling), and other categories as developed above (see Strategic Goal 1) to ensure that predictions are broadly applicable. The wealth of existing data from NTP assays, published reports, and previous EPA intramural studies will be leveraged wherever possible with additional experiments designed to fill data gaps. Such efforts will also help answer how well *in vitro* experimental systems represent the full range of diverse cells present in the human body, how variability observed in the human population can modify quantitative predictions of *in vivo* dose-response, how exposure conditions influence outcomes, and how well the virtual tissue models represent the underlying processes.

Not all toxicity pathways are likely to be expressed in every tissue, and likewise not all tissues are likely to manifest adverse outcomes following chemical perturbation. Chemicals that affect the same toxicity pathway can do so via a number of different (and overlapping) mechanisms, and development of assays across toxicity pathways leading to the same outcome is a necessary component of the proposed strategy. Some toxicities are manifest only when multiple cell types and specific cell-cell interactions are present. Other toxicities may be dependent upon tissue geometry and three-dimensional architecture. Examples include signaling between hepatocytes and Kupffer cells, or the many forms of signaling between epithelial and mesenchymal cells. As such, developers of virtual cells, tissues, organs, and systems must always bear in mind the need to remain relevant to the processes critical to expressions of toxicity *in vivo.* Consistent with the NRC vision (2007), this need will likely entail a continued although decreasing role for *in vivo* systems for the foreseeable future.

A premise of the new toxicity testing strategy is that computational methods combined with an understanding of biological and exposure processes can be used to develop a more efficient and accurate approach for predicting risks from many chemicals. On the exposure side, models have been developed and are available that predict fate and transport, environmental concentration, exposures, and doses. These models work at multiple scales; for multiple sources, routes, and pathways; and for multiple chemicals, although each model only addresses a single process or compartment. Research is needed so that such models can take into account weathering of contaminants, differences in bioavailability of contaminants, variations in exposures with age, and variability in exposures within populations. Research is also needed to combine these models across various scales to develop a linked source-to-outcome modeling framework, to evaluate the framework using multiple chemicals and exposure scenarios, and to improve the computational

efficiency for the approach. Ultimately, these exposure models will be linked to the virtual tissue models for utilizing *in vitro* toxicity test results in quantitative risk assessments. Given the complexity of the challenges present in addressing each of these components, this effort represents a long-term goal of the strategy. However, efforts must begin now to put us on the path to achieving the ultimate vision of *Toxicity Testing in the 21st Century* (NRC, 2007).

The derived computational models must accurately describe the processes and mechanisms that determine exposure and effect. They must have reliable input parameters in order to quantify these processes. On the exposure side, our current understanding of processes and factors for many classes of chemicals and pathways (i.e., dermal and incidental ingestion) is limited. New approaches will be evaluated that will allow us to address the most significant uncertainties. Relational databases populated with data on exposures, exposure factors, activity patterns, and biomarkers will be developed as described. Informatic approaches or applications of network theory could potentially be used to provide a better understanding of important exposures, as well as exposure/response relationships. In the 2007 NRC report, emphasis was placed on biomarkers and their role in relating real world exposure to *in vivo* and *in vitro* biological response. They were also proposed as primary indicators in surveillance programs for tracking predicted exposures and health outcomes. Because of this emphasis, novel approaches for using biomarkers and integrating them into new risk assessment approaches will be investigated for chemicals already existing in the human environment. Perhaps such biomarker data can be used to improve predictive exposure models that will be relied upon for new chemicals not yet introduced into the environment.

4.3. Strategic Goal 5: Human Evaluation and Quantitative Risk Assessment

The critical challenge of this new vision for toxicity testing using mechanistic *in vitro* assays, targeted *in vitro* or *in vivo* testing, and computational models is to demonstrate that it successfully and adequately predicts human toxicological responses. Proof of concept efforts need to address this challenge both retrospectively and prospectively. Existing human data from pharmaceutical and environmental studies will be used to the extent possible. Human data could come from a range of sources including case reports, epidemiological studies (e.g., from the National Children's Study), and clinical trials. EPA has extensive experience obtaining human clinical data following exposure to the criteria air pollutants (e.g., ozone, particulate matter) and other chemicals (e.g., MTBE)[11]. Engagement of the pharmaceutical industry and the Food and Drug Administration to access toxicity findings from clinical trials of drugs that were successfully registered or that failed to be registered would be a desirable component of this effort. Limited data may be available for some nutrients or dietary supplements as well.

Such efforts will help address the question of the extent to which key events (critical perturbations) that are predictive of health endpoints (e.g., cancer, immunosuppression, kidney disease) must be demonstrated or whether the perturbation of baseline biological processes sufficient to induce substantial cellular level response (e.g., a stress response) should be considered an adequate endpoint for risk assessment. Linking a specific pathway perturbation to

[11] All EPA conducted or supported research is subject to and must comply with EPA regulations on the protection of human subjects. See http://www.epa.gov/fedrgstr/EPA-GENERAL/2006/February/Day-06/g1045.htm; http://www.epa.gov/oamrtpnc/forms/1000_17a.pdf

a particular target organ endpoint has the advantage of predicting outcomes that are already used in risk assessment, while alternative approaches raise issues of which endpoints should and should not be considered for risk assessment. This approach is relatively straightforward for some effects (e.g., hemolysis of red blood cells by EGBE, where the effect and the mechanism of action leading to it are qualitatively the same, even if quantitatively different). Linkage is more complicated for effects observed in animals that may predict human effects that are related, but not identical to, the outcomes in animals (e.g., developmental effects in an animal model may predict developmental effects in humans, but the exact manifestation might be different). On the other hand, as knowledge is gained about the interaction of chemicals with molecular targets, and this knowledge is combined with information on how perturbations of those targets are translated to responses in species-specific patterns (e.g., how activation of certain transcription factors lead to species-specific tissues responses), it will be increasingly possible to predict human outcomes from *in vitro* studies that identify mechanism of action. Clearly this aspect will need to be addressed on a case-by-case basis as we gain experience.

To be most useful in evaluation of risk to humans, the pathway-based efforts should ideally be tied to a known mechanism of action, such as via the use of quantitative biologically based, dose-response models. Understanding of the relevant mechanism of action will enable the identification of biomarkers for key event parameters (linked to toxicity pathways) that can be monitored in human studies for those chemicals already released into the environment at significant levels. These biomarkers could be measured in observational human studies to provide *in vivo* data to support the underlying pathway-based model. In addition, genetic susceptibility in humans identified via whole genome association studies will provide support for pathway-based models when genes critical for a key toxicity pathway are associated with susceptibility. Finally, the use of quantitative models requires estimation of uncertainty and variability in the predictions from *in vitro* assays and computational models. Formal methods for model evaluation are essential for demonstrating the success of this new approach to toxicity testing and risk assessment.

5. INSTITUTIONAL TRANSITION

Implementing major changes in toxicity testing of environmental contaminants and incorporating new types of toxicity data into risk assessment will require significant institutional changes. This section will touch upon three major thrusts of implementing institutional transition: operational transition, organizational transition, and outreach.

5.1. Strategic Goal 6: Operational Transition

Operational transition covers the technical aspects associated with EPA's implementation of a new toxicity testing paradigm and associated changes in risk assessment. It will consider such disparate topics as the importance of grounding the science, ensuring consistency of approaches within EPA, and working with outside partners and issues associated with the use of new models and tools.

The NRC "envision[s] a future in which tests based on human cell systems can serve as better models of human biologic responses than apical studies in different species." Achieving such a future, however, will require substantial research to study and define various toxicity pathways. In evaluating possible options for the future of toxicity testing, the NRC eventually chose an option involving both *in vitro* and *in vivo* tests but based primarily upon human biology and the attendant use of substantially fewer animal studies that would be focused on mechanism and metabolism. Their vision for the next 10 to 20 years relies on understanding perturbations of critical cellular responses and the use of computational approaches for assessing hazard and risk.

A paradigm shift in toxicity testing based on pathway perturbation will likely require significant methodological advances and future changes to EPA's risk assessment guidelines. Although it is infeasible to denote a specific timeline for how long it will take to substantially complete the strategic goals associated with toxicity pathway identification, chemical screening and prioritization, and toxicity pathway-based risk assessment, this plan takes the view that advances are likely to be gradual over the next decade or two. The good news is that toxicity testing research efforts have already begun moving EPA and others towards the use of *in silico* technologies and high throughput testing systems. The speed at which we are able to complete this transition will depend on the availability of increased research funding. It is important to note that our understanding of toxicity pathways for some apical endpoints (e.g., hepatotoxicity) may be developed at a faster pace than others (e.g., neurotoxicity) thus, allowing more rapid introduction of newer high-throughput *in vitro* testing methods.

Grounding the Science – From a broad regulatory perspective, data used by EPA to support regulatory decisions will be shaped by the statutory language covering the action, regulatory policies, and the resulting time and resources allocated to the assessment. Where appropriate, use of data should be consistent with the EPA guidance articulated in a number of science policy and guidance documents, including toxicity testing guidelines, risk assessment guidelines[12], information quality guidelines[13], and peer review guidance.[14]

[12] http://www.epa.gov/risk/guidance.htm
[13] http://www.epa.gov/quality/informationguidelines/
[14] http://www.epa.gov/peerreview/pdfs/Peer%20Review%20HandbookMay06.pdf

To implement this new paradigm, regulators, stakeholders, and the public will need to develop confidence that the data generated can be used effectively and that public health will continue to be protected. A step-wise implementation is envisioned: first, experience will be gained from proof of concept studies using data from chemicals (e.g., pesticides) with a large set of toxicity data developed using the current paradigm. Availability of both new and traditional types of data will allow extrapolation and comparison of results across methodologies.

Optimally, early success stories that meet programmatic needs in specific areas such as mechanism of action analyses or cumulative risk assessments will demonstrate the broader applicability of computational toxicology within the Agency. Reliability of the testing paradigm will need to be evaluated via a comprehensive development and review process, involving public comment, harmonization with other agencies and international organizations, and peer review by experts in the field. Bringing new methods into regulatory practice will require several phases starting from the development of the science and technologies, to technology transfer and building the regulatory infrastructure, to incorporation of the new tools into decision making.

Because this transformative paradigm will rely on new and complex science and will likely be surrounded by some controversy, an important part of regulatory acceptance will be to conduct research that will verify the approaches and models that will come to replace much of the way toxicity testing and risk assessments are conducted in the Agency today. An important component of the effort to develop new approaches to testing will be to translate the research into regulatory applications.

Issues Associated With the Use of New Methods and Models – For this new paradigm to be successful, new methods and models should be thoroughly evaluated prior to their application and use in regulatory decision making. The computer-based models used by the Agency should be publicly available. Testing methods should be accompanied by documentation that describes (1) the method and its theoretical basis, (2) the techniques used to verify that the method is accurate, and (3) the process used to evaluate whether the method and the results are sufficient to provide an adequate basis for its use in regulatory decision making. Access to data to allow for third party independent replication of results, to the extent practicable, is essential. Such review is appropriate before the Agency relies on data from such a method.[15]

Working With Outside Partners –The appendix provides details about the many outside parties EPA will need to partner with in order to implement this strategic plan including:

- Other federal bodies such as the National Toxicology Program (NTP) and the NIH Chemical Genomics Center (NCGC), with whom EPA has a memorandum of understanding to collaborate;
- The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), which is made up of representatives from 15 federal agencies that generate or use toxicological data;
- Foreign governmental parties and programs such as REACH, which is the new European Union Regulation on **R**egistration, **E**valuation, **A**uthorization, and Restriction of **Ch**emicals that went into effect June 1, 2007;

[15] See http://epa.gov/crem/library/CREMguidancedraft12_03.pdf

- The OECD (Organization for Economic Co-Operation and Development), which represents over 30 countries in the Americas, Europe and Asia;
- Academia;
- Chemical industry; and
- Non-governmental organizations.

Case Study Development – Significant challenges, such as interpretation and communication of data obtained using new toxicity testing approaches, will emerge under a new paradigm for toxicity testing. A key feature of a successful communication strategy will be to develop case studies using new kinds of data that can serve as a basis to explore, evaluate, and most importantly explain hazard, dose-response, and exposure information in a risk assessment framework. Characterization of risk information, both qualitative and quantitative, in a manner suitable for communication to risk managers will be a significant challenge for the research and risk assessment community, but it will be crucial if the new toxicity testing paradigm is to reach its potential.

5.2. Strategic Goal 7: Organizational Transition

Organizational transition is meant to cover changes in direction over time with regard to deployment of human capital resources necessary to implement the new toxicity testing paradigm such as hiring of scientists with particular scientific expertise and training of existing scientific staff. For example, EPA has hired key new scientific staff and initiated training including three new training courses in genomics designed and implemented by EPA's Risk Assessment Forum. Additional resources and training programs will be needed in both EPA's research program as well as its regulatory and regional programs.

As noted in Section 2, several intra-agency, interagency, and international activities are already underway to begin the transformation that will change the nature of toxicity data generated and how it is used to assess chemically induced risks to human health. Substantial funding will be needed to provide the scientific basis for creating new testing tools; to verify the utility of new testing tools including conducting peer review; to develop and standardize data-storage, data-access, and data-management systems; to evaluate predictive power for humans; and to improve the understanding of the implications of test results and how they can be applied in risk assessments used in environmental decision-making.

EPA expects that the use of less expensive, high-throughput testing methods will allow for the generation of toxicity data for thousands of currently untested or under-tested chemicals. The availability of these new data will likely lead to the need for more staff to interpret the data for many more chemicals and manage their risks. Additionally, toxicity databases such as EPA's IRIS and models used to assess risks may need to undergo substantial changes in the long term requiring future resources.

5.3. Strategic Goal 8: Outreach

Outreach consists of those efforts that will be used to help educate the public and stakeholders as well as improve risk communication.

In reaching out to the public, it will be important to re-emphasize points made by EPA Administrator Carol Browner in a 1995 memorandum to senior Agency staff about the Agency's policy related to its new Risk Characterization Program. This memorandum described the importance of adhering to the "core values of **transparency, clarity, consistency, and reasonableness** (which) need to guide each of us in our day-to-day work; from the toxicologist reviewing the individual (scientific) study, to the exposure and risk assessors, to the risk manager, and through to the ultimate decision-maker." Further, "because transparency in decision-making and clarity in communication will likely lead to more outside questioning of our assumptions and science policies, we must be more vigilant about ensuring that our core assumptions and science policies are consistent and comparable across programs, well grounded in science, and that they fall within a 'zone of reasonableness.'"[16]

Stakeholder Involvement – Implementation of a paradigm shift in toxicity testing and related changes to risk assessment methods and practices will require a sustained effort over many years – remember that the NRC envisioned some 10 to 20 years to reach their goal. This transition to new methods and approaches will need to be transparent, including efforts to share information with both the public and risk managers. It will be critical to effectively communicate with stakeholders (the public, scientists, federal and state agencies, industry, the mass media, nongovernmental organizations) about the new tools and the overall program regarding its strengths, limitations, and uncertainties. One way to enhance stakeholder involvement and ensure cooperation is to hold periodic workshops where all parties can gather to share information and progress; another tool is for EPA to establish a web portal to detail advancements in the science and relate these to improvements in risk assessment methods and practice.

Collaboration among different elements in the research community involved in relevant research on new testing approaches will be needed to take advantage of the new knowledge, technologies, and analytical tools as they are developed, and collaboration between research and regulatory scientists will be vital to ensure that the methods developed can be reliably used in risk assessments of various types (initially qualitative, but ultimately both qualitative and quantitative). Mechanisms for ensuring sustained communication and collaboration, such as data sharing, will also be needed. Independent review and evaluation of the new toxicity testing paradigm should be conducted to provide advice for midcourse corrections, weigh progress, evaluate new and emerging methods, and make any necessary refinements in light of new scientific challenges/advances. This may be accomplished using existing EPA mechanisms for peer review, e.g., through reviews by the Board of Scientific Counselors, the Science Advisory Board, and the FIFRA Scientific Advisory Panel. For testing that the Agency may wish to require, performance standards should be considered so that individual methods from any qualified source may be used. The NRC (2007) stressed that "in vitro tests would be developed not to predict the results of current [animal] apical toxicity tests but rather as [human] cell-based assays that are informative about mechanistic responses of human tissues to toxic chemicals. The [NRC] committee is aware of the implementation challenges that the new toxicity-testing paradigm would face." Presumably, establishing regulatory confidence that the new approaches are robust and protective of human health will be at the forefront of future challenges for EPA and its partners.

[16] http://www.epa.gov/oswer/riskassessment/pdf/1995_0521_risk_characterization_program.pdf

Risk Communication – Communicating with policy makers and the public is an important part of any risk management exercise. The complexity of the emerging toxicity testing paradigm and how new types of data and information will be used to assess risk will make communication of results challenging; consequently, the Agency must work to build public trust in the adopted technologies. As the science moves away from well-established animal models, a significant effort must be made to share information with risk assessors/managers and the public by clearly describing test results and methodologies in a transparent manner. A fundamental aspect of gaining public trust is transparency. Therefore, education and effective communication with stakeholders (the public, scientists, regulatory authorities, industry, the mass media, and nongovernmental organizations) on the strengths, limitations, and uncertainties of the new tools/paradigm will be critical.

Given that these new methods will be less intuitive than looking for traditional effects in whole animal studies, communication strategies will be very important. At this time, much of EPA's effort in this area is presented on the Agency's National Center for Computational Toxicology Web site.[17] As the new toxicity testing paradigm continues to evolve, the Agency will need to be vigilant in maintaining an interactive Web site to describe each individual assay or method in use and where it fits into the exposure-response continuum.

When communicating about risk, it is important for the Agency to address the source, cause, variability, uncertainty, and the potential adversity of the risks, including the degree of confidence in the risk assessment methodology, the rationale for the risk management decision, and the options for reducing risk (U.S. EPA, 1995; U.S. EPA, 1998). EPA will continue to interact with stakeholders in order to develop and maintain effective informational tools.

[17] http://www.epa.gov/comptox/

6. FUTURE STEPS

This strategic plan describes an ambitious and substantive change in the process by which chemicals are evaluated for their toxicity. The NRC (2007) suggested that such a transformation would require up to $100M per year in funding over a 10-20 year period to have a reasonable chance of reaching the goals. Even including the resources of sister agencies, the overall federal budget for the collaborative efforts does not approach the NRC proposed level of funding. Decision on the relative role of EPA vis-à-vis other partners will have a major impact on the resources that EPA needs to dedicate to this effort. These decisions will have to be made as the strategy is implemented. Explanation of these decisions, their rationale, and implications will be included in a subsequent implementation plan.

Regardless of whatever level of funding is ultimately applied to the vision of a more efficient and effective chemical safety evaluation effort, translation of this strategy into research and activities related to operational and organizational change will require development of an implementation plan as well as periodic peer review of directions and progress. Representatives from those EPA organizations most involved and impacted by the new vision will play key roles in the implementation program. The Science Advisory Board and/or the Board of Scientific Counselors will play key roles in the scientific peer review of the program. As noted in Section 4, there will be a progression in the implementation efforts from an early focus on hazard identification to a growing emphasis on the use of toxicity pathway characterization in risk assessment. Support for institutional transitions is also expected to increase over time as the tools and technologies emerge out of the research programs and become available for regulatory use. Figure 6 depicts one potential way that the level of effort of the three main activities involved in this strategy could change over time.

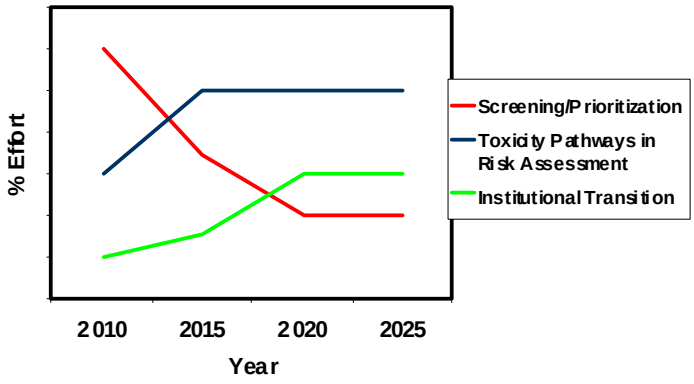

Figure 6. Relative (%) Emphasis of the Three Main Components of this Strategic Plan over its Expected 20-year Duration.

APPENDIX: OTHER RELATED ACTIVITIES

Other US Government Activities

The **National Toxicology Program (NTP)** at the National Institute of Environmental Health Sciences (NIEHS) coordinates toxicological testing programs within the Department of Health and Human Services[18]. Similar to EPA, NTP is developing the use of computational models, *in vitro* assays, and non-mammalian *in vivo* assays targeting key pathways, molecular events, or processes linked to disease or injury for incorporation into a transformed chemical testing paradigm.

The **NIH Chemical Genomics Center (NCGC)** of the National Human Genome Research Institute conducts ultra high throughput screening assays as part of the NIH's Molecular Libraries Initiative within the NIH Roadmap

A **Memorandum of Understanding**[19] was recently signed by EPA, the NTP, and the NCGC to collaborate on generating a comprehensive map of the biological pathways affected by environmental chemical exposures and use this map to predict how potential chemical toxicants will affect various types of cells, tissues, and individuals. The hope is to refine many of the toxicity tests performed on animals and eventually supplant them with *in vitro* testing and computational prediction (Collins et al., 2008).

In 2004 the **Food and Drug Administration (FDA)** produced a report[20] addressing the need to translate the rapid advances in basic biomedical sciences into new preventions, treatments and cures. FDA holds large databases of human, animal, and *in vitro* data for screening drug candidates for toxicity that may also be useful for screening environmental chemicals. The FDA's National Center for Toxicological Research (NCTR) aims to develop methods for the analysis and integration of genomic, transcriptomic, proteomic, and metabolomic data to elucidate mechanisms of toxicity[21]. NCTR has coordinated the Microarray Quality Control (MAQC) project, with numerous partners including EPA (Shi et al., 2006). In addition, NCTR has provided its ArrayTrack database to EPA for storage of genomics data for research and possible regulatory use.

The **Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)** was established by law in 2000 to promote development, validation, and regulatory acceptance of alternative safety testing methods. ICCVAM is made up of representatives from 15 federal agencies that generate or use toxicological data. Emphasis is on alternative methods that will reduce, refine, and/or replace the use of animals in testing while maintaining and promoting scientific quality and the protection of human health and the environment[22]. The NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) administers and provides scientific support for ICCVAM. ICCVAM/NICEATM evaluates test method submissions and nominations, prepares technical review documents, and organizes

[18] http://ntp.niehs.nih.gov/ntp/main_pages/NTPVision.pdf
[19] http://www.epa.gov/ncct/articles/comptox_mou.html;
[20] http://69.20.19.211/oc/initiatives/criticalpath/whitepaper.html
[21] http://www.fda.gov/nctr/overview/mission.htm
[22] http://iccvam.niehs.nih.gov/about/ni_QA.htm

scientific workshops and peer review meetings. For example, ICCVAM/NICEATM recently released a report[23] that describes two *in vitro* cytotoxicity tests that can be used for estimating starting doses for acute oral toxicity tests, thereby reducing the number of animals used.

Related Activities by Foreign Governments

A new **European Union Regulation on Registration, Evaluation, Authorization, and Restriction of Chemicals (REACH)** went into effect June 1, 2007. The main goals of REACH are (1) to improve the protection of human health and the environment from risks associated with chemicals in commerce and (2) to promote alternative test methods. REACH requires manufacturers and importers to demonstrate they have appropriately identified and managed the risks of substances produced or imported in quantities of one ton or more per year per company. The new European Chemicals Agency (ECHA)[24] will manage the system databases, coordinate evaluation of chemicals, and run a public database of hazard information[25].

The European Centre for the Validation of Alternative Methods (ECVAM)[26] coordinates the validation of alternative test methods in the European Union. ECVAM develops, maintains, and manages a database on alternative procedures and promotes the development, validation, and international recognition of alternative test methods.

The Japanese Center for the Validation of Alternative Methods (JaCVAM) is part of the Japanese National Institute of Health Sciences. JaCVAM has conducted validation studies for alternative test methods and participates in international validation efforts[27].

The Korean Center for the Validation of Alternative Methods (KoCVAM) is a branch of NITR, the National Institute of Toxicological Research. NITR is collaborating with the Korean Society for Alternatives to Animal Experiments (KSAAE) to refine methods in acute oral, reproductive/development, genetic, and endocrine toxicity testing[28].

The Organization for Economic Co-Operation and Development (OECD) represents 30 countries in the Americas (including the United States), Europe, and Asia. The OECD "Guidelines for the Testing of Chemicals" provides a collection of internationally harmonized testing methods for a number of toxicological endpoints using *in vivo*, *in vitro*, and even alternative approaches.[29] Test guidelines can be updated to reflect scientific advances and the state of the science if member countries agree to do so. A few OECD workgroups and efforts address issues relevant to this EPA strategy, e.g., the OECD QSAR Toolbox[30] and the joint OECD/IPCS (International Programme for Chemical Safety) Toxicogenomics Working Group, which has developed a proposal for a Molecular Screening Project, modeled after EPA's ToxCast™ program.

---

[23] http://iccvam.niehs.nih.gov/methods/acutetox/inv_nru_tmer.htm
[24] http://echa.europa.eu/reach_en.asp
[25] http://ec.europa.eu/environment/chemicals/reach/reach_intro.htm
[26] http://ecvam.jrc.it/
[27] http://www.nihs.go.jp/english/index.html
[28] http://wwwsoc.nii.ac.jp/jsaae/PARK.pdf
[29] http://titania.sourceoecd.org/vl=856000/cl=23/nw=1/rpsv/periodical/p15_about.htm?jnlissn=1607310x
[30] http://www.oecd.org/document/23/0,3343,en_2649_37465_33957015_1_1_1_37465,00.html

Academia

Numerous U.S. academic researchers and centers are funded by NIH or EPA's National Center for Environmental Research to develop assays and analysis methods that might be helpful to the goals of this EPA research strategy. This includes two Bioinformatics Centers funded by EPA in 2006.

The European Commission funds several large academic, government, and industry consortia that are conducting research that could lead to effective *in vitro* toxicity tests. **The CASCADE Network of Excellence**[31] studies human health effects of chemical residues and contaminants in food and drinking water, designing assays to elucidate estrogen, testosterone, and thyroid hormone pathways for the development of mechanism- and disease-based test methods. The aim of the **carcinoGENOMICS**[32] project is to develop *in vitro* methods for assessing the carcinogenic potential of compounds. **ReProTect**[33] is optimizing an integrated set of reproductive/developmental tests for a detailed understanding of gametogenesis, steroidogenesis, and embryogenesis that can support regulatory decisions.

Industry

The European Partnership for Alternative Approaches to Animal Testing (EPAA)[34] is a joint initiative from the European Commission and a number of companies and trade federations. Its purpose is to promote the development of alternative approaches to safety testing. The EPAA focuses on mapping existing research; developing new alternative approaches and strategies; and promoting communication, education, validation, and acceptance of alternative approaches.

Non-Governmental Organizations (NGOs)

The Comparative Toxicogenomics Database[35] (CTD) elucidates molecular mechanisms by which environmental chemicals affect human disease. CTD includes manually curated data describing cross-species chemical–gene/protein interactions and chemical– and gene–disease relationships to illuminate molecular mechanisms underlying variable susceptibility and environmentally influenced diseases. These data will also provide insights into complex chemical–gene and protein interaction networks.

The Johns Hopkins Center for Alternatives to Animal Testing[36] supports the creation, development, validation, and use of alternatives to animals in research, product safety testing, and education. Similarly, **AltTox.org**[37] provides information on non-animal methods for toxicity testing including a table[38] that summarizes the alternative testing methods by endpoint that have been approved or endorsed internationally by at least one regulatory agency.

---

[31] http://www.cascadenet.org/
[32] http://www.carcinogenomics.eu/
[33] http://www.reprotect.eu/
[34] http://ec.europa.eu/enterprise/epaa/index_en.htm
[35] http://ctd.mdibl.org/
[36] http://altweb.jhsph.edu/index.htm
[37] http://www.alttox.org/about/
[38] http://www.alttox.org/ttrc/validation-ra/validated-ra-methods.html

REFERENCES

Coecke S, Goldberg AM, Allen S, Buzanska L, Calamandrei G, Crofton K, Hareng L, Hartung T, Knaut H, Honegger P, Jacobs M, Lein P, Li A, Mundy W, Owen D, Schneider S, Silbergeld E, Reum T, Trnovec T, Monnet-Tschudi F, Bal-Price A. (2007) Workgroup report: incorporating *in vitro* alternative methods for developmental neurotoxicity into international hazard and risk assessment strategies. *Environ Health Perspect.* 115(6):924-31.

Collins FS, Gray GM, Bucher JR. (2008) Transforming Environmental Health Protection. *Science* 319:906-7.

Dix DJ, Houck KA, Martin MT, Richard AM, Setzer RW, Kavlock RJ. (2007) The ToxCast program for prioritizing toxicity testing of environmental chemicals. *Toxicol Sci.* 95(1):5-12.

Edwards SW, Preston RJ. Systems Biology and Mode of Action Based Risk Assessment. *Toxicol. Sci.* in press, doi:10.1093/toxsci/kfn190.

Guyton KZ, Kyle AD, Aubrecht J, Cogliano VJ, Eastmond DA, Jackson M, Keshava N, Sandy MS, Sonawane B, Zhang L, Waters MD, Smith MT. (2008) Improving prediction of chemical carcinogenicity by considering multiple mechanisms and applying toxicogenomic approaches. *Mutat. Res.* In press, doi:10.1016/j.mrrev.2008.10.001.

Judson R, Richard A, Dix D, Houck K, Elloumi F, Martin M, Cathey T, Transue TR, Spencer R, Wolf M. (2008) ACToR--Aggregated Computational Toxicology Resource. *Toxicol Appl Pharmacol.* 233(1):7-13.

Kavlock RJ, Ankley G, Blancato J, Breen M, Conolly R, Dix D, Houck K, Hubal E, Judson R, Rabinowitz J, Richard A, Setzer RW, Shah I, Villeneuve D, Weber E. (2007) Computational Toxicology A State of the Science Mini Review. *Toxicol Sci.* Dec 7.

Kavlock RJ, Dix DJ, Houck KA, Judson RS, Martin MT, Richard AM. (2008). ToxCast™: Developing predictive signatures for chemical toxicity. *Proceedings of the 6th World Congress on Alternatives in the Life Sciences*.T8-4-1. In Press.

Lein P, Locke P, Goldberg A. (2007) Meeting report: alternatives for developmental neurotoxicity testing. *Environ Health Perspect.* 115(5):764-8.

National Research Council of the National Academies (NRC). (2006) Toxicity Testing for Assessment of Environmental Agents. The National Academies Press. Washington, DC.

National Research Council of the National Academies (NRC). (2007) Toxicity Testing in the 21st Century: A Vision and A Strategy. The National Academies Press. Washington, DC.

Shi L et al. (2006) The MicroArray Quality Control (MAQC) project shows inter- and intraplatform reproducibility of gene expression measurements. *Nat Biotechnol.* 24(9):1151-61.

U.S. Environmental Protection Agency. (1995) Ecological risk: a primer for risk managers. Washington, DC: U.S. Environmental Protection Agency. EPA/734/R-95/001. Available at http://www.epa.gov/nscep/.

U.S. Environmental Protection Agency. (1998) Guidelines for Ecological Risk Assessment. Washington, DC: U.S. Environmental Protection Agency. EPA/630/R-95/002F. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=12460