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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