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Review

Alternative approaches for identifying acute systemic toxicity: Moving from research to regulatory testing

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ABSTRACT

Acute systemic toxicity testing provides the basis for hazard labeling and risk management of chemicals. A number of international efforts have been directed at identifying non-animal alternatives for *in vivo* acute systemic toxicity tests. A September 2015 workshop, 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. 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.

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1. Introduction

All substances are potentially toxic at sufficiently high doses. Regulatory authorities often require testing of substances to characterize their toxicity. Testing information is then used to assign substances to toxicity categories, which determine the content of product labels to indicate precautions to be taken while handling. Acute systemic toxicity tests are conducted for these purposes *via* three routes of exposure: oral, dermal, and/or inhalation.

The traditional *in vivo* tests commonly included in regulatory test guidelines generate an LD_{50}, which is the dose that produces lethality in 50% of the animals tested. However, substantial progress has been made in recent decades in reducing and refining animal use for these tests. The first acute oral toxicity test accepted as a test guideline by the Organisation for Economic Co-Operation and Development (OECD) in 1987 (Test Guideline 401) was deleted in 2002 in favor of versions designed to use fewer animals (Test Guidelines 420, 423, and 425; (OECD, 2002a; OECD, 2002b; OECD, 2008). Two acute inhalation OECD test guidelines exist, with Test Guideline 436 being a reduction alternative to Test Guideline 403; in addition, a draft Test Guideline 433 is currently being developed as a reduction and refinement alternative to Test Guideline 403 (OECD, 2009a; OECD, 2009b; OECD, 2015). OECD Test Guideline 402 for acute dermal toxicity testing is generally used only following confirmation of dermal penetration by Test Guideline 428 or other method (OECD, 1987; OECD, 2004). In March 2016, EPA published a draft policy to waive all acute lethality dermal studies for formulated pesticide products and registrants can submit waiver requests through existing processes (www.epa.gov/pesticides/new-epa-guidance-testing-pesticides-will-reduce-animal-testing). Additionally, in 2016, the OECD published a guidance document on considerations for waiving or bridging of mammalian acute toxicity tests that is applicable to chemical pesticides and other substances (OECD, 2016).

More recent international efforts have been directed at identifying non-animal alternatives for *in vivo* acute systemic toxicity tests. The multiple mechanisms associated with acute systemic toxicity preclude a single cell- or biochemical-based assay serving as a full replacement, but integrating data from multiple assays with other physiologically relevant information (*e.g.*, water solubility, molecular weight) about the test substance or a structurally similar substance could provide sufficient information to predict toxicity. Computational models to integrate these data and information, built using machine learning approaches that leverage existing data, physicochemical properties, and other information to predict a substance's toxicity, are becoming widely used in toxicology.

With the goal of identifying approaches that could potentially replace animal use for required acute systemic toxicity testing, an international group of experts convened in September 2015 to discuss progress and challenges associated with the development, validation, and implementation of alternatives. The workshop on Alternative Approaches for Identifying Acute Systemic Toxicity: Moving from Research to Regulatory Testing included >60 representatives from regulatory agencies, academia, nongovernmental organizations, and industry.

The objectives of the workshop were to:

- review the regulatory landscape in order to define when and how acute systemic toxicity data are used,
- review the state-of-the-science for alternative approaches to identifying acute systemic toxicity, including mechanism-based models, *in vitro* and *in silico* approaches, lower vertebrate and invertebrate models, and adverse outcome pathway (AOP)-based approaches,
- identify mechanisms of acute systemic toxicity and relevant AOP data and data gaps in order to promote development of AOPs for acute