

# Three-Phase Testing of Agrochemical Formulations: Developing Defined Approaches for Eye Irritation Potential A.B. Daniel<sup>1</sup>, A.J. van der Zalm<sup>2</sup>, A.J. Clippinger<sup>2</sup>, N.C. Kleinstreuer<sup>3</sup>, and D.G. Allen<sup>1</sup>

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## 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<sup>™</sup> 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 (Dol) method.
  - A subset of 13 formulations spanning the full range of ocular irritancy has been tested in the EyeIRR-IS method.

Table 1. GH and	S and EPA Associate	Hazard Cl ed PPE Sta	assificatio Itements	n Systems	Table 3. Non-Animal Classification Criteria for Ocular Irritancy Categories									
GHS EPA					Table 3A. Non-Animal Classification Criteria for GHS Ocular Irritancy Categories									
Effects	Classification PPE		Classification	PPE	Test Method/	GHS Classification								
Corrosive	Category 1	Eye protection	Category I	Eye protection	Protocol	NC 2B		2A	1		NPCBM			
Moderate irritant	rate irritant Category 2A Eye protection		Category II	Eye protection		IVIS ≤ 55	IVIS ≤ 55	IVIS ≤ 55	IVIS > 55;		,			
Mild irritant	Category 2B	Eye protection	Category III	No minimum	BCOP-OECD	and histo = minimal	and histo = mild	and histo = moderate	or histo = s	severe	NA			
Non-corrosive/ minimal irritant	Not Classified	None noted	Category IV	No minimum			1.1S > 30 and $1.0x/7 < 145$	1.1S > 30 and $1.0x/7 < 145$	LIS > 30 and lux/7 ≤ 145					
Abbreviations: PPE = pers	onal protective equipme	ent			BCOP-LIS	LIS ≤ 30 and	and OD490 $\leq 2.5$	and OD490 $\leq 2.5$	or		NA			
Table 2. Tes	st Methods	Evaluated	in Phase 3	3		histo = minimal	and histo = mild	and histo = moderate	or histo = severe					
					EO-OECD	Viability > 60%	NA	NA	NA		Viability ≤ 60%			
Test Method	Pro	Protocol		Testing Lab	IVDoI-10%*	Dol = 0% and meta test = neg		Dol = 0% and	Dol > 20%		NA			
	Standard proto based on IV findings (B	Standard protocol, predictions based on IVIS and histo findings (BCOP-OECD)		Institute for In Vitro Sciences	IVDol-Neat*		0% < Dol < 15%	meta test = pos; or 15% ≤ Dol ≤ 20%						
Bovine corneal opaci	y Standard proto based on LIS a	Standard protocol, predictions based on LIS and histo findings			TTL-OECD**	Viability > 50% for all three exposure times Any other combination		ombination	Viability ≤ 5 three expos	0% for all sure times	NA			
(BCOP) with histopathology	(BCC) Predictions ba described in	Predictions based on IVIS as described in EPA Alternate Framework for AMCP (2015) and histo findings (BCOP-EPA)			EyelRR-IS**	LII < 10 at 30% and LII < 10 at 100%	LII < 10 ar LII ≥ 10	at 30% nd at 100%	LII ≥ 10 at 30% (independently of the LII value obtained at 100%)		NA			
	Framework fo and hist (BCO				Abbreviations: Dol = meta = metabolic; N/ *Consensus classific	eviations: Dol = stromal depth of injury; histo = histopathology; IVIS = in vitro irritancy score; LII = liquid irritation index; LIS = laser light-based opacitometer irritancy score a = metabolic; NA = not applicable; NC = not classified; neg = negative; NPCBM = no prediction can be made; pos = positive asensus classification based on 2 of 3 runs; **Prediction model does not distinguish GHS 2A/2B subcategories								
EpiOcular (EO)	Standard proto	col (EO-OECD)	OECD TG 492	MatTek	Table 3B. Non	Table 3B. Non-Animal Classification Criteria for EPA Ocular Irritancy Categories								
· · · · · · · · · · · · · · · · · · ·	Standard prote				Test Method/		EPA Classification							
In vitro depth of injur	tested at 10%	tested at 10% (IVDoI-10%)			Protocol	IV		"	II		I.			
(IVDol)	All test articles tested neat		-	Lebrun Labs		ΝΔ	IVIS < 25	IVIS <	< 75 d	١٧	′IS ≥ 75;			
SkinEthic		Standard protocol (TTL-OECD) Standard protocol (EyeIRR-IS)					histo = minimal o	r mild histo = m	histo = moderatehistoromal Dol = 0% and meta test = pos; or $15\% \le Dol \le 20\%$ Stromatic		o = severe			
Time-to-Toxicity for liquids (TTL)	Standar (TTL-			EpiSkin	IVDol-10%	Stromal Dol = 0% and	Stromal Dol < 2	Stromal Dol = 0 test =			nal Dol > 20%			
EyelRR-IS	Standard proto			ImmunoSearch	IVDol-Neat	meta test = neg		or 15% ≤ Do						
Abbreviations: histo = hist score; OECD = Organisat	opathology; IVIS = in vit on for Economic Co-op	tro irritancy score; LIS peration and Developm	= laser light-based opa nent; TG = Test Guideli	acitometer irritancy	Abbreviations: Dol =	depth of injury; histo = histopat	hology; IVIS = in vitro irritancy score	; meta = metabolic; NA = not appli	cable; neg = negati	ve; pos = positiv	/e			

The Classification of the Classificat	Table 1. GHS and	and EPA Associate	Hazard Cl d PPE Sta	assificatio tements	n Systems	Table 3. Non-Animal Classification Criteria for Ocular Irritancy Categories									
Effects     Classification     PPE     Classification     PPE     Classification     PPE     Control in the production     Category 1     Eye production     Category 1     No	GHS EPA				Table 3A. Non-Animal Classification Criteria for GHS Ocular Irritancy Categories										
Conversion Modurate initial Modurate initial Mill initial Mi	Effects (	Classification	PPE	Classification	PPE	Test Method/		GHS Classification	lassification						
Model relation in the Category 2A     Eye protection (All price on only and local seguery 2B)     Eye protection (All price on only and local seguery 2B)     Eye protection (All price on only and local seguery 2B)     Eye protection (All price on only and local seguery 2B)     Eye protection (All price on only and local seguery 2B)     INIS s 55 (All price on only and local segu	Corrosive	Category 1	Eye protection	Category I	Eye protection	Protocol	NC	2B	2A	1		NPCBM			
Midi mitaniani     Categopy 28     Eye protoccion     Categopy 111     No minimum       Mid mitaniani     Nort-corrective minimad intraser     Nort Classified     Nore noted     Categopy 111     No minimum       Mid minimad intraser     Nort Classified     Nore noted     Categopy 111     No minimum       Attendiation: PPE - personal question equipment     Exercision equipment <td>Moderate irritant</td> <td>Category 2A</td> <td>Eye protection</td> <td>Category II</td> <td>Eye protection</td> <td></td> <td>IVIS ≤ 55</td> <td>IVIS ≤ 55</td> <td>IVIS ≤ 55</td> <td>IVIS &gt;</td> <td><b>&gt;</b> 55;</td> <td></td>	Moderate irritant	Category 2A	Eye protection	Category II	Eye protection		IVIS ≤ 55	IVIS ≤ 55	IVIS ≤ 55	IVIS >	<b>&gt;</b> 55;				
Non-encode log     Note Classified     None noted     Category IV     Non-minimum       Addresesting     None noted     Category IV     Non-minimum     Non-minim <td< td=""><td>Mild irritant</td><td>Category 2B</td><td>Eye protection</td><td>Category III</td><td>No minimum</td><td>BCOP-OECD</td><td>and histo = minimal</td><td>and histo = mild</td><td>and histo = moderate</td><td colspan="2">or histo = severe</td><td>NA</td></td<>	Mild irritant	Category 2B	Eye protection	Category III	No minimum	BCOP-OECD	and histo = minimal	and histo = mild	and histo = moderate	or histo = severe		NA			
Abbrevietions: PPE - personal productive equipment   BCOP-LIS   LIS 4.30 and histo = minimal   and OD490 S.2.3 and histo = minimal	Non-corrosive/ minimal irritant	Not Classified	None noted	Category IV	No minimum		_	$1.1S > 30$ and $1_{1}/7 \le 145$	$1.1S > 30$ and $1/2/7 \le 145$	LIS > 30 and	LIS > 30 and lux/7 ≤ 145 and OD490 > 2.5; or				
Table 2. Test Methods Evaluated in Phase 3   Initial Phase = minimal   and histo = minimal   and histo = minimal   LIS > 30 and hull /> 145; or moderate   LIS > 30 and hull /> 145; or moderate     Test Method   Protocol   QECD TG   Testing Lab   NA	Abbreviations: PPE = persor	al protective equipme	ent			BCOP-LIS	LIS ≤ 30 and	and OD490 $\leq 2.5$	and OD490 ≤ 2.5	or					
ControlProtocolOECD TGTesting LabStandard protocol, predictions based on IVIS and frator findings (BCOP-UECD)OECD TG 4.37 (2020)NA $Dol = 0\%$ and metatest = neg $Dol = 0\%$ and $Dol = 0\%$ $Dol = 0\%$ and metatest = neg $Dol = 0\%$ and $Dol = 0\%$ $Dol = 0\%$ and $Dol = 0\%$ $Dol = 0\%$ and $Dol = 0\%$ $NA$ Finderport (BCOP-LIS)CECD TG 432 (2020)Institute for in Vitro Steiners $Dol = 0\%$ (2020) $Dol = 0\%$ and $Dol = 0\%$ $Dol = 0\%$ and $Ul = 10 a 100\%$ $NA$ $NA$ Finderport (BCOP-LIS)CECD TG 432 (2020)MatTek (2019) $Dol = 0\%$ and distor findings $Dol = 0\%$ and $Ul = 10 a 100\%$ $NA$ $NA$ $NA$ Finderport (BCOP-LIS)Standard protocol (BCOP-LIS) $OECD TG 492$ (2019)MatTek (2019) $Epolenion (NS = not metateopic$	Table 2, Test	Methods	Fvaluated	in Phase ?	3		histo = minimal	and histo = mild	and histo = moderate	or histo = severe					
Test Method     Protocol     OECD TG     Testing Lab       Standard protocol, predictions based on IVS and histo findings (BCOP-DECD)     Standard protocol, predictions based on IVS and histo findings (BCOP-DECD)     OECD TG 437 (2020)     Image and permetable (2020)     Image and permetable (2020)     Dol = 0% and meta test = nog     Dol = 0% and meta test = nog     Dol = 0% and meta test = nog     Dol > 20%     NA       Bovine corneal opacity is dead on LIS and histo findings (BCOP-DED)     Standard protocol, predictions based on IVS as described in EPA Attemate Framework for AMCP (2015)     OECD TG 437 (2020)     Institute for In Vitro Sciences     Institute for In Vitro Sciences     UI = 10 at 30% (UDol-10%)     UI = 10 at 30% UI = 10 at 100%     UI = 10 at 30% (Independentity in EVA and genetice exposure times     NA       EpiOcular (EO)     Standard protocol, greating (BCOP-EPA)     OECD TG 492 (2019)     MatTek     Lil = 10 at 100%     UI = 10 at 100%     UI = 10 at 100%     UI = 10 at 100%     III = 10						EO-OECD	Viability > 60%	NA	NA	NA		Viability ≤ 60%			
Standard protocol, predictions findings (BCOP-OEDD)     OED TG 437 (2020)     Name     Name     Name     Name       Bovine correat opacity and permeability and permeability based on IVIS and histo indings (BCOP-UED)     Standard protocol, predictions based on IVIS and (2020)     OED TG 437 (2020)     Institute for In Vito (2020)     Intel (1 of 1 30% (2020)     Intel (1 of 1 30% (20	Test Method	Protocol		OECD TG	Testing Lab	IVDoI-10%*	Dol = 0%		Dol = 0% and	Dol > 20%					
Bowine corneal opcoding and permeability (BCOP) with histopathology     Standard protocol, predictions based on LVS and described in EPA Atternate Framework for AMCP (2015) and histo findings and histo finding and histo finding and histo finding and histo finding and histo finding and histo findings		Standard protocol, predictions based on IVIS and histo findings (BCOP-OECD)		OECD TG 437 (2020)		IVDol-Neat*	and meta test = neg	0% < Dol < 15%	meta test = pos; or 15% ≤ Dol ≤ 20%			NA			
(BCOP) with histopathology   (BCOP-LIS)   (BCOP)	Bovine corneal opacity and permeability	Standard protocol, predictions based on LIS and histo findings		OECD TG 437 (2020)	Institute for In Vitro Sciences	TTL-OECD**	Viability > 50% for all three exposure times	Any other of	combination	Viability ≤ 50% for all three exposure times		NA			
Framework for AMCP (2015) and histo findings (BCOP-EPA)   -   Abbreviations: Dol = 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; NPCBMuis = not rediscipative index pose = positive "consensus classification model dees not distinguistic index; Prediction model dees not model dees note index; PREDICTION model dees not index; PREDICTION	(BCOP) with histopathology	(BCO Predictions ba described in	Predictions based on IVIS as described in EPA Alternate			EyelRR-IS**	LII < 10 at 30% and LII < 10 at 100%	LII < 10 a LII ≥ 10	) at 30% nd at 100%	LII ≥ 10 at 30% (independently of the LII value obtained at 100%)		NA			
EpiOcular (EO)   Bandard protocol (EO-OECD)   OECD TG 492 (2019)   MaTek   Table 3B. Non-Atimal Classification Criteria for EPA Ocular Irritancy Categories     In vitro depth of injury (IVDol)   Standard protocol, surfactants tested at 10% (IVDol-Neat)   1   I   I     All test arrices tested neat (IVDol-Neat)   -		⊢ramework for AMCP (2015) and histo findings (BCOP-EPA)		-		Abbreviations: Dol = meta = metabolic; NA *Consensus classific	Abbreviations: Dol = 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								
Leproduct (gb)   Lebrun Labs   Test Method/ Protocol   Test Method/ Protocol   EPA Classification     In vitro depth of injury (IVDol)   Standard protocol, surfactants tested at 10% (IVDol-10%)   -   Lebrun Labs   IV   III   II   I   I     All test articles tested neat (IVDol)-Neat)   -   Lebrun Labs   BCOP-EPA   NA   IVIS < 25	EpiOcular (EO)	Standard proto	col (EO-OECD)	OECD TG 492	MatTek	Table 3B. Non-Animal Classification Criteria for EPA Ocular Irritancy Categories									
In vitro depth of injury (IVDol) Calculation protocol tested at 10% (IVDol-10%) Calculation tested at 10% (IVDol-10%) Calculation (IVDol-Neat) Calculation Calculation (IVDol-Neat) Calculation Calculation (IVDol-Neat) Calculation Calculation (IVDol-Neat) Calculation Calculation (IVDol-Neat) Calculation Calculation (IVDol-Neat) Calculation Calculation (IVDol-Neat) Calculation Calculation (IVDol-Neat) Calculation Calculation (IVDol-Neat) Protocol Calculation (IVDol-Neat) Protocol Calculation (IVDol-Neat) Protocol Calculation (IVDol-Neat) IVIS < 75 (IVIS < 75) (IVIS < 75)	,			(2019)		Test Method/		EPA Classification							
(IVDol)   All test articles tested neat (IVDol-Neat)   -   Lebitit Labs   BCOP-EPA   NA   IVIS < 25 and   IVIS < 75 and   IVIS ≥ 75; and     SkinEthic Time-to-Toxicity for liquids (TTL)   Standard protocol (TTL-OECD)   OECD TG 492B (2022)   EpiSkin   IVDol-10%   Stromal Dol = 0% and   Stromal Dol = 0% and   Stromal Dol = 0% and   Stromal Dol < 15%	In vitro depth of injury	All test articles tested neat		-	Lakawa Laka	Protocol BCOP-EPA	IV	III	II	II IVIS < 75 and		1			
Skine thic Time-to-Toxicity for liquids (TTL)   Standard protocol (TTL-OECD)   OECD TG 492B (2022)   EpiSkin   EpiSkin   IVDol-10%   Stromal Dol = 0% and meta test = neg   Stromal Dol = 0% and meta test = neg   Stromal Dol < 15%   Stromal Dol < 15%   Stromal Dol < 20%   Stromal Dol > 20%     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   ImmunoSearch   IVDol-Neat   meta test = neg   Stromal Dol < 15%   Stromal Dol ≤ 20%   Stromal Dol ≤ 20%	(IVDol)			-	Lebrun Labs		NA	IVIS < 25 and	IVIS -			IS ≥ 75; or			
Time-to-Toxicity for liquids (TTL)   Standard protocol (TTL-OECD)   OECD 1G 492B (2022)   EpiSkin   IVDol-10%   Stromal Dol = 0% and   Stromal Dol = 0% and meta test = pos;   Stromal Dol = 0% and meta test = pos;   Stromal Dol > 20%     EyeIRR-IS   Standard protocol (EyeIRR-IS)   -   ImmunoSearch   IVDol-Neat   meta test = neg   Stromal Dol < 15%	SkinEthic		(IVD0I-Neal)					histo = minimal o	or mild histo = m	histo = moderatehisStromal Dol = 0% and meta test = pos; or $15\% \le Dol \le 20\%$ Strom		o = severe			
EyeIRR-IS   Standard protocol (EyeIRR-IS)   -   ImmunoSearch     Abbreviations: histo = histopathology; IVIS = in vitro irritancy score; LIS = laser light-based opacitometer irritancy   IVDol-Neat   meta test = neg   0r     Abbreviations: histo = histopathology; IVIS = in vitro irritancy score; LIS = laser light-based opacitometer irritancy   Abbreviations: Dol = depth of injury; histo = histopathology; IVIS = in vitro irritancy score; meta = metabolic; NA = not applicable; neg = negative; pos = positive	Time-to-Toxicity for liquids (TTL)	(TTL-OECD)		(2022)	EpiSkin	IVDoI-10%	Stromal Dol = 0% and	Stromal Dol <	15% Stromal Dol =			nal Dol > 20%			
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 Abbreviations: DoI = depth of injury; histo = histopathology; IVIS = in vitro irritancy score; meta = metabolic; NA = not applicable; neg = negative; pos = positive	EyelRR-IS	Standard protocol (EyeIRR-IS)		-	ImmunoSearch	IVDoI-Neat	meta test = neg		o 15% ≤ Do						
	Abbreviations: histo = histop score; OECD = Organisation	athology; IVIS = in vit	ro irritancy score; LIS eration and Developm	= laser light-based opa ent; TG = Test Guidelin	acitometer irritancy	Abbreviations: Dol =	depth of injury; histo = histopath	nology; IVIS = in vitro irritancy scor	e; meta = metabolic; NA = not appl	icable; neg = negati	ive; pos = positiv	/e			

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

Formulation				GHS Predictions								EPA Predictions				
Code	Туре	BCOP-LIS <sup>#</sup>	IVDoI-10% <sup>#</sup>	EO-OECD	TTL-OECD	BCOP-OECD	IVDol-Neat	EyelRR-IS	Historical In Vivo	Consensus	IVDoI-10% <sup>#</sup>	IVDol-Neat	BCOP-EPA	Historical In Vivo	Consensus	Кеу
A	EC/ME	-	NC <sup>†</sup>	NC	NC	NC	NC <sup>†</sup>	-	NC	NC (5/5)	IV	IV	III	IV	IV (2/3)	
В	SC	-	NC <sup>†</sup>	NC	NC	NC	NC <sup>†</sup>	-	NC	NC (5/5)	IV	IV	III	IV	IV (2/3)	Consensus prediction
С	SC	-	NC <sup>†</sup>	NC	NC	NC	NC <sup>†</sup>	-	NC	NC (5/5)	IV	IV		IV	IV (2/3)	alignment between 3+
D	EC	-	1†	NPCBM	2	1	1 <sup>†</sup>	-	1	1 (3/4)	I	l I	l l		l (3/3)	methods
E	EC	-	1†	NPCBM	2	2B	1†	1	1	1 (3/5)	I	l l	III	l l	l (2/3)	
F	SL	-	1†	NPCBM	1	1	1†	1	1	1 (5/5)	I	l I	l I	l l	l (3/3)	Consensus prediction
G	EC	-	1†	NPCBM	2	1	1†	1	1	1 (4/5)	I	l I	l l	l l	l (3/3)	determined based on
Н	SL	-	1†	NPCBM	1	1	1†	-	1	1 (4/4)	I	l I	l l	I	l (3/3)	alignment between 2
I	SL	-	1†	NPCBM	2	1	1†	-	1	1 (3/4)	I	l I	l l	l l	l (3/3)	methods
J	EC	-	1†	NPCBM	2	1	1 <sup>†</sup>	-	1	1 (3/4)	I	l I	l l	-	l (3/3)	
К	SL	-	2A <sup>†</sup>	NPCBM	2	NC	NC <sup>†</sup>	2	2A	2A (3/5)	II	IV	III	=	<b>Inconclusive</b>	Inconclusive: unclear
L	EC	-	NC <sup>†</sup>	NPCBM	2	NC	NC <sup>†</sup>	NC	NC	NC (4/5)	NC	IV	III	Ш	III (2/3)	or insufficient data to
М	SL	-	NC <sup>†</sup>	NC	NC	NC	NC <sup>†</sup>	NC	NC	NC (6/6)	IV	IV	III	IV	IV (2/3)	determine a
N	SC	-	NC <sup>†</sup>	NC	NC	NC	NC <sup>†</sup>	NC	NC	NC (6/6)	IV	IV		IV	IV (2/3)	consensus prediction
0	SL	-	2A <sup>†</sup>	NPCBM	2	NC	2A <sup>†</sup>	NC	NC	NC (3/5)	II	II	III	IV	<b>Inconclusive</b>	
Р	SC	-	NC <sup>†</sup>	NC	NC	NC	NC <sup>†</sup>	-	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)	Alignment with
R	SL	2A	1	NPCBM	1	2A	1	1	2A	1 (3/5)	I	I	II	II	II (2/3)	consensus prediction
S	SL	2B	NC	NPCBM	2	2B	2A	-	2B	2B (3/4)	IV	ll ll	III	III	III (2/3)	
Т	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	I	II (3/3)	Misalignment with
V	SL	1	NC	NPCBM	1	1	1	1	2B	1 (4/5)	IV	I	II	III	Inconclusive	consensus prediction;
W	SL	2B	2A	NPCBM	2	2B	NC	-	NC	Inconclusive		IV	III	III	III (2/3)	would not change
Х	EC	2A	1	NPCBM	2	2A	1	1	2A	2A (3/5)	I	l	II	II	II (2/3)	PPE labeling
Y	EC	2B	NC	NPCBM	2	2B	2B	-	2A	2B (3/4)	IV	III		I	III (2/3)	
Z	EC	2B	NC	NC	NC	2B	NC	NC	NC	NC (5/6)	IV	IV		III	III (2/3)	Misalignment with
AA	EC	2B	NC	NPCBM	2	2B	2A	-	2A	2A (3/4)	IV	II	III	II	II (2/3)	consensus prediction;
AB	EC	2A	-	NPCBM	-	2A	-	-	2B	Inconclusive	-	-			Inconclusive	
AC	EC	2B	1	NPCBM	2	2B	1	-	NC	2B (2/4)				III	III (2/3)	laboling
Abbroviation	e: EC – omulai	- iable concentrate	ME – microoncor	oulated: NC - n	at alassified: NDC	PM – no prodiction	an ha mada: Si		noontroto: SI		- act tootod					

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

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# Abstract 3137 Poster P245

## **Results**

#### <u>GHS:</u>

- 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 eve 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 nonanimal 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.

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