Developing a Defined Approach for Eye Irritation Testing

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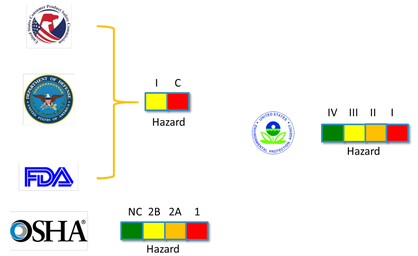
Introduction

- Establishing confidence in new methods requires public-private partnerships that allow cross-sector communication and cooperation among federal agencies and the private sector. These partnerships facilitate sharing knowledge, experience, and data.
- Eye irritation testing is conducted as part of the overall safety assessment of chemicals.
- There are several in vitro and ex vivo methods that can identify severe eye irritant and corrosive chemicals and chemicals that do not require hazard classification (i.e., "nonirritants"). However, no methods are available that can identify all eye irritation hazard categories.
- While validation of in vitro methods included a wide variety of regulated chemicals, results from prospective testing of agrochemicals have reported discordant results (Settivari et al. 2016; Kolle et al. 2017).
- PETA International Science Consortium Ltd., CropLife America companies, and the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) are collaborating to:
  - Assess the applicability of in vitro eye irritation/corrosion methods to agrochemical formulations
  - Develop a defined testing approach for prediction of U.S. and international irritancy classifications

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



- Color coding indicates relative level of human hazard.
  - Red = corrosive
  - Orange = moderate irritant
  - Yellow = mild irritant
  - Green = non-corrosive/minimal irritant
- Different classification schemes are used by agencies based on different regulatory needs.
  - The U.S. Environmental Protection Agency (EPA) uses its own four-tier classification system (Categories I-IV).
  - The four-tier system used by the Occupational Safety and Health Administration (OSHA) follows the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS; Categories 1, 2A, 2B, and Not Classified [NC]).
  - The other agencies pictured only require identification of irritant (I) and corrosive (C) chemicals.

Study Design

Table 1 lists the three phases of the study, the goals, activities, and completion dates.

- Agrochemical formulations tested in the study were selected to:
  - Include a range of hazard classifications
  - Focus on common formulation types, including:
    - Suspension concentrates
    - Emulsifiable concentrates
    - Soluble liquid
  - Support comparisons to high-quality in vivo data
- Coded formulations, donated by companies listed below, were distributed by NTP.
  - BASF
  - Bayer (and Monsanto)
  - FMC
  - Corteva Agriscience (formerly Dow-DuPont)
  - Syngenta
- Formulations were categorized using the EPA and GHS classification systems based on historical in vivo animal data.

Table 2 lists evaluated in vitro methods, applicable Organisation for Economic Co-operation and Development (OECD) test guidelines (TG), and laboratories that conducted the testing.

Table 3 provides the classification criteria for each in vitro test method. Most methods are not designed to distinguish mild/moderate irritants ("NA" in "Concordant" column for Categories II/2A and III/NC).

Table 1. Study Phases

Phase	Phase Goal	Activities	Completion Dates
Phase 1	Initial testing with Category I/Category 1 and Category IV/Not Classified formulations to assess validity of included assays	Testing six formulations in all in vitro test methods	September 2018
Phase 2	Expand testing to include formulations classified as Category II, III, or Category 2 to refine test methods for potential use in defined approach	Testing 10 formulations in all in vitro test methods	March 2019
Phase 3	Greater expansion of formulation categories in test methods identified for incorporation in a potential defined approach for eye irritation classification	Testing 30 formulations in selected in vitro test methods	September 2020 (projected)

Table 2. Evaluated In Vitro Methods

Test Method	OECD TG	Testing Laboratory
Bovine Corneal Opacity and Permeability (BCOP)	OECD TG 437 (2017)	Institute for In Vitro Sciences
BCOP – Extended Incubation Period*	-	Institute for In Vitro Sciences
Neutral Red Release (NRR)	-	Institute for In Vitro Sciences
Isolated Chicken Eye (ICE)	OECD TG 438 (2018)	Citoxlab
Porcine Cornea Reversibility Assay (PorCORA)	-	MB Research Labs
EpiOcular (EO) (EIT method)	OECD TG 492 (2019)	MatTek
EO (Time-to-toxicity method; ET50-neat protocol)	-	MatTek
EO (Time-to-toxicity method; ET50-dilution protocol)	-	MatTek

*Method introduced in Phase 2 only.

Table 3. Phase 1 and 2 Results Classification Key for EPA and GHS Ocular Irritation Categories

	Category IV/Category NC			Category III/Category NC			Category II/Category 2A			Category I/Category 1		
	Concordant†	NPCBM†	Discordant†	Concordant†	NPCBM†	Discordant†	Concordant†	NPCBM†	Discordant†	Concordant†	NPCBM†	Discordant†
BCOP-OECD*	IVIS ≤3 and histopath as III or IV/NC, or negative	IVIS ≤3 and histopath as negative-slight	IVIS >3	NA	IVIS >3 and ≤55	IVIS <3 or >55	NA	IVIS >3 and ≤55	IVIS <3 or >55	IVIS >55 or histopath as I/1, severe, or moderate-severe	NA	IVIS <55
BCOP-Extended*	IVIS <15	NA	IVIS >15	NA	IVIS >15 and ≤55	IVIS <15 or >55	NA	IVIS >15 and ≤55	IVIS <15 or >55	IVIS >55	NA	IVIS <55
NRR*	NRR50 >250 mg/mL	NA	NRR50 ≤250 mg/mL	NA	NRR50 >50 mg/mL	NRR50 <50 mg/mL	NA	NRR50 >50 mg/mL	NRR50 <50 mg/mL	NRR50 <50 mg/mL	NA	NRR50 >50 mg/mL
ICE-OECD*	NC and histopath as NP	NP and histopath as NP	Any other combo	NA	NP and histopath as NP	Any other combo	NA	NP and histopath as NP	Any other combo	Cat 1 or histopath as Cat 1	NA	NC or NP and histopath as NP
PorCORA*	NA	Revers.	Irrevers.	NA	Revers.	Irrevers.	NA	Revers.	Irrevers.	Irrevers.	Revers.	NA
EO-OECD*	Viability >60%	NA	Viability ≤60%	NA	Viability ≤60%	Viability >60%	NA	Viability ≤60%	Viability >60%	NA	Viability ≤60%	Viability >60%
EO-neat ET50*	ET50 ≥70 min	NA	ET50 <70 min	ET50 ≥4 and <70	NA	ET50 <4 or ≥70	NA	Any ET50	NA	ET50 <4 min	NA	ET50 ≥4 min
EO-dil. ET50*	ET50 ≥256 min	ET50 >64 and <256 min	ET50 <64 min	NA	ET50 ≥16 and <256 min	ET50 <16 or >256 min	NA	ET50 ≥4 and <64 min	ET50 <4 or >64 min	ET50 <4 min	ET50 >4 and <16 min	ET50 ≥16 min
EO-CON4EI*	NC	NA	Cat 1 or 2	NA	Cat 2 or NC	Cat 1	NA	Cat 2 or NC	Cat 1	Cat 1	NA	Cat 2 or NC

Abbreviations: Cat = Category; CON4EI = Consortium for in vitro Eye Irritation Testing Strategy Project; combo = combination; dil. = dilution protocol; ET50 = exposure time required to reduce tissue viability to 50%; histopath = histopathology; Irrevers. = irritation did not reverse during 21-day observation period; IVIS = in vitro irritation score; NA = not applicable; NC = not classified; NP = no prediction; NPCBM = no prediction can be made; NRR50 = concentration of test substance that causes 50% release of incorporated neutral red dye; Revers. = irritation reversed during 21-day observation period.

*BCOP-OECD, ICE-OECD, and EO-OECD classifications are based on decision criteria defined in OECD TGs for individual test methods with modifications to accommodate the EPA classification system. Additionally, BCOP-OECD classification system was modified to incorporate histopathology results. Histopathology classification criteria for BCOP and ICE, and classification criteria for BCOP-extended, NRR, EO-neat ET50, and EO-dil. ET50 are based on criteria utilized by individual testing laboratories. EO-CON4EI classification criteria are described in Kandarova et al. (2018).

†Term key: Concordant result = classification based on in vitro results are concordant with classification based on in vivo data (color coded as green in **Tables 4** and **5**); Discordant result = classification based on in vitro results are discordant with classification based on in vivo data (color coded as red in **Tables 4** and **5**); NPCBM result = in vitro classification criteria does not allow for definitive classification of formulation (e.g., EO-OECD classification system indicates no classification prediction can be made when tissue viability ≤60%; therefore, formulations that produce this response cannot be classified) (color coded as orange in **Tables 4** and **5**).

Table 4. Phase 1 In Vitro Classification Results Relative to In Vivo Classification Results

	Category IV/Category NC			Category I/Category 1		
	Formulation A	Formulation B	Formulation C	Formulation D	Formulation E	Formulation F
BCOP-OECD[1]	Concordant	Concordant	Concordant	Concordant	Discordant	Concordant
NRR[2]	Discordant	Concordant	Concordant	Concordant	Concordant	Concordant
ICE-OECD[3]	NPCBM	Concordant	NPCBM	Discordant	Discordant	Concordant
PorCORA[4]	NPCBM	NPCBM	NPCBM	Concordant	Concordant	NPCBM
EO-OECD[2]	Concordant	Concordant	Concordant	NPCBM	NPCBM	NPCBM
EO-neat ET50[5]	Concordant	Concordant	Concordant	Concordant	Discordant	Concordant
EO-dil. ET50[5]	Concordant	Concordant	Concordant	Discordant	Discordant	Concordant
EO-CON4EI[6]	Concordant	Concordant	Concordant	Discordant	Discordant	Concordant

Abbreviations: CON4EI = Consortium for In Vitro Eye Irritation Testing Strategy Project; dil. = dilution protocol; ET50 = exposure time required to reduce tissue viability to 50%; NPCBM = no prediction can be made (see color/term key below).
Color/Term key: Green/Concordant = classification based on in vitro results are concordant with classification based on in vivo data; Red/Discord. = classification based on in vitro results are discordant with classification based on in vivo data; Orange/NPCBM = in vitro classification criteria does not allow for definitive classification of formulation (e.g., EO-OECD classification system indicates no classification prediction can be made when tissue viability ≤60%; therefore, formulations that produce this response cannot be classified).
[1]Classification based on most severe response obtained from IVIS or histopathology results.
[2]Classification based on most severe response obtained in two runs.
[3]Classification based on most severe response obtained from ICE score or histopathology results.
[4]Classification based on reversibility.
[5]Classification based on most severe response obtained in 2-3 runs.
[6]Classification presented in Kandarova et al. (2018). Mean of all runs used for decision tree calculations.

Table 5. Phase 2 In Vitro Classification Results Relative to In Vivo Classification Results

	Category IV/Category NC				Category III/Category NC	Category II/Category 2A	Category I/Category 1			
	Formulation G	Formulation H	Formulation I	Formulation J	Formulation K	Formulation L	Formulation M	Formulation N	Formulation O	Formulation P
BCOP-OECD[1]	NPCBM	NPCBM	NPCBM	Concordant	Discordant	Discordant	Concordant	Concordant	Concordant	Concordant
BCOP-Extended[2]	Concordant	Concordant	Concordant	Concordant	Discordant	Discordant	Concordant	Discordant	Concordant	Discordant
NRR[3]	Discordant	Concordant	Discordant	Discordant	Discordant	Discordant	Concordant	Concordant	Discordant	Discordant
ICE-OECD[4]	Concordant	Concordant	NPCBM	Concordant	NPCBM	Discordant	Discordant	Concordant	Concordant	Concordant
PorCORA[5]	NPCBM	NPCBM	NPCBM	NPCBM	NPCBM	NPCBM	NPCBM	Concordant	NPCBM	Concordant
EO-OECD[3]	Concordant	Concordant	Discordant	Concordant	NPCBM	NPCBM	NPCBM	NPCBM	NPCBM	NPCBM
EO-neat ET50[6]	Discordant	Concordant	Discordant	Concordant	Concordant	NPCBM	Concordant	Concordant	Concordant	Discordant
EO-dil. ET50[6]	NPCBM	Concordant	Discordant	Concordant	NPCBM	NPCBM	NPCBM	Discordant	Discordant	Discordant
EO-CON4EI[7]	Discordant	Concordant	Discordant	Concordant	NPCBM	NPCBM	Concordant	Discordant	Discordant	Discordant

Abbreviations: CON4EI = Consortium for In Vitro Eye Irritation Testing Strategy Project; dil. = dilution protocol; ET50 = exposure time required to reduce tissue viability to 50%; NPCBM = no prediction can be made (see color/term key below).
Color/Term key: Green/Concordant = classification based on in vitro results agreed with classification based on in vivo data; Red/Discordant = classification based on in vitro results did not agree with classification based on in vivo data; Orange/NPCBM = in vitro classification criteria does not allow for definitive classification of formulation (e.g., EO-OECD classification system indicates no classification prediction can be made when tissue viability ≤60%; therefore, formulations that produce this response cannot be classified).
[1]Classification based on most severe response obtained from IVIS or histopathology results.
[2]Classification based on IVIS.
[3]Classification based on most severe response obtained in two runs.
[4]Classification based on most severe response obtained from ICE score or histopathology results.
[5]Classification based on reversibility.
[6]Classification based on most severe response obtained in 2-3 runs.
[7]Classification presented in Kandarova et al. (2018). Mean of all runs used for decision tree calculations.

Results

Phase 1

- No single test method agreed with the in vivo data classification for all six pesticide formulations (**Table 4**).
- All methods were included in Phase 2.

Phase 2

- No single test method agreed with the in vivo data classification for all 10 pesticide formulations (**Table 5**).
- Lack of decision criteria for Category II and III ocular irritants currently limits classification of formulations in these hazard categories.

Conclusions and Future Directions

- Results suggest that combining results of multiple tests in an integrated testing strategy may be useful in classifying these formulations (e.g., BCOP and NRR, or BCOP and EO).
- Additional analyses are underway to include physicochemical properties and composition of tested formulations in integrated approaches and determine if there are any common features that impact in vitro test method accuracy.
- NICEATM is conducting an analysis of in vivo test method variability to establish a confidence interval for consideration when using these data for comparison to new approach methodologies.
- Adverse outcome pathways (AOPs) are currently under development for each of the major corneal and conjunctival tissue layers. Efforts are also underway to better understand the human-relevance of each of the available alternative test methods and to establish how each method aligns with each AOP (and where gaps in test method coverage exist).

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