

## Replacing Animals for Acute Systemic Toxicity Testing: A U.S. Strategy and Roadmap

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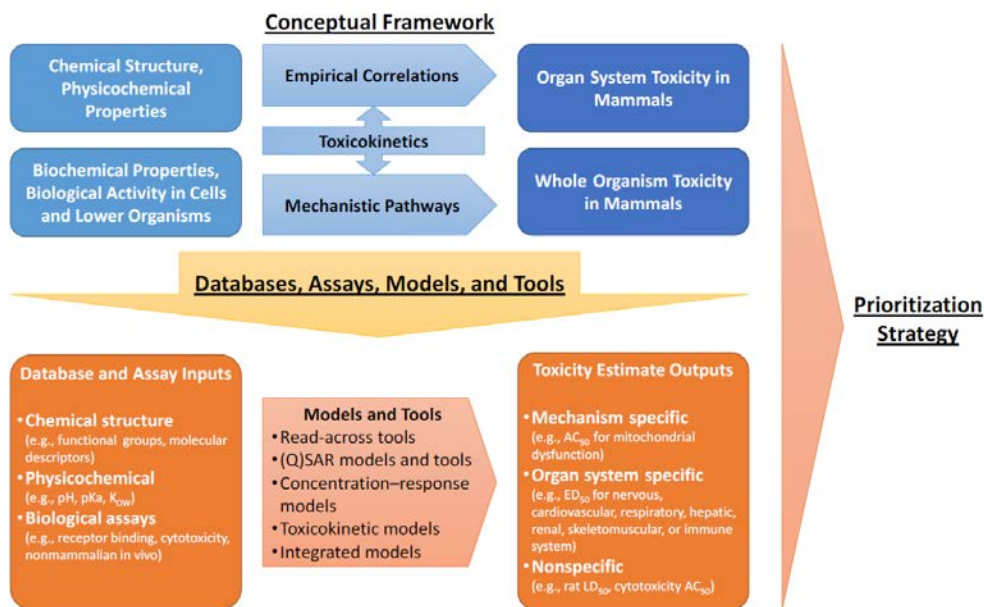
### Introduction

- The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) is developing a Roadmap to New Approaches for Safety Testing.
- The Roadmap will establish a new framework evaluating the safety of chemicals and medical products in the United States that will increase confidence and improve relevance to human health outcomes while maximizing efficiency and maintaining a commitment to replace, reduce, and refine animal use.
- Federal agencies, technical experts, and the regulated community will work together to develop this framework that:
  1. Guides the development of new science and technology to align with needs of Federal agencies
  2. Uses knowledge of human biology as the basis for establishing confidence in new approaches for decision making
  3. Ensures the implementation and use of these new approaches by Federal agencies and regulated industries
- A critical part of this effort will be focused activities associated with endpoints of regulatory concern, such as acute systemic toxicity.
- This poster describes ICCVAM's current and planned activities to support implementation of new approaches for acute systemic toxicity assessment.
- These activities are intended to address each of the three strategic themes included in the roadmap, and include: defining testing needs, identifying available alternatives, developing integrated approaches to testing and assessment (IATA), and addressing both scientific and non-scientific challenges.

## The Need for a Plan for Acute Systemic Toxicity

- To support its efforts to prevent debilitating acute exposures to deployed personnel, the U.S. Department of Defense (DoD) asked the National Research Council (NRC) to determine how DoD could use new approaches for predicting chemical toxicity.
- To address this request, the NRC prepared a report that provides a conceptual approach that DoD could use to develop a predictive toxicology system (**Figure 1**).

**Figure 1. Conceptual Framework and Strategy Proposed by NRC**



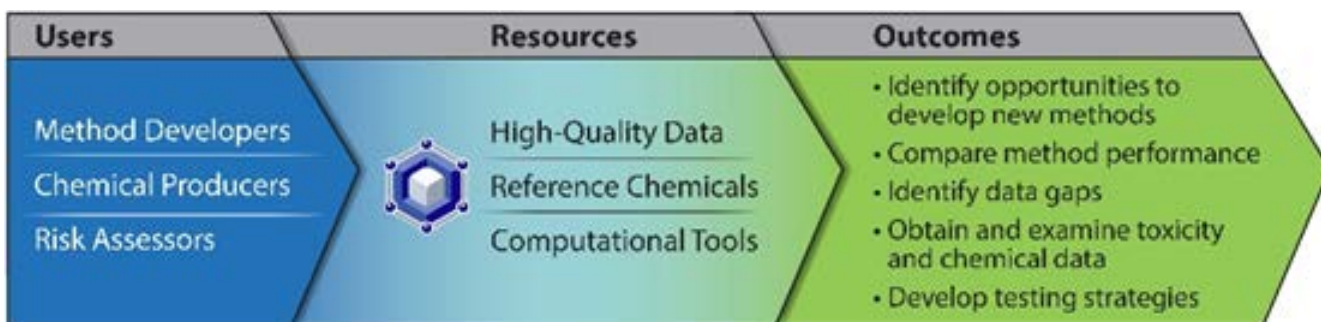
The conceptual framework and strategy proposed by NRC consists of three components: (1) a conceptual framework that links chemical structure, physicochemical properties, biochemical properties, and biological activity to acute toxicity; (2) a suite of databases, assays, models, and tools that are based on modern in vitro, nonmammalian in vivo, and in silico approaches that are applicable for prediction of acute toxicity; and (3) a tiered prioritization strategy for using databases, assays, models, and tools to predict acute toxicity in a manner that balances the need for accuracy and timeliness (NRC 2015).

- The U.S. Environmental Protection Agency's (EPA) Office of Pesticide Programs (OPP) is evaluating and implementing new technologies in molecular, cellular, computational sciences to supplement or replace more traditional methods of toxicity testing and risk assessment to better protect human health and the environment (EPA 2016a).
- Excerpt from Letter to Stakeholders on OPP's Goal to Reduce Animal Testing:

- “OPP’s immediate goal is to significantly reduce the use of animals in acute effects testing (the “6-pack” studies). Over 50 animals are used for a complete set of 6-pack studies. Annually, we receive over 500 acute toxicity 6-pack submissions.”
- ICCVAM establishes temporary ad hoc working groups to perform specific tasks that have been identified by the committee as being important for the development or validation of alternative test methods.
- In response to a request from EPA and DoD, an ICCVAM working group was established to draft an ICCVAM strategy and roadmap for acute systemic toxicity testing.

### General Approach to the Roadmap to New Approaches for Acute Systemic Toxicity Assessment

- Coordination of activities via the ICCVAM Acute Toxicity Working Group to:
  - Prioritize activities based on agency needs, expected impact on animal usage, ability to mitigate obstacles, and available resources
  - Coordinate efforts with international partners
  - Build from the NRC report for DoD
- Development of a Draft Scoping Document that:
  - Identifies all agency requirements / expectations
    - Classification and labeling system(s) (Globally Harmonized System of Classification and Labeling of Chemicals [GHS; UN 2015], EPA, Consumer Product Safety Commission)
    - Legal framework for acceptance of alternative methods
  - Defines chemical and regulatory space for each agency
  - Identifies validated alternatives and status of acceptance by agency
  - Identifies obstacles to implementation
- Identification, acquisition, and curation of high quality data (in vitro and in vivo) for building and evaluating new approaches

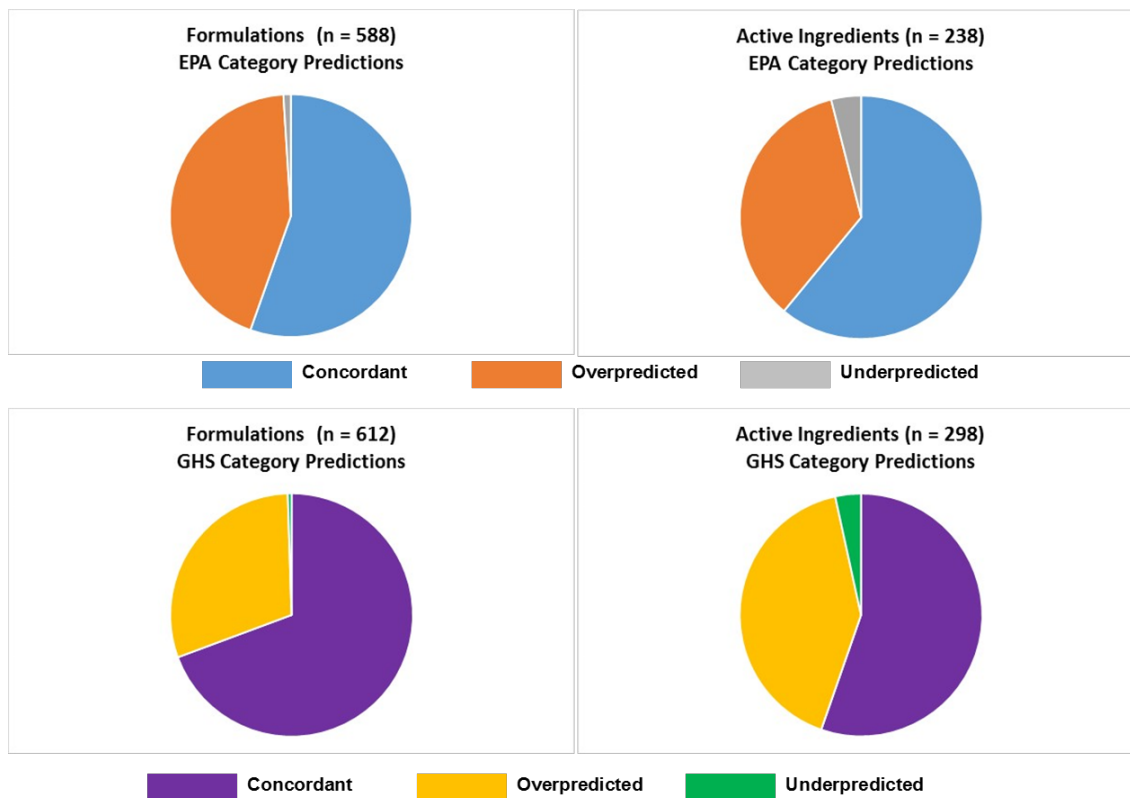


- One example is the **Integrated Chemical Environment (ICE)**, which NICEATM is developing to provide data and tools for development and evaluation of new chemical safety testing methods. The public release of ICE will be announced during SOT and details will be presented on March 15:
  - Bell et al., “An Integrated Chemical Environment to Support 21<sup>st</sup> Century Toxicology,” Abstract 2935/Poster P429, presented 1:15-4:30 p.m.
  - Exhibitor-hosted session, “ICCVAM Tools for Validation and Regulatory Application of Alternatives” 1:30-2:30 p.m., CC Room 337

### **Planned and Current Activities**

- Evaluate the usefulness of acute oral LD50 data for classifying dermal systemic hazard of potential toxicants such as pesticides, industrial chemicals, chemical warfare agents, and household chemicals
  - Status update: EPA OPP guidance for pesticides in place based on retrospective analysis (EPA 2016b) (**Figure 2**)

**Figure 2. Concordance of Oral and Dermal Hazard Classifications for Pesticide Formulations and Active Ingredients**



Charts from Paris et al. 2016.

- Evaluate the usefulness of the GHS additivity formulas for classifying formulations and mixtures for acute systemic toxicity tests
  - Status update: ongoing – EPA pilot program in progress to accept oral and inhalation toxicity data paired with calculations done in accordance with the GHS additivity formula

$$\frac{100}{ATE_{mix}} = \sum_{i=1}^n \frac{C_i}{ATE_i}$$

Weight % of Ingredient	Tox data (mg/kg)
45%	Oral LD50 = 500
20%	Oral LD50 = 1500
5%	Oral LD50 = 200
30%	Oral LD50 > 5000

$$\text{Acute Toxicity Estimate} = \frac{100}{\frac{45}{500} + \frac{20}{1500} + \frac{5}{200}} = 779 \text{ mg/kg}$$

- Evaluate in vitro and in silico approaches for predicting acute oral, dermal, and/or inhalation systemic toxicity
  - Status update: workshops convened in September 2015 and 2016 (see below); follow-up activities in progress
  - Planned activities:
    - Evaluate the performance of existing quantitative structure-activity relationship (QSAR) models and their relevance for the chemistries/inventories of interest
    - Incorporate high throughput screening assay data; evaluate the relevance of this data to the extent to which it is informative in characterizing specific modes of action (MOAs)
    - Investigate the feasibility of developing new models, particularly for classes of substances that are poorly predicted by the existing models
- Contribute to a scoping document that outlines the current requirements and testing needs for DoD and U.S. regulatory agencies, as well as international regulatory authorities
  - Status update: information for this document is being collected through interactions with ICCVAM agencies and International Cooperation on Alternative Methods (ICATM) partners.
    - ICATM was established by international validation organizations (**Figure 3**) to enhance international cooperation in validation and promotion of alternative test methods and strategies for regulatory use.

**Figure 3 ICATM Members and Partners**



## Outcomes of 2015 and 2016 Workshops

### September 2015 Workshop on Acute Systemic Toxicity

The poster features a dark green header with the National Toxicology Program logo and text. The main title is in large, bold, blue font. Below the title, the dates and time are listed. The location is Porter Neuroscience Research Center at the National Institutes of Health. A photograph shows a person in a lab coat and safety goggles holding a petri dish. At the bottom, there is a QR code, a small text block about accommodations, and logos for PCRM and PETA International Science Consortium.

- “Alternative Approaches for Identifying Acute Systemic Toxicity: Moving from Research to Regulatory Testing” reviewed the state-of-the-science of non-animal alternatives for this testing and explored ways to facilitate implementation of alternatives. (More information: <https://ntp.niehs.nih.gov/go/atwksp-2015>.)
- Workshop attendees included representatives from international regulatory agencies, academia, nongovernmental organizations, and industry.
- Resources identified as necessary for meaningful progress in implementing alternatives included compiling and making available high-quality reference data; training on use and interpretation of in vitro and in silico approaches; and global harmonization of testing requirements.
- Attendees particularly noted the need to characterize variability in reference data to evaluate new approaches. They also noted the importance of understanding the mechanisms of acute toxicity, which could be facilitated by the development of adverse outcome pathways.

- Workshop breakout groups explored different approaches to reducing or replacing animal use for acute toxicity testing, with each group crafting a roadmap and strategy to accomplish near-term progress.
- The workshop steering committee has organized efforts to implement the recommendations of the workshop participants.

### **September 2016 Workshop on Acute Inhalation Toxicity**



- Attendees at “Alternative Approaches for Acute Inhalation Toxicity to Address Global Regulatory and Non-regulatory Data Requirements” explored and discussed alternative approaches that could replace, reduce, or refine the use of animals for identifying chemicals that may cause acute systemic toxicity when inhaled. (More information: <https://ntp.niehs.nih.gov/go/inhalation-2016>.)
- A webinar series preceding the workshop reviewed:
  - Regulatory guidelines to define when and how acute systemic toxicity data are used for assessing inhalation toxicity hazard potential
  - Existing alternative approaches for identifying chemicals likely to cause acute systemic toxicity via inhalation, which could include mechanism-based models or in vitro and in silico approaches
  - Identified mechanisms of acute toxicity that may constitute key events in adverse outcome pathways for acute inhalation toxicity
- At the workshop, a series of breakout groups resulted in four primary recommendations for moving forward new approaches for acute inhalation toxicity testing:
  - Develop a database of existing acute systemic toxicity data



- Prepare a state-of-the-science review on mechanisms and assays for acute inhalation toxicity
  - Develop an in silico decision tree for determining when acute inhalation testing should be performed
  - Optimize in vitro assays and standardized protocols that can be used across laboratories
- The workshop steering committee comprised members from government and nongovernment stakeholder organizations including NICEATM, PETA International Science Consortium Ltd. (PISC), The Dow Chemical Company, Simulations Plus, Inc., the Netherlands Organisation for Applied Scientific Research, and EPA.
  - Workshop participants were tasked with establishing working groups that would be responsible for addressing the breakout group recommendations. These groups are meeting regularly via teleconference to provide updates and track progress.

### **Actions and Input Needed**

- While there are regional differences in specific testing requirements, all currently accepted guidelines for these tests share core principles, including essential testing needs to be addressed by alternative approaches and opportunities for existing information to enable waivers of required testing.
- While a variety of available alternative test methods can reliably identify potential cytotoxicants, none can single-handedly assess the multiple mechanisms of acute systemic toxicity following oral, dermal, or inhalation exposure.
- IATA will need to be developed to address the breadth of different mechanisms, ensure good coverage of the chemical landscape of interest, and leverage the collective strengths of the most promising test and non-test methods.
- To realize success, input will be needed from industrial sectors, academic disciplines, federal agencies, stakeholder organizations, and international partners.

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