National Institute of Environmental Health Sciences
Division of Translational Toxicology

Three-Phase Testing of Agrochemical Formulations: Developing Defined Approaches for Eye Irritation Potential

A.B. Daniel[1], A.J. van der Zalm[2], A.J. Clippinger[2], N.C. Kleinstreuer[3], and D.G. Allen[1]

[1]Inotiv, RTP, NC; [2]PETA Science Consortium International e.V., Stuttgart, Germany; [3]NIH/NIEHS/DTT/PTB/NICEATM, RTP, NC

Abstract 3137
Poster P245

Introduction

- Regulators require that agrochemical manufacturers provide information about potential harmful effects of their products.
- The accuracy of data from new methods for eye irritation testing has historically been determined solely through direct comparison to the Draize rabbit eye test, despite its demonstrated lack of reproducibility and relevance to humans (Luechtefeld et al. 2016, Clippinger et al. 2021).
- Data from non-animal test methods may be used in the development of defined approaches to predict the eye irritation potential of chemicals. Defined approaches are intended to overcome limitations of individual test methods by using information from multiple selected sources in a specific combination.
- The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and PETA Science Consortium International e.V. are collaborating to test agrochemical formulations in a multi-phase study using a common set of non-animal test methods.
- Our objectives are to assess the applicability of non-animal methods to agrochemical formulations and develop defined approaches that leverage strengths of these methods to predict the complete spectrum of eye irritancy potential.

Study Design

Test Substances:

- Formulations were donated by agrochemical companies and coded and distributed by NTP.
- Formulations were selected for testing based on the following criteria:
  - Availability of historical rabbit data or ocular irritancy classification information to enable the identification of drivers of classification (i.e., severity or persistence of a response) and to understand potential reasons for lack of reliability of the in vivo data.
  - Representation of common agrochemical formulation types.
  - Representation of a range of United Nations Globally Harmonised System of Classification and Labelling of Chemicals (GHS) and U.S. Environmental Protection Agency (EPA) hazard classifications (Table 1).

Testing Phases:

- **Phase 1:** Six formulations classified as GHS Category (Cat.) 1 or NC / EPA Cat. I or IV based on the in vivo rabbit test were tested in eight test methods/protocols to assess validity of test methods.
- **Phase 2:** Ten formulations classified as GHS Cat. 2A or 2B / EPA Cat. II or III based on the in vivo rabbit test were tested in eight test methods/protocols to refine test methods for potential use in defined approaches.
- **Phase 3:** Testing to expand the number of formulations classified as GHS Cat. 2A or 2B / EPA Cat. II or III based on the in vivo rabbit test.

Test Methods:

- Test methods included in Phase 3 were selected based on an assessment of Phase 1 and 2 results (see Choksi et al. 2021) and considering the relevance of each method to humans.
  - The EpiOcular™ standard protocol and the bovine corneal opacity and permeability (BCOP) standard protocol (with histopathology) were selected to proceed with Phase 3 testing of an additional 13 formulations classified as GHS Cat. 2A or 2B / EPA Cat. II or III based on the in vivo rabbit test.
  - Other test methods/protocols evaluated in Phase 1 and 2 (i.e., BCOP extended incubation period, neutral red release, isolated chicken eye, porcine cornea reversibility assay, and EpiOcular time-to-toxicity neat and diluted protocols) did not move forward (but may still be useful models).
  - In Phase 3, the common set of test methods was expanded to include newer methods (i.e., methods developed, optimized, or validated after initiation of this study):
    - All formulations were tested in SkinEthic Time-to-Toxicity approach for liquids, except Formulation AB for which the donated volume was insufficient.
    - Twelve GHS Cat. 2A or 2B / EPA Cat. II or III formulations were tested in the in vitro depth of injury (DoI) method.
    - A subset of 13 formulations spanning the full range of ocular irritancy has been tested in the EyeIRR-IS method.

Table 1. GHS and EPA Hazard Classification Systems and Associated PPE Statements

	GHS		EPA	
Effects	**Classification**	**PPE**	**Classification**	**PPE**
Corrosive	Category 1	Eye protection	Category I	Eye protection
Moderate irritant	Category 2A	Eye protection	Category II	Eye protection
Mild irritant	Category 2B	Eye protection	Category III	No minimum
Non-corrosive/ minimal irritant	Not Classified	None noted	Category IV	No minimum

Abbreviations: PPE = personal protective equipment

Table 2. Test Methods Evaluated in Phase 3

Test Method	Protocol	OECD TG	Testing Lab
Bovine corneal opacity and permeability (BCOP) with histopathology	Standard protocol, predictions based on IVIS and histo findings (BCOP-OECD)	OECD TG 437 (2020)	Institute for In Vitro Sciences
	Standard protocol, predictions based on LIS and histo findings (BCOP-LIS)	OECD TG 437 (2020)	
	Predictions based on IVIS as described in EPA Alternate Framework for AMCP (2015) and histo findings (BCOP-EPA)	-	
EpiOcular (EO)	Standard protocol (EO-OECD)	OECD TG 492 (2019)	MatTek
In vitro depth of injury (IVDoI)	Standard protocol, surfactants tested at 10% (IVDoI-10%)	-	Lebrun Labs
	All test articles tested neat (IVDoI-Neat)	-	
SkinEthic Time-to-Toxicity for liquids (TTL)	Standard protocol (TTL-OECD)	OECD TG 492B (2022)	EpiSkin
EyeIRR-IS	Standard protocol (EyeIRR-IS)	-	ImmunoSearch

Abbreviations: histo = histopathology; IVIS = in vitro irritancy score; LIS = laser light-based opacitometer irritancy score; OECD = Organisation for Economic Co-operation and Development; TG = Test Guideline

Table 3. Non-Animal Classification Criteria for Ocular Irritancy Categories

Table 3A. Non-Animal Classification Criteria for GHS Ocular Irritancy Categories

Test Method/ Protocol	GHS Classification				
	NC	**2B**	**2A**	**1**	**NPCBM**
BCOP-OECD	IVIS ≤ 55 and histo = minimal	IVIS ≤ 55 and histo = mild	IVIS ≤ 55 and histo = moderate	IVIS > 55; or histo = severe	NA
BCOP-LIS	LIS ≤ 30 and histo = minimal	LIS > 30 and lux/7 ≤ 145 and OD490 ≤ 2.5 and histo = mild	LIS > 30 and lux/7 ≤ 145 and OD490 ≤ 2.5 and histo = moderate	LIS > 30 and lux/7 ≤ 145 and OD490 > 2.5; or LIS > 30 and lux/7 > 145; or histo = severe	NA
EO-OECD	Viability > 60%	NA	NA	NA	Viability ≤ 60%
IVDoI-10%*	DoI = 0% and meta test = neg	0% < DoI < 15%	DoI = 0% and meta test = pos; or 15% ≤ DoI ≤ 20%	DoI > 20%	NA
IVDoI-Neat*					
TTL-OECD**	Viability > 50% for all three exposure times	Any other combination		Viability ≤ 50% for all three exposure times	NA
EyeIRR-IS**	LII < 10 at 30% and LII < 10 at 100%	LII < 10 at 30% and LII ≥ 10 at 100%		LII ≥ 10 at 30% (independently of the LII value obtained at 100%)	NA

Abbreviations: DoI = stromal depth of injury; histo = histopathology; IVIS = in vitro irritancy score; LII = liquid irritation index; LIS = laser light-based opacitometer irritancy score; meta = metabolic; NA = not applicable; NC = not classified; neg = negative; NPCBM = no prediction can be made; pos = positive
*Consensus classification based on 2 of 3 runs; **Prediction model does not distinguish GHS 2A/2B subcategories

Table 3B. Non-Animal Classification Criteria for EPA Ocular Irritancy Categories

Test Method/ Protocol	EPA Classification			
	IV	**III**	**II**	**I**
BCOP-EPA	NA	IVIS < 25 and histo = minimal or mild	IVIS < 75 and histo = moderate	IVIS ≥ 75; or histo = severe
IVDoI-10%	Stromal DoI = 0% and meta test = neg	Stromal DoI < 15%	Stromal DoI = 0% and meta test = pos; or 15% ≤ DoI ≤ 20%	Stromal DoI > 20%
IVDoI-Neat				

Abbreviations: DoI = depth of injury; histo = histopathology; IVIS = in vitro irritancy score; meta = metabolic; NA = not applicable; neg = negative; pos = positive

Table 4. Alignment of Predictions Across Non-Animal and In Vivo Test Methods

Formulation Information		GHS Predictions								EPA Predictions				
Code	**Type**	**BCOP-LIS#**	**IVDoI-10%#**	**EO-OECD**	**TTL-OECD**	**BCOP-OECD**	**IVDoI-Neat**	**EyeIRR-IS**	**Historical In Vivo**	**Consensus**	**IVDoI-10%#**	**IVDoI-Neat**	**BCOP-EPA**	**Historical In Vivo**	**Consensus**
A	EC/ME	-	NC†	NC	NC	NC	NC†	-	NC	**NC (5/5)**	IV	IV	III	IV	**IV (2/3)**
B	SC	-	NC†	NC	NC	NC	NC†	-	NC	**NC (5/5)**	IV	IV	III	IV	**IV (2/3)**
C	SC	-	NC†	NC	NC	NC	NC†	-	NC	**NC (5/5)**	IV	IV	III	IV	**IV (2/3)**
D	EC	-	1†	NPCBM	2	1	1†	-	1	**1 (3/4)**	I	I	I	I	**I (3/3)**
E	EC	-	1†	NPCBM	2	2B	1†	1	1	**1 (3/5)**	I	I	III	I	**I (2/3)**
F	SL	-	1†	NPCBM	1	1	1†	1	1	**1 (5/5)**	I	I	I	I	**I (3/3)**
G	EC	-	1†	NPCBM	2	1	1†	1	1	**1 (4/5)**	I	I	I	I	**I (3/3)**
H	SL	-	1†	NPCBM	1	1	1†	-	1	**1 (4/4)**	I	I	I	I	**I (3/3)**
I	SL	-	1†	NPCBM	2	1	1†	-	1	**1 (3/4)**	I	I	I	I	**I (3/3)**
J	EC	-	1†	NPCBM	2	1	1†	-	1	**1 (3/4)**	I	I	I	I	**I (3/3)**
K	SL	-	2A†	NPCBM	2	NC	NC†	2	2A	**2A (3/5)**	II	IV	III	II	**Inconclusive**
L	EC	-	NC†	NPCBM	2	NC	NC†	NC	NC	**NC (4/5)**	NC	IV	III	III	**III (2/3)**
M	SL	-	NC†	NC	NC	NC	NC†	NC	NC	**NC (6/6)**	IV	IV	III	IV	**IV (2/3)**
N	SC	-	NC†	NC	NC	NC	NC†	NC	NC	**NC (6/6)**	IV	IV	III	IV	**IV (2/3)**
O	SL	-	2A†	NPCBM	2	NC	2A†	NC	NC	**NC (3/5)**	II	II	III	IV	**Inconclusive**
P	SC	-	NC†	NC	NC	NC	NC†	-	NC	**NC (5/5)**	IV	IV	III	IV	**IV (2/3)**
Q	SL	2A	2A	NPCBM	2	2A	2A	-	NC	**2A (3/4)**	II	II	II	II	**II (3/3)**
R	SL	2A	1	NPCBM	1	2A	1	1	2A	**1 (3/5)**	I	I	II	II	**II (2/3)**
S	SL	2B	NC	NPCBM	2	2B	2A	-	2B	**2B (3/4)**	IV	II	III	III	**III (2/3)**
T	SC	2B	NC	NC	2	2B	NC	NC	NC	**NC (4/6)**	IV	IV	III	III	**III (2/3)**
U	EC	1	2A	NPCBM	2	2A	2A	-	2A	**2A (4/4)**	II	II	II	II	**II (3/3)**
V	SL	1	NC	NPCBM	1	1	1	1	2B	**1 (4/5)**	IV	I	II	III	**Inconclusive**
W	SL	2B	2A	NPCBM	2	2B	NC	-	NC	**Inconclusive**	II	IV	III	III	**III (2/3)**
X	EC	2A	1	NPCBM	2	2A	1	1	2A	**2A (3/5)**	I	I	II	II	**II (2/3)**
Y	EC	2B	NC	NPCBM	2	2B	2B	-	2A	**2B (3/4)**	IV	III	III	II	**III (2/3)**
Z	EC	2B	NC	NC	NC	2B	NC	NC	NC	**NC (5/6)**	IV	IV	III	III	**III (2/3)**
AA	EC	2B	NC	NPCBM	2	2B	2A	-	2A	**2A (3/4)**	IV	II	III	II	**II (2/3)**
AB	EC	2A	-	NPCBM	-	2A	-	-	2B	**Inconclusive**	-	-	II	III	**Inconclusive**
AC	EC	2B	1	NPCBM	2	2B	1	-	NC	**2B (2/4)**	I	I	III	III	**III (2/3)**

Abbreviations: EC = emulsifiable concentrate; ME = microencapsulated; NC = not classified; NPCBM = no prediction can be made; SC = suspension concentrate; SL = soluble liquid; - = not tested
#Data not used for consensus analysis; †Data generated in an independent study

Key

- Consensus prediction determined based on alignment between 3+ methods
- Consensus prediction determined based on alignment between 2 methods
- Inconclusive; unclear or insufficient data to determine a consensus prediction
- Alignment with consensus prediction
- Misalignment with consensus prediction; would not change PPE labeling
- Misalignment with consensus prediction; would change to PPE labeling

Results

GHS:

- Of the seven non-animal test methods/protocols evaluated in Phase 3 that predict GHS classification, data from five protocols (i.e., EO-OECD, TTL-OECD, BCOP-OECD, IVDoI-Neat, and EyeIRR-IS) were used to determine consensus predictions and to assess alignment across non-animal methods and the in vivo rabbit test. BCOP-LIS and IVDoI-10% protocols were excluded from this analysis to prevent consensus predictions being weighted toward a method with multiple protocols.
- Consensus predictions were achieved for 27 of 29 formulations for the GHS classification system.
- No single non-animal test method/protocol produced a result that aligned with the consensus prediction for all formulations.
- The historical in vivo rabbit test classification differed from the consensus prediction for five formulations: Q, R, V, Y, and AC.

EPA:

- Of the three non-animal test methods/protocols evaluated in Phase 3 that predict EPA classification, data from two protocols (i.e., IVDoI-Neat and BCOP-EPA) were used to determine consensus predictions and to assess alignment across non-animal methods and the in vivo rabbit test. The IVDoI-10% protocol was excluded from this analysis to prevent consensus predictions being weighted toward a method with multiple protocols.
- Consensus predictions were achieved for 25 of 29 formulations for the EPA classification system.
- No single non-animal test method/protocol produced a result that aligned with the consensus prediction for all formulations.
- The historical in vivo rabbit test classification differed from the consensus prediction for one formulation (formulation Y).

Conclusion and Future Directions

- The historical in vivo rabbit test classification did not concur with the GHS consensus prediction for five formulations and with the EPA consensus prediction for one formulation.
- The non-animal methods included in this evaluation offer equivalent or greater relevance to mechanisms associated with human eye irritation compared with the in vivo rabbit test.
- Results suggest that combining results of multiple non-animal tests in an integrated testing strategy may achieve an equivalent or superior predictive capacity than that of the in vivo rabbit test for eye irritation hazard classification of agrochemical formulations.
  - Defined approaches are being developed for the prediction of EPA eye irritation classification using the EO-OECD and/or BCOP-OECD methods, and for GHS eye irritation classification using different non-animal methods (e.g., TTL-OECD and BCOP-OECD). Based on initial analyses, the performance of these defined approaches for predicting the complete spectrum of eye irritancy potential are promising (manuscripts in preparation).

References and Acknowledgements

Choksi et al. 2021. NICEATM Report 01. DOI: 10.22427/NTP-NICEATM-1.

Clippinger et al. 2021. Cutaneous and Ocular Toxicology 40(2):145-167. DOI: 10.1080/15569527.2021.1910291.

EPA 2015. Office of Pesticide Programs, U.S. EPA. Available: https://www.epa.gov/sites/default/files/2015-05/documents/eye_policy2015update.pdf.

Luechtefeld et al. 2016. ALTEX 33(2): 123-134. DOI: 10.14573/altex.1510053.

OECD 2020. Test No. 437. OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects. DOI: 10.1787/9789264203846-en.

OECD 2019. Test No. 492. OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects. DOI: 10.1787/9789264242548-en.

OECD 2022. Test No. 492B. OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects. DOI: 10.1787/0d603916-en.

Test formulations were donated by BASF, Bayer (and Monsanto), FMC, Corteva Agriscience (formerly Dow-DuPont), and Syngenta.

This project was funded with federal funds from NIEHS, NIH under Contract No. HHSN273201500010C.

The views expressed above do not necessarily represent the official positions of any federal agency.

Subscribe to the NICEATM News email list
https://npt.niehs.nih.gov/go/niceatm