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Report of an ISRTP Workshop: Progress and barriers to incorporating alternative toxicological methods in the U.S.

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Abstract

The workshop objectives were to explore progress in implementing new, revised and alternative toxicological test methods across regulatory evaluation frameworks and decision-making programs in the United States, to identify barriers and to develop recommendations to further promote adoption of approaches that reduce, refine, or replace the use of animal methods. The workshop included sessions on: (1) current research, development, and validation of alternative methods within the U.S. federal government; (2) emerging alternative methodologies with potential applications to a broad spectrum of toxicity evaluation strategies; (3) tiered evaluation ("intelligent testing") strategies; and (4) identification of, and recommendations to address, critical barriers that affect adoption and use of new, revised alternative toxicological test methods by U.S. regulatory agencies. Through facilitated discussion, a list of barriers and recommendations were developed and grouped into categories of economic/financial, scientific/technical, and regulatory/policy. Overall, participants from all sectors collectively supported catalyzing actions to promote more meaningful and rapid progress for research to develop alternative methods focused for use in regulatory programs, accelerated lab investigations to validate such alternative methods and adoption of regulatory frameworks which embrace and incorporate these validated alternatives.
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The International Society of Regulatory Toxicology and Pharmacology (ISRTP) hosted a workshop in November 2005 that explored progress to date in implementing new, revised and alternative toxicological test methods to reduce, refine, or replace the use of animals across regulatory evaluation frameworks and decision-making programs in the U.S. (see http://www.isrtp.org for workshop program, speaker's slides, and available workshop CDs). In addition to providing a better understanding of current alternatives research and validation efforts, the workshop focused on

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identifying barriers that impede progress, and explored bridges to overcome such barriers. In opening remarks, Dr. Christopher Portier (NIEHS/NTP) called attention to the range of challenges alternative methods face, which include technical/scientific (method development, validation and implementation), regulatory/policy (timeframe of changing policy requirements), economic (international funding cooperation), and political/societal (impact of European initiatives, activism, inertia of the status quo). Dr. Portier concluded that for alternative methods to substantially advance, a better environment must be created by active dialogue and engagement of the scientific and regulatory communities with stakeholders, avoidance of extreme positions, and focused research. Dr. Portier suggested that these efforts could substantially improve regulatory toxicology by creating a phylogenic tiered testing framework, consisting of an initial tier of mechanistic, high through-put screening, followed, when warranted, with higher tiered testing comprised of methods based on increasing biologic complexity.

Richard Becker (American Chemistry Council) and Sara Amundson (Doris Day Animal League) co-chaired the first workshop session, which covered current research, development, and validation of alternative methods within the U.S. federal government. Speakers included Dr. David Dix (U.S. EPA/National Center for Computational Toxicology), Dr. Christopher Portier (NIEHS/National Toxicology Program), Dr. Abigail Jacobs (FDA/Center for Drug Evaluation and Research), and Dr. William Stokes (NIEHS/NICEATM/Interagency Coordinating Committee on the Validation of Alternative Methods). Each speaker described the strategies within their respective programs for identifying potential hazards. Topics covered included prioritizing and grouping chemicals by common chemical structure or mode of action, screening for chemical and pharmaceutical toxicity or bioactivity using alternative (*in vitro*) methods that were often designed for high-throughput operation, analysis of data using computational methods, database development for data storage and management, and employing data for risk assessment and hazard identification. Approaches to increase the capacity and efficiency, such as validation of high-throughput screening via robotic technology, were discussed. With such an approach comes the acknowledgment that simultaneous screening of hundreds of chemicals by such methods is inherently an exercise in hypothesis generation, further *in vitro* or *in vivo* studies would be needed to determine the conditions where toxicity develops and the toxic mode of action.

To date, in the U.S., the only endpoints for which alternative methods have been validated and accepted for regulatory use are dermal sensitization, dermal corrosivity, and acute oral toxicity. Other methods have been submitted and reviewed by the Interagency Coordinating Committee on Validation of Alternative Methods (ICCVAM), but have not proven to be sufficiently accurate or reproducible. Programmatic similarities and differences were apparent. For example, in contrast to industrial chemical regulation, FDA does not require full method validation, as defined by ICCVAM, for new data in support of drug applications and will accept data generated from any alternative method providing it is, in the opinion of FDA reviewers, scientifically valid and addresses fundamental questions of human drug safety and efficacy.

Following individual presentations, the speakers collectively participated as a panel to address questions from the workshop participants. The speaker panel recognized that it is difficult for *in vitro* methods to capture the complexity of the toxic response generated in an intact organism and that the lack of reference data from a sufficiently diverse set of substances for alternative methods is a barrier to validation. Nonetheless, the panel encouraged development of alternative methods for toxicity testing and recommended focusing on methods that address very specific regulatory purposes. To move forward in developing and incorporating alternative toxicological testing methods in the federal framework, the panel recommended collaboration with stakeholders, routine consideration and use of the 3Rs principle (Reduce, Refine, and Replace), identification and collection of quantitative objective data from *in vivo* studies for use in modeling toxic mechanism in *in vitro* systems, generation of parallel data from *in vivo* and *in vitro* studies for comparison and method development, identification and validation of biomarkers of early toxicity. Dissemination of information and education of all stakeholders about alternative methods was also emphasized; this was seen as a critical step in the translational stage in taking a method from validation to regulatory acceptance and test guideline development.

The second workshop session, chaired by William Stott (Dow Chemical Company), focused on emerging alternative methodologies with potential applications to a broad spectrum of toxicity evaluation strategies. Specific methodologies detailed in this session included structure-activity relationships (SAR) (Ann Richard, U.S. EPA/National Center for Computational Toxicology), systems toxicology (Pieter Muntendam, BG Medicine), high-throughput screening (HTS) (William Janzen, Amphora), and 'organs on a chip' technology (Albert Li, The ADMET Group). Each of these methods aims to identify bioactive compounds, and collectively provide unique opportunities for identifying biomarkers of toxicity, creating molecular profiles of systemic perturbations, improving predictive toxicology based on chemical structure, or advancing the ability to predict human toxicity from animal and *in vitro* methods. These methods have the potential to greatly increase the speed and capacity for screening compounds, and as envisioned could generate a wealth of screening and mechanistic data. However, speakers cautioned that experimental parameters (chemical structure, concentration, etc.) must be closely monitored, especially in high-throughput mode, as variations in protocols and variability in study conduct generates large potential errors. It was suggested that data generated from alternative screening and testing

batteries should be standardized and deposited into publicly accessible and continually updated databases, like those hosted by NIH, NTP and EPA (DSSTox), so that it may be applied for future model development and human risk analysis. To evaluate and expand the robustness of predictive models, speakers strongly recommended incorporating a predictive component in the method validation phase to enable proper comparison against actual results.

These emerging technologies face several barriers, including limited to non-existent funding to support translation of methods from the development stage at the research lab bench into standardization and through to completion of method validation. Validation is necessary because this process provides the scientific basis to judge reliability across labs and over time, and relevance to endpoints (and/or to existing methods). Other barriers identified included resistance to acceptance of *in vitro* methods by 'classical' toxicologists and the significant challenge of gaining adoption at the regulatory level. One hurdle is the need for an alternative method to provide equivalent, more, or better, information than an animal testing method to be accepted for regulatory purposes as a replacement test. Without regulatory acceptance, regulated parties and regulatory agencies are limited in their ability to rely on alternative methods due to statutory constraints and potential product liability concerns. In the near-term, alternative methods may help to reduce and refine animal use through revision of current methods, improved study designs which integrate 2 tests into a single protocol, and enhanced utilization and integration into tiered testing approaches.

The third workshop session, chaired by Jay Ansell (Yves Rocher North America, Inc.) and Simon Webb (Procter & Gamble), focused on tiered evaluation strategies and featured presentations on High Production Volume (HPV) programs (Larry Rampy, American Chemistry Council), ILSI's Agricultural Chemical Safety Assessment (ACSA) project (Ann Blacker, Bayer Crop Science), Europe's Registration Evaluation and Authorisation of Chemicals (REACH) program (David Owen, Shell Chemicals), the Canadian Environmental Protection Act (CEPA)/Domestic Substances List (DSL) (Bette Meek, Health Canada), and concluded with an animal protection perspective (Martin Stephens, Humane Society of the United States). Tiered evaluation strategies were described as "intelligent" testing strategies, which employ a hierarchical series of assays, starting with non-animal methods, designed to generate data sufficient for human hazard identification within a specified degree of scientific certainty dictated by the programs' regulatory focus. Tiered evaluation strategies were contrasted to the existing toxicity testing approach, which all acknowledge is time consuming and both resource and animal intensive. This "traditional" approach has come to be perceived by some as a "one size fits all' framework consisting of a check box scheme, whereby completion of all studies is held to be essential for support of decision making with the requisite degree of certainty. The objective of the ILSI ACSA project was to completely re-evaluate the existing strategy for testing pesticide active ingredients to develop a newer, efficient, tiered testing approach that incorporates alternative testing methods to reduce or refine animal use, while at the same time providing sufficient data to support regulatory decision making.

Addressing the scale of a regulatory program is integral to design of a tiered testing framework and process. As discussed by Rampy, although the U.S. TSCA inventory contains more than 90,000 chemicals, the inventory does not represent chemicals actually in commerce. Estimates presented indicated there are approximately 9000 chemicals today in the U.S. with usage exceeding 10,000 lbs. annually, and of these, there are approximately 2500 that are High Production Volume (HPV) substances (defined as substances produced or imported in excess of 10^6 lbs. annually and which account for 95–98% by volume of annual use). The U.S. HPV program, which focuses on these some 2500 substances, as well as the Canadian DSL and E.U. REACH processes, are designed to initially screen this larger set of chemicals. The challenges faced in designing and implementing tiered screening and testing programs at this scale were presented. These strategies were developed through multi-stakeholder involvement and aimed to be transparent, timely, publicly accountable, and to generate data that can be used for regulatory hazard identification.

While there are unique features and differences across the U.S., Canadian and E.U. approaches, some common features were identified. In general, all three approaches for developing tiered testing strategies commence with an initial step that promotes, when scientifically appropriate, grouping of substances with like characteristics and has as a first step compilation and review of all available relevant existing data. An evaluative process is used to both judge whether existing data is sufficient, and if not, to identify data gaps and to set priorities for further, specific testing. Shared objectives of the U.S. HPV program, Canadian DSL and E.U. REACH includes implementation of efficient, accurate, and flexible testing strategies with improved methodologies that reduce and refine animal use, *i.e.* use categories and other methods to minimize the use of laboratory animal tests to the extent practicable and necessary for scientifically sound decision making. Although an enormous amount of data exists in various archives (more than initially predicted), these data need to be made more accessible through standardization and incorporation into public databases (as is currently underway through the U.S. HPV program). The methodological challenges for implementing tiered testing at this scale are numerous. Since production/import volume is a poor surrogate for human exposure, developing better estimation methods should be given high priority. Speakers acknowledged that the difficulty of *proving absence* of toxicity hinders progress, and suggested the development of a battery of mechanistic tests that model living systems to address this limitation. Lack of data sharing, limited funding for method development and translational research, and regulatory resistance to change create significant challenges to progress. Nonetheless,

implementation of tiered testing strategies is growing and with international cooperation, method convergence is likely to provide efficient and accurate frameworks for screening and testing chemical toxicity using fewer and fewer animals.

The final session was a roundtable discussion facilitated by Leon Bruner (Gillette) that included representatives from academia (Alan Goldberg, John Hopkins University), government (William Stokes, NIEHS/NICEATM), animal welfare (Andrew Rowan, HSUS), industry (William Stott, Dow Chemical), and private contract research (Rodger Curren, Institute for In Vitro Sciences) sectors. The objective of this session was to identify the critical barriers that impede implementation of new, revised alternative toxicological test methods by regulatory agencies in the U.S., and to identify courses of action to overcome those barriers. The main barriers identified throughout the workshop and their corresponding recommended actions are summarized in Table 1.

Recognizing that many federal agencies have implemented numerous programs aimed at the development of new methods, the question was asked "Is it clear what

Table 1
Critical barriers impeding progress in development, validation, regulatory acceptance and use of alternative methods in the United States and recommendations to overcome these barriers

Critical barriers impeding progress	Consensus recommendation to overcome barriers
Economic/financial	
• Research on alternative methods is diverse, encompasses many different disciplines, and is catalyzed by a broad spectrum of objectives. Research with specific focus on development and standardization and validation of alternative methods is rarely a priority for government funding.	• Individuals and organizations need to maintain and coordinate advocacy initiatives that target responsible agencies to promote focused research on validating and implementing alternative methods.
Scientific/technical	
• Generally, peer-reviewed literature does not provide the underlying data required for method development and validation. A lack of reference data from a sufficiently diverse set of substances can limit applicability and/or validation. Limitations on access to proprietary methods and data, although sometimes reasonable and appropriate based on business considerations, can impede progress in development and validation of alternative methods, especially *in vitro* and *in silico* methods/models. • There is recognition that it is potentially difficult for *in vitro* methods to fully capture the complexity of the toxic response in an intact organism. • When there is lack of information on the reliability and predictivity of currently accepted *in vivo* regulatory test methods for human responses, comparison of the performance of alternative methods is impeded. • Although much academic research focuses on *in vitro* methodologies, because funding for research specifically focused on standardization and validation of alternative methods for regulatory use is atypical, academic involvement is generally lacking.	• Mechanisms that may facilitate access to existing data need to be explored. Cataloging of data in publicly accessible databases should be promoted. • Research needs to be designed to focus efforts on (i) elucidating mechanisms and modes of action, (ii) method development, identification and validation of biomarkers and (iii) translating such methods into appropriate levels of decision making within risk assessment. • Applied research is needed to generate reference data, to demonstrate proof-of-concept models and to evaluate prediction models. • More research and evaluation of the predictive capability and reproducibility of existing animal models to develop data sets to permit side-by-side comparison with alternative methods. • Consideration should be given to use of alternative assays for defined chemistries, or sub-sets of chemistries (*i.e.* specific and/or limited applicability domains). There should be acceptance of the fact that some assays may not be applicable for all chemistries to be useful. • With the remarkable advances in molecular biology and genomics, it is worthwhile to consider the potential for launching a large coordinated and accelerated program (similar to the Human Genome Project) to investigate and develop molecular biological approaches to human health and environmental risk assessment.
Regulatory/policy	
• There is a low level of comfort or acceptance of alternative assays in both the regulatory and regulated communities. Considerable efforts are needed to overcome the inertia to maintain the status quo. • There are differences between agencies with respect to their standards for validation that impacts interpretation, use and acceptance of alternative assays (cf. chemical regulation versus pharmaceuticals). • Certain testing requirements are exceedingly prescriptive, especially those that require complex, long term animal intensive studies within a use of base set. Such "*check box*" approaches may entail considerable animal use. • The number of submissions or nominations to ICCVAM for method validation has been less than expected. • Objectives in method development and standardization phases are not always clear or tied to the broader perspective of ultimate use in a regulatory framework.	• Stakeholders should cooperate to create venues for the dissemination of information and education on the use of alternatives to catalyze the acceptance of validated methods by the relevant regulatory communities. • When protocols can be aligned, as is the case in formalized validation studies, parallel generation and submission of *in vivo* and alternative (including *in vitro*) studies will reinforce longer-term acceptance of alternative methods. • There is a need to develop, evaluate and promote the use of integrated or tiered testing strategies (which incorporate scientifically based decision triggers that signal the need for additional testing) Evaluation should be capable of demonstrating that such approaches can provide sufficient data and certainty to support regulatory decision-making. • There is a need for an organization (e.g., NTP) to serve as a coordinating agency within the federal government to encourage development and use of alternative methods. There is a need to identify both short- and long-term objectives.

agency strategies in this area are? Is there a need for an overarching federal strategy for prioritizing this research?" Panelists agreed that a few agencies (EPA, FDA, NTP) have begun to implement alternative methods programs. However, the view was expressed that there is a need for an overarching strategy (that could potentially be provided through linkage and integration into NTP's Roadmap (http://ntp.niehs.nih.gov/go/vision)), that would include both short- and long-term goals.

The issue of funding in the U.S. for alternatives methods was also discussed. The panel concluded that, at present, funding sources for this area are unclear, both within specific agencies and across the federal government as a whole. There is a need for a leadership within the federal programs to develop both overarching and specific objectives and to develop and implement a process to identify priorities, link these to program plans and resource these efforts sufficiently. Objectives and resources must address efforts to carry methods from development all the way through validation. Rowan, for example, citing the notable advances in molecular biology and genomics, proposed the time has come to consider a well funded, coordinated and accelerated program (similar to the Human Genome Project) to develop molecular biological approaches to human health and environmental risk assessment

The panel also expressed the opinion that current regulatory frameworks are too rigid to allow the substitution of validated alternative methods. To a certain extent, the rigidity is provided by current law and guidelines, and furthered by the fact that regulators and the regulated community are uncomfortable in changing the structured framework. However, the panel felt that flexibility is needed in several spheres, including flexibility to consider employment of alternatives for specific uses, and not solely as entire or full replacement of an assay for all situations and substances.

The rich discussion of the workshop illustrated a collective interest across all sectors for catalyzing more meaningful and rapid progress for research to develop alternative methods, for lab investigations to validate alternative methods and for regulatory frameworks to adopt validated alternatives. The discussion noted that across the scientific, regulatory and stakeholder communities, there is a lack of understanding of the breadth and depth of ongoing efforts in the U.S. of research now in progress on alternative methods. This has arisen because, in the U.S., alternatives research is not housed in a single entity nor described in an agency's communication or budget as specifically focused on development, standardization or validation of alternative methods. Panelists agreed that it would be very beneficial to have a nationally coordinated effort to gather this type of information and to disseminate it widely.

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