Public-Private Partnerships to Advance In Vitro Eye Irritation Testing Methods and Approaches

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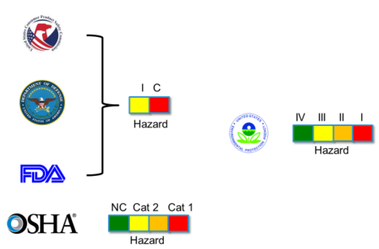
Introduction

- U.S. agencies have various requirements for eye irritation hazard classification and labeling (**Figure 1**).
- The Draize rabbit eye test is currently used to assess eye irritation and corrosion potential.
  - Test substance is instilled in one rabbit eye; the other eye serves as control.
  - Effects on the cornea and conjunctiva are subjectively evaluated for up to 21 days after instillation.
- In January 2018, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) developed "A Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States" (ICCVAM 2018).
- The roadmap describes three strategic goals:
  - Connect end users with developers of new approach methodologies
  - Foster the use of efficient, flexible, and robust practices to establish confidence in new methods
  - Encourage the adoption and use of new methods and approaches by federal agencies and regulated industries

- One approach to establishing confidence in new methods is through public-private partnerships. These allow cross-sector communication and cooperation among federal agencies and the private sector, to facilitate sharing knowledge, experience, and data.
- Public-private partnerships have been successfully implemented to advance new approaches for eye irritation and corrosion testing. These partnerships supported an alternate testing framework for U.S. Environmental Protection Agency classification of eye irritation potential for antimicrobial cleaning products (EPA 2015) and are continuing to move the science forward.
- To increase acceptance of novel in vitro and in silico methods, government, industry, and non-governmental stakeholders are collaborating to develop approaches for eye irritation testing that can be applied to specific substance and mixture types.

Figure 1. Eye Irritation Hazard Classification by U.S. Agencies



Crop Life America – EPA-NICEATM Collaboration: Data Set

- Paired data for over 200 agrochemical formulations were submitted by BASF, Dow, Bayer, Syngenta, and Dupont (**Table 1**).
- Paired data included rabbit eye test data and in vitro data from one or more assays:
  - Bovine corneal opacity and permeability (BCOP)
  - Isolated chicken eye (ICE)
  - EpiOcular (EO)
  - Neutral red release (NRR)
  - Chorioallantoic membrane vascular assay (CAMVA)
- Data were analyzed to determine if a defined approach could be used to assess eye irritation and corrosion potential of agrochemical formulations.
  - Defined approaches may incorporate data from multiple sources to predict eye irritation and corrosion potential of a formulation. Defined approaches apply a fixed data interpretation procedure (e.g., statistical model) to data generated with a defined set of information sources (e.g., a specific in vitro assay).
  - Predictions obtained from a defined approach are rule-based and not based on expert judgment.

Table 1. Distribution of Paired Data for Submitted Agrochemical Formulations

Company	Total Formulations in Data Set[a]	Formulations	A.I.s	Intermed.	BCOP	ICE	EO	NRR	CAMVA
A	68	68	-	-	-	-	52	68	-
B	104	97	7	-	97	10	97	-	-
C	14	-	14	-	14	-	-	-	4
D	56	42	8	6	14	56	5	-	-
E	25	25	-	-	-	25	-	-	-
Total	267	232	29	6	132	91	172	68	4

Abbreviations: A.I.s = active ingredients; Intermed. = intermediates

[a]Each formulation in a data set had in vivo data and data from one or more of the five in vitro assays in the five right-hand columns.

Crop Life America – EPA-NICEATM Collaboration: Results

- A tiered approach using EO and NRR was promising, but was not sufficient to identify all hazard categories.
- BCOP optimization and/or inclusion of a histopathology endpoint may be needed for accurate classification of agrochemical formulations.
- The ICE and CAMVA data sets were too small for definitive assessments.
- It was determined that additional prospective in vitro testing would be needed. Prospective testing would include:
  - Protocol optimization
  - Data generation for specific agrochemical formulation types

Prospective Testing of Agrochemical Formulations

- Co-organized by NICEATM and PETA-ISC
- Validation Management Team members from:
  - EPA Office of Pesticide Products
  - ICCVAM
  - European Union Reference Laboratory for Alternatives to Animal Testing
  - Canada's Pesticide Management Regulatory Authority
  - Industry

Study Design

- Two testing phases:
  - Phase 1: six formulations tested in five test methods (seven protocols) to demonstrate proof-of-concept
  - Phase 2: comprehensive assessment of applicability using a set of 40 formulations
    - Phase 2A: 10 formulations tested
    - Phase 2B: 30 formulations tested
- Coded formulations donated by:
  - BASF
  - Bayer
  - FMC
  - Corteva Agriscience (Agriculture Division of DowDuPont)
  - Monsanto
  - Syngenta
- Test substances were selected to:
  - Include a balance of all hazard classifications
  - Represent the most common agrochemical formulation types (suspension concentrates, emulsifiable concentrates, and soluble liquids)
  - Support comparison to high-quality in vivo data

Study Logistics

- **Table 2** lists the methods utilized, the applicable OECD test guidelines, and the laboratories conducting each test.
- Chemicals were distributed by the National Toxicology Program.
  - Coded substances to be forwarded to testing laboratories
  - Remaining materials may be archived for future use

Table 2. In Vitro Methods Used in Prospective Testing

Test Method	OECD Test Guideline	Testing Laboratory
BCOP	OECD TG 437	Institute for In Vitro Sciences
NRR	-	Institute for In Vitro Sciences
ICE	OECD TG 438	Citoxlab
EO (EIT method)	OECD TG 492	MatTek Corporation
EO (Time-to-toxicity method; ET50-neat)	-	MatTek Corporation
EO (Time-to-toxicity method; ET50-dilution)	-	MatTek Corporation
Porcine cornea reversibility assay (PorCORA)	-	MB Research Labs

In Vitro Methods Background

BCOP

- Bovine corneal tissue, obtained as a byproduct from a slaughterhouse, is mounted in chamber.
- Formulations are applied to the epithelial surface of the cornea.
- After designated exposure period, two endpoints are assessed:
  - Opacity – determined by light transmission through cornea
  - Permeability – determined by amount of fluorescein dye that penetrates through cornea
- Histopathology is used to analyze the degree and depth of corneal damage.

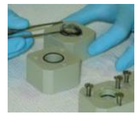


Photo courtesy Institute for In Vitro Sciences

NRR

- Cultured normal human epidermal keratinocytes are pre-exposed to neutral red medium.
- After pre-exposure, dilution series of test formulation is applied for 1 minute to culture surface and then removed.
- Neutral red retention by cells is measured spectrophotometrically.
- Cytotoxicity is measured as the concentration that causes 50% neutral red release.

Photo courtesy Institute for In Vitro Sciences

ICE

- Freshly isolated chicken corneas, obtained as byproducts from a slaughterhouse, are mounted.
- Formulation is applied for 10 seconds to the corneal surface and then rinsed off.
- Four endpoints are assessed at pre-defined time points up to 240 minutes after exposure:
  - Thickness – determined by amount of swelling using an optical pachymeter on a slit-lamp microscope
  - Opacity – determined by light transmission through cornea
  - Integrity – determined by fluorescein retention
  - Morphology – determined by visual inspection of the eye
- Histopathology is used to analyze the degree and depth of corneal damage.

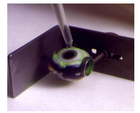


Photo courtesy Menk Prinsen, TNO

EO (EIT method)

- Nonkeratinized epithelium is prepared from normal human keratinocytes.
- Cells are seeded in an insert that contains a porous membrane to allow nutrients to reach the cells.
- Formulation is applied for a pre-defined exposure period and then rinsed off.
- Tissue viability is measured after exposure and a post-exposure incubation period using a vital dye (e.g., MTT).

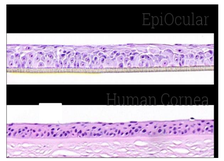


Photo courtesy MatTek Corporation

EO (Time-to-toxicity method)

- Same construct is used as for EO EIT method.
- Two different protocols used:
  - Neat Protocol: Formulations tested undiluted and tissue viability measured at pre-defined time points up to 60 minutes after administration
  - Dilution Protocol: Formulations tested at 20% concentration and tissue viability measured at pre-defined time points up to 256 minutes after administration
- Cell viability is measured at different time points for each protocol; data are used in a decision tree to determine hazard labeling.

PorCORA

- Excised porcine corneal tissues, obtained as byproducts from a slaughterhouse, are maintained in culture for up to 3 weeks.
- Tissues are exposed to test article for 5 minutes and then rinsed.
- Tissues are stained with fluorescein to visualize damage.
- Area of damage is repeatedly assessed over 3 weeks to determine potential reversibility of formulation-induced damage.

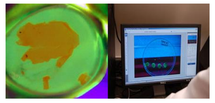


Photo courtesy MB Research Labs

Table 3. Testing Timeline

Milestone	Completed Date/Target Deadline
Laboratory testing of Phase 1 formulations completed	August 2018
Identification of methods for evaluation in Phase 2	September 2018
Shipment of Phase 2 formulations to testing laboratories	November 2018
Laboratory testing of Phase 2 formulations completed	February 2019
Data analysis and report preparation	May 2019

ICCVAM Agency Engagement with Stakeholders

- EPA established a stakeholder group to discuss development, evaluation, and implementation of alternative test methods for toxicity tests required for pesticide registration.
- EPA published a strategic plan to promote the development and implementation of alternative test methods for chemicals covered by the Toxic Substance Control Act (EPA 2018). The strategic plan was developed in collaboration with various stakeholders, including animal protection groups, regulated community, and non-governmental agencies.
- The 2018 FDA Predictive Toxicology Roadmap promotes development, acceptance, and implementation of cutting-edge science to assess safety and effectiveness of regulated products (FDA 2018). The Roadmap notes that the acceptance of methods requires continuous dialogue and feedback among all relevant U.S. Food and Drug Administration stakeholders.

Discussion and Conclusion

- Ethical concerns and a desire to increase human relevance of test data have increased interest in use of non-animal methods by regulatory agencies, regulated communities, and other stakeholders.
- Collaboration among these groups led to a successful implementation of a non-animal testing strategy for antimicrobial cleaning products.
- Building off this success, additional efforts are ongoing to broaden the application of non-animal eye irritation testing to agrochemical formulations.
- This multi-stakeholder collaboration model is being applied to other toxicity testing areas to further the use of in vitro and in silico test methods.

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