Testing of Agrochemical Formulations Using In Vitro and Ex Vivo Eye Irritation Test Methods

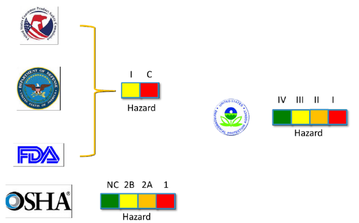
N Choksi[1], AJ Clippinger[2], S Gehen[3], M Corvaro[4], SN Kolle[5], K Bentley[6], A Hofstra[7], M Inforzato[8], N Ryan[9], E Webb[9], W Casey[10], N Kleinstreuer[10], D Allen[1]

[1]ILS, RTP, NC, USA; [2]PETA International Science Consortium Ltd., London, UK; [3]Corteva Agriscience, Indianapolis, IN, USA; [4]Corteva Agriscience, Rome, Italy; [5]BASF SE, Ludwigshafen, Germany; [6]FMC Agricultural Sciences, Newark, DE, USA; [7]Syngenta Canada Inc., Guelph, ON, Canada; [8]Syngenta Crop Protection LLC, Greensboro, NC, USA; [9]Bayer CropScience, St. Louis, MO, USA; [10]NIH/NIEHS/DNTP/NICEATM, RTP, NC, USA

Introduction

- Establishing confidence in new methods requires public-private partnerships. These partnerships facilitate sharing knowledge, experience, and data.
- Eye irritation testing is conducted as part of the overall safety assessment of chemicals.
- In vitro and ex vivo methods can identify severe eye irritants and chemicals that do not require hazard classification. However, no existing non-animal methods can identify all hazard categories.
- Prospective testing of agrochemicals using this methods has produced 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 defined approaches using these methods 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.

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Current affiliation for Natalia Ryan is Syngenta Crop Protection, LLC.

Study Design

Table 1 lists the study phases, goals, and activities.

- Agrochemical formulations tested in the study were selected to:
  - Include a range of hazard classifications.
  - Focus on common formulation types.
  - Support comparisons to historical high-quality in vivo data that allowed for categorization using the EPA and GHS classification systems.
- Donated formulations from companies listed below were distributed by NTP.
  - BASF
  - Bayer (and Monsanto)
  - FMC
  - Corteva Agriscience (formerly Dow-DuPont)
  - Syngenta

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

Table 3 provides the classification criteria for each in vitro test method. Most methods are not designed to distinguish mild/moderate irritants.

Table 1. Study Phases

	Phase Goal	Activities
Phase 1	Testing with EPA Category I/GHS Category 1 and EPA Category IV/GHS Not Classified formulations to assess validity of included assays	Testing 6 formulations in all in vitro test methods
Phase 2	Testing included formulations classified as EPA Category II/III or GHS Category 2 to refine test methods for potential use in defined approach	Testing 10 formulations in all in vitro test methods

EPA = U.S. Environmental Protection Agency; GHS = United Nations Globally Harmonized System of Classification and Labelling of Chemicals.

Table 2. Evaluated In Vitro Methods

Test Method	OECD TG	Testing Laboratory
Bovine Corneal Opacity and Permeability (BCOP)	OECD TG 437 (2020)	Institute for In Vitro Sciences (IIVS)
BCOP – Extended Incubation Period*	-	IIVS
Neutral Red Release (NRR)	-	IIVS
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		
Method[1]	**Concordant[2]**	**NPCBM[2]**	**Discordant[2]**	**Concordant**	**NPCBM**	**Discordant**	**Concordant**	**NPCBM**	**Discordant**	**Concordant**	**NPCBM**	**Discordant**
BCOP-OECD (IVIS and histo)	≤3 and histo as III or IV/NC, or negative	≤3 and histo as negative-slight	>3	NA	>3 and ≤55	<3 or >55	NA	>3 and ≤55	<3 or >55	>55 or histo as I/1, severe, or mod-severe	NA	<55
BCOP-Extend (IVIS)	<15	NA	>15	NA	>15 and ≤55	<15 or >55	NA	>15 and ≤55	<15 or >55	>55	NA	<55
NRR (NRR50)	>250 mg/mL	NA	≤250 mg/mL	NA	>50 mg/mL	<50 mg/mL	NA	>50 mg/mL	<50 mg/mL	<50 mg/mL	NA	>50 mg/mL
ICE-OECD	NC and histo as NP	NP and histo as NP	Any other combo	NA	NP and histo as NP	Any other combo	NA	NP and histo as NP	Any other combo	Cat 1 or histo as Cat 1	NA	NC or NP and histo 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	≥70 min	NA	<70 min	≥4 and <70	NA	<4 or ≥70	NA	Any ET50	NA	<4 min	NA	≥4 min
EO-dil. (ET50)	≥256 min	>64 and <256 min	<64 min	NA	≥16 and <256 min	<16 or >256 min	NA	≥4 and <64 min	<4 or >64 min	<4 min	>4 and <16 min	≥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%; histo = 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.
[1]BCOP-OECD, ICE-OECD, and EO-OECD classifications based on criteria in OECD TGs for test methods modified to accommodate EPA classifications. BCOP-OECD classification also 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 used criteria of individual testing laboratories. EO-CON4EI classification criteria described in Kandarova et al. (2018).
[2]Term key: Concordant result = classification based on in vitro results 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 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 EPA/GHS 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[6]	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 = in vitro classification agreed with in vivo classification; Red/Discordant = in vitro classification did not agree with in vivo classification; Orange/NPCBM = in vitro classification criteria does not allow for classification (e.g., EO-OECD criteria states no classification can be made when ≤60% tissue viability).
[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 EPA/GHS In Vivo Classification Results

	Category IV/Category NC				Category III/Category NC	Category II/Category 2A	Category I/Category 1			
	Form G	**Form H**	**Form I**	**Form J**	**Form K**	**Form L**	**Form M**	**Form N**	**Form O**	**Form P**
BCOP-OECD[1]	NPCBM	NPCBM	NPCBM	Concordant	Discordant	Discordant	Concordant	Concordant	Concordant	Concordant
BCOP-Extend[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 = in vitro classification agreed with in vivo classification; Red/Discordant = in vitro classification did not agree with in vivo classification; Orange/NPCBM = in vitro classification criteria does not allow for classification (e.g., EO-OECD criteria states no classification can be made when ≤60% tissue viability).
[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 was fully concordant with the in vivo data (**Table 4**).
- All methods were included in Phase 2.

Phase 2

- No single test method was fully concordant with the in vivo data (**Table 5**).
- Lack of decision criteria for EPA 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 may be useful in classifying these formulations (e.g., BCOP with histology and NRR, or BCOP and EO-OECD).
- Additional analyses are underway to include physicochemical properties and composition of tested formulations and determine if there are any common features that impact in vitro test method accuracy.
- NICEATM is evaluating in vivo test method variability to establish a confidence interval for consideration when using these data for comparison to new approach methodologies.
- Phase 1 and 2 results could be used to identify methods for evaluation of ≤30 formulations in a third phase of testing which could support developing defined approaches for testing agrochemical formulations for eye irritation potential.
- Consideration of human eye irritation mechanisms and the extent to which available in vitro/ex vivo methods align with these mechanisms will aid in developing integrated testing strategies that will be useful in classifying the eye irritation potential of agrochemicals following exposure.

References

Kandarova H, Letasiova S, Adriaens E, Guest R, Willoughby JA Sr, Drzewiecka A, Gruszka K, Alépée N, Verstraelen S, Van Rompay AR. 2018. Toxicol In Vitro. 49:34-52.

Kolle SN, Van Cott A, van Ravenzwaay B, Landsiedel R. 2017. Regul Toxicol Pharmacol. 85:33-47.

OECD. 2018. In: OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects. Paris: OECD Publishing. Available: https://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788.

OECD. 2019. In: OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects. Paris: OECD Publishing. Available: https://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788.

OECD. 2020. In: OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects. Paris: OECD Publishing. Available: https://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788

Settivari RS, Amado RA, Corvaro M, Visconti NR, Kan L, Carney EW, Boverhof DR, Gehen SC. 2016. Regul Toxicol Pharmacol. 81:407-420

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