Developing a Defined Approach for Eye Irritation Testing

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Introduction

§ Eye irritation testing is conducted as part of the overall safety assessment of chemicals.

§ While several in vitro and ex vivo methods can identify severe eye irritant and corrosive chemicals and chemicals that do not require hazard classification (i.e., “nonirritants”), no methods are available that can identify all eye irritation hazard categories.

§ Results from prospective testing of agrochemicals using in vitro methods have reported discordant results relative to in vivo tests.

§ Establishing confidence in new methods requires public-private partnerships that allow cross-sector communication and cooperation. PETA Science Consortium International, CropLife America companies, and the National Toxicology Program 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.

Study Design

§ 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 liquids.
  – Support comparisons to high-quality in vivo data.

§ Formulations were categorized using the EPA and GHS classification systems based on historical in vivo animal data.

§ **Table 1** lists evaluated in vitro methods, applicable Organisation for Economic Co-operation and Development (OECD) test guidelines (TG), and laboratories that conducted the testing.

Conclusions and Future Directions

- No single test method agreed with the in vivo data classification for all tested formulations (Tables 2 and 3).
- Combining multiple tests (e.g., BCOP and NRR, or BCOP and EO) in an integrated testing strategy may be useful in classifying these formulations.
- Additional testing with formulations identified as mild and moderate eye irritants is planned to further identify methods that may be complementary for hazard classification.
- Efforts are also underway to:
  - Better understand the human relevance of each of the available alternative test methods.
  - Establish how each method aligns with the mechanisms of human eye irritation and where gaps in test method coverage exist.

Table 1. 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
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)	Charles River (formerly 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 2. 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

[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 3. 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			
	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-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

[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.

Table Abbreviations and Color Key

Abbreviations
CON4EI = Consortium for In Vitro Eye Irritation Testing Strategy Project
dil. = dilution protocol
ET50 = exposure time required to reduce tissue viability to 50%
Form. = formulation
IVIS = in vitro irritation score
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).

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A summary of NICEATM and ICCVAM activities at the Eleventh World Congress is available on the National Toxicology Program website athttps://ntp.niehs.nih.gov/go/wc11.