Presentation Abstracts and Background Materials

SCIENTIFIC ADVISORY COMMITTEE ON ALTERNATIVE TOXICOLOGICAL METHODS

Session III: Validation and Establishing Scientific Confidence in New Approach Methodologies
Thursday, September 22, 2022

ICCVAM Validation Work Group: Updating the ICCVAM Guidance on Validation – Progress Report

Presenter: Dr. Suzanne Fitzpatrick, U.S. Food and Drug Administration

The ICCVAM Validation Work Group (VWG) was formed to update the ICCVAM Validation Document and to give guidance on developing and evaluating flexible practices that consider context of use (COU) to build confidence in new methods. Since not all uses or purposes (COUs) of alternative methods will be the same, the data required for validation will be, to a certain extent, dependent on the purpose. Different agencies often use different validation terms. Sometimes the meaning has overlap but there can be distinctions. The VWG has been trying to reconcile the definitions of these terms to have more unified terminology where possible. In updating the guidelines, the VWG has discussed several issues including how new principles of validation can fit into a globally harmonized approach to allow for continued mutual acceptance of data and what to recommend for best practices for quality and quality systems. The format and organization of the document is still under consideration. VWG members and staff of the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) will continue to contribute to the drafting and editing. When the draft is completed, the federal agencies involved in ICCVAM will review and provide comments. Once this is finished, stakeholders will have opportunity to comment on the document.

Background

- Validation and Regulatory Acceptance of Toxicological Test Methods: A Report of the ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods

Technical Framework for Enabling High Quality Measurements in NAMs

Presenter: Dr. Elijah Petersen, National Institute of Standards and Technology

New approach methodologies (NAMs) are in vitro, in chemico, and computational approaches that can potentially be used to reduce animal testing. For NAMs that require laboratory experiments, it is critical that they provide consistent and reliable results. While guidance has been provided on improving the reproducibility of NAMs that require laboratory experiments, there is not yet an overarching technical framework that details how to add measurement quality features into a protocol. In this manuscript, we discuss such a framework and provide a step-by step process describing how to refine a protocol using basic quality tools: cause-and-effect analysis, flowcharts, check sheets, control charts, histograms, and scatterplots. The steps in this framework include 1) conceptual analysis of sources of technical variability in the assay, 2) within-laboratory evaluation of assay performance, 3) statistical data analysis, and 4) determination of method transferability (if needed). While each of these steps has discrete components, they are all inter-related and insights from any step can influence the others. Following the steps in this framework can help reveal the advantages and limitations of different choices during the design of an assay such as the what in-process control measurements to include and how

many replicates to use for each control measurement and for each test substance. Overall, the use of this technical framework can support optimizing NAM reproducibility, thereby supporting meeting research and regulatory needs.

Background

- Characteristics to Consider When Selecting a Positive Control Material for an In Vitro Assay
- Development of a 96-Well Electrophilic Allergen Screening Assay for Skin Sensitization Using a Measurement Science Approach
- Technical Framework for Enabling High-Quality Measurements in New Approach Methodologies (NAMs)

Scientific Confidence Framework

Biological Relevance: A Better Benchmark

Presenter: Dr. Nicole Kleinstreuer, National Institute of Environmental Health Sciences

As regulatory decision makers evaluate how to apply the best science to protect human health and the environment across a diversity of chemical exposures, there is an increasing need to rely on NAMs that provide more rapid, efficient, and, above all, human-relevant insights into potential toxicities. Scientific confidence in NAMs can be achieved via demonstrating that the NAM captures key aspects of human biological information as well as or better than the traditional animal test method and provides information that allows regulators to make protective decisions. Establishing biological relevance of NAMs offers an alternative to benchmarking the performance of the NAM using results from traditional animal test methods that may not adequately represent the human scenario. Substantial progress has been made in the area of topical toxicities by evaluating coverage of human anatomy, physiology, exposure, and toxicity mechanisms across all available in vitro and in vivo test methods. Skin sensitization and skin/eye irritation will be discussed as proof-of-concept case studies demonstrating how mechanistic insight, e.g. via adverse outcome pathways, and assessing human biological relevance can support establishing scientific confidence in NAMs. Considerations for moving beyond topical toxicities to more complex endpoints will be discussed.

Background

- Human-relevant Approaches to Assess Eye Corrosion/Irritation Potential of Agrochemical Formulations
- A Framework for Establishing Scientific Confidence in New Approach Methodologies

Variability of Reference Data

Presenter: Dr. Agnes Karmaus, Inotiv

Historically, toxicity testing has been conducted using in vivo test methods. Confidence in data from these methods is such that regulatory hazard classification and labeling systems have been designed around their results and the methods are used as the benchmark against which NAMs that replace or reduce animal use are compared. For many toxicity endpoints there is no NAM accepted as a complete replacement for animal use because hazard categorizations based on data from the NAM do not always agree with hazard categorizations based on in vivo data for the same chemical set. However, discordance with in vivo results may not always indicate that the NAM is generating an incorrect prediction. Variability of results from in vivo test methods could be an important contributor to such discordance and therefore should be carefully considered when comparing in vivo and NAM results. To establish confidence in NAMs, it is critical to understand any variability inherent to the in vivo test a NAM is intended to replace, as this variability will directly affect the expectations for performance of NAMs that seek to replace it. Sources of such variability might include both the inherent variability among animals and the subjective nature of observational in vivo endpoints. Recent analyses indicate that in

many cases data from in vivo test methods are highly variable. These efforts provide the basis for redefining benchmarks against which to evaluate NAMs and thereby set appropriate expectations for NAM performance.

Major points:

- To establish confidence in NAMs, we must demonstrate that they are as good or better than the existing in vivo test method.
- Establishing confidence in NAMs includes considerations of test method variability. It is unrealistic to expect a NAM to achieve a level of concordance with an in vivo test higher than the intrinsic level of concordance exhibited by that test.
- Human relevance should also be considered in such assessments.

Background

- Evaluation of Variability Across Rat Acute Oral Systemic Toxicity Studies
- Analysis of Variability in the Rabbit Skin Irritation Assay

Transparency, Data Integrity, and External Review

Presenter: Dr. João Barroso, European Union Reference Laboratory for Alternatives to Animal Testing

Establishing scientific confidence in a NAM is an indispensable step to facilitate its regulatory acceptance. To increase efficiency, this process needs to be streamlined, understood, and accessible to all stakeholders. However, as more validation studies are sponsored and managed by method developers, ensuring data integrity, transparency, and independent review of the study becomes paramount. This talk provides an overview of the challenges and opportunities for adapting validation practices to keep pace with scientific progress whilst ensuring scientific confidence.

Background

- The Role of Validation in Establishing the Scientific Credibility of Predictive Toxicology Approaches Intended for Regulatory Application
- ESAC Opinion on the Scientific Validity of the GARDskin and GARDpotency Test Methods

Understanding Context of Use

Understanding Context of Use for Medical Devices: Case Study – U.S. Food and Drug Administration

Presenter: Dr. Shelby Skoog, U.S. Food and Drug Administration

This presentation will provide an overview of critical aspects for qualification of NAMs for biocompatibility evaluation of medical devices, with a focus on the importance of context of use. When assessing the applicability of a NAM for medical devices, the data needed for qualification depend on how the method is intended to be used in a regulatory context (e.g., the context of use), such as if it is proposed as a stand-alone replacement for an in vivo test and/or to provide supplementary data in an integrated approach. Evaluation of the NAM should include a comprehensive investigation of existing data and identification of any scientific gaps. For test methods that have been validated for neat chemicals, additional information may be needed to qualify the method for use in medical devices. For products evaluated by the U.S. Food and Drug Administration's Center for Devices and Radiological Health, there is a need to understand a NAM's performance with regard to detection of low concentrations of medical device-relevant weak to moderate toxicants in a mixture as compared to animal or human data and how a yes/no determination in a NAM correlates to a multi-point

grading scale in the in vivo method, if applicable. This presentation will discuss major concepts related to qualification of NAMs for use in medical devices, including chemical applicability domain and in vitro-in vivo correlation. Key concepts for designing qualification studies will be presented, including justification for test chemicals and controls, optimization of medical device-specific methods, and assessing performance.

Background

- Round Robin Study to Evaluate the Reconstructed Human Epidermis (RhE) Model as an In Vitro Skin Irritation Test for Detection of Irritant Activity in Medical Device Extracts

Context of Use of Mammalian Median Lethal Dose (LD_{50}) in Ecological Assessment at U.S. Environmental Protection Agency

Presenter: Dr. William Eckel, U.S. Environmental Protection Agency

Many program offices in the U.S. Environmental Protection Agency (EPA) conduct assessments of the risks of chemical stressors to mammalian species (https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/models-pesticide-risk-assessment#terrestrial). While exposure routes and context vary by program, acute toxicity endpoints usually come from median lethal dose (LD_{50}) tests on rats or mice. The Office of Pesticide Programs (OPP) uses an exposure model (T-REX) that converts application rates (lb/acre) to standard dietary exposures including plant tissues, invertebrates, and pesticide-treated granules and seeds, and then compares these exposures to the LD_{50}.

Over the past 40 years, OPP has amassed a database of rat LD_{50} data for hundreds of pesticide active ingredients and formulations. As part of EPA's commitment to reduce animal testing, OPP has worked with NICEATM (https://www.niehs.nih.gov/research/atniehs/labs/bmsb/niceatm/index.cfm) to compare OPP's data holdings to LD_{50} predictions from the Collaborative Acute Toxicity Modeling Suite (CATMoS) model (https://ehp.niehs.nih.gov/doi/full/10.1289/EHP8495) to build confidence in the CATMoS predictions, and to determine the circumstances in which these predictions might be used to replace or waive required animal data in pesticide risk assessments. This talk will present our progress to date.

Background

- CATMoS: Collaborative Acute Toxicity Modeling Suite
- T-REX Version 1.5 User's Guide for Calculating Pesticide Residues on Avian and Mammalian Food Items

Development of a Rapid Risk Assessment Process and Software Tools to Support Air Force Operational Decision-Making and Technology Acquisition)

Presenter: Dr. Rebecca Clewell, Henry Jackson Foundation for the Advancement of Military Medicine

The predictive toxicology team of the 711th Human Performance Wing of the Air Force Research Laboratory is developing a rapid risk assessment process to support operational decision-making and technology acquisition for the Air Force. This process will leverage existing toxicological data and exposure limits, as well as in silico and in vitro new approach methods to streamline risk assessment and enable quick turnaround chemical risk consults. The process is being designed to be flexible in order to support diverse scenarios – from in the field and emergency response, to in-garrison occupational health assessments. It can also be used to rapidly assess chemical hazards associated with technology under development, with a goal of doing so within a viable scope of time and cost to be able to assist decision-making on the potential toxicological drawbacks or benefits of the acquisition of that technology. The proposed assessment workflow incorporates traditional animal data, predictive models, and in vitro testing toward qualitative and quantitative risk estimates, but new capabilities are also being developed to support rapid assessments. For example, novel quantitative structure-activity relationship (QSAR) models for lung injury and neurotoxicity are currently being developed for use in the

workflow. Software tools for toxicological assessment (ToxAdvisor and other guided assessment software) are also being created and optimized to enhance rapid operational decision-making about chemical hazards.

Background

- Developing Context Appropriate Toxicity Testing Approaches Using New Approach Methodologies (NAMs)
- Reverse Molecular Docking and Deep-learning to Make Predictions of Receptor Activity for Neurotoxicology