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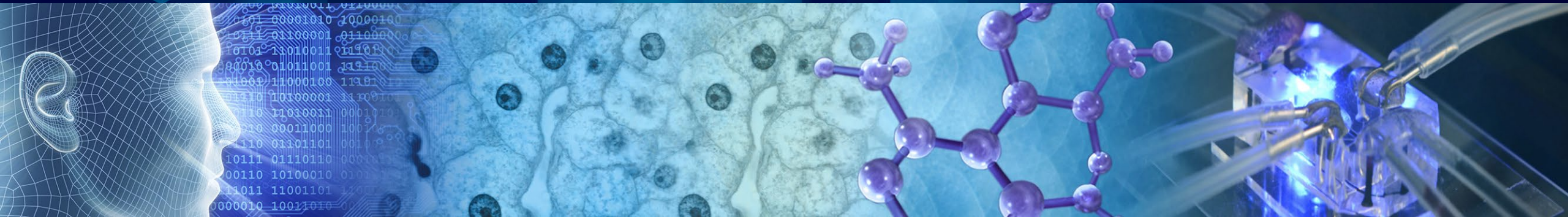

Defined Approaches for Predicting GHS and EPA Eye Irritation Classification of Agrochemicals

Amber Daniel, MTox

Inotiv, contractor supporting the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

ASCCT-ESTIV Award Winners Webinar
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Outline

INTRODUCTION

STUDY DESIGN

RESULTS & ANALYSES

CONCLUSIONS

Conflict of Interest Statement

The authors declare that there exist no actual or potential conflicts of interest.

Introduction

Alternatives to animal testing for eye irritation have historically been assessed by direct comparison with Draize rabbit eye test

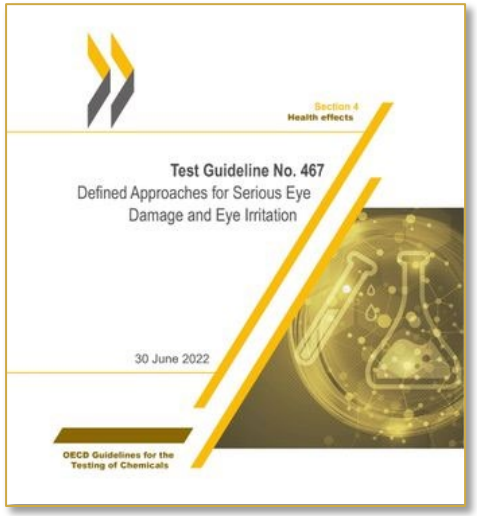
Rabbit test has been demonstrated to lack reproducibility and human relevance

Movement away from direct comparisons in favor of evaluating based on reliability and human-relevance of the method

Luechtefeld et al. 2016 ALTEX
Clippinger et al. 2021 Cutan Ocul Toxicol
van der Zalm et al. 2022 Arch Toxicol

Defined Approaches (DAs): A rule-based data interpretation procedure that is applied to data generated with a defined method(s) to derive a prediction.

- No expert judgment is required.
- DAs can achieve an equivalent or better predictive capacity than that of the animal test to predict responses in humans.



US EPA Office of Pesticide Programs

PETA Science Consortium International, e.V.

NIEHS/DTT/NICEATM

1. **Assess the applicability of in vitro methods to agrochemical formulations**
2. **Develop DAs that leverage strengths of these methods to predict the complete spectrum of eye irritancy potential**

Study Design

Phase 1

Assess validity of test methods

Phase 2

Refine test methods for potential use in defined approaches

Phase 3

Expand the number of formulations classified as mild or moderate irritants based on the in vivo test

29 agrochemical formulations selected based on:

- Availability of historical rabbit data or ocular irritancy classification information.
- Representation of common agrochemical formulation types (i.e., emulsifiable concentrate, suspension concentrate, soluble liquids).
- Representation of the full range of GHS and EPA hazard classifications.

Assays/protocols evaluated:

- BCOP - OP-KIT opacitometer in vitro irritancy score (w/ histopathology)
- BCOP - extended incubation in vitro irritancy score (w/ histopathology)
- BCOP - laser light-based opacitometer irritation score (w/ histopathology)
- EpiOcular - standard protocol
- EpiOcular - time-to-toxicity neat
- EpiOcular - time-to-toxicity diluted
- neutral red release
- isolated chicken eye
- porcine cornea reversibility assay
- SkinEthic time-to-toxicity for liquids
- EyeIRR-IS
- in vitro depth of injury - neat protocol
- in vitro depth of injury - diluted protocol

Full NICEATM report available at:
https://doi.org/10.22427/NTP-NICEATM-1

Selection of Assays for Inclusion in DAs

Test Method	OECD TG	Human Relevant
Bovine corneal opacity and permeability (BCOP) with histopathological depth of injury evaluation	437	-
EpiOcular™ Eye Irritation Test (EO)	492	Yes
SkinEthic™ time-to-toxicity for liquids (TTL)	492B	Yes
EyeIRR-IS	-	Yes

Developing DAs

DAs for **EPA** Classification of Agrochemicals:

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2024, VOL. 43, NO. 1, 58–68
https://doi.org/10.1080/15569527.2023.2275029

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RESEARCH ARTICLE

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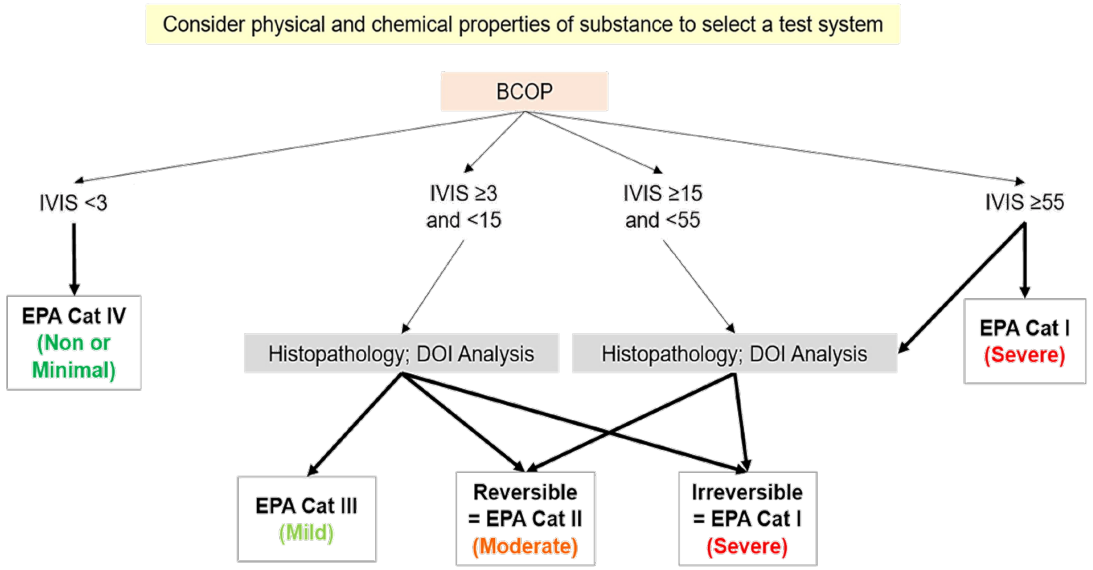
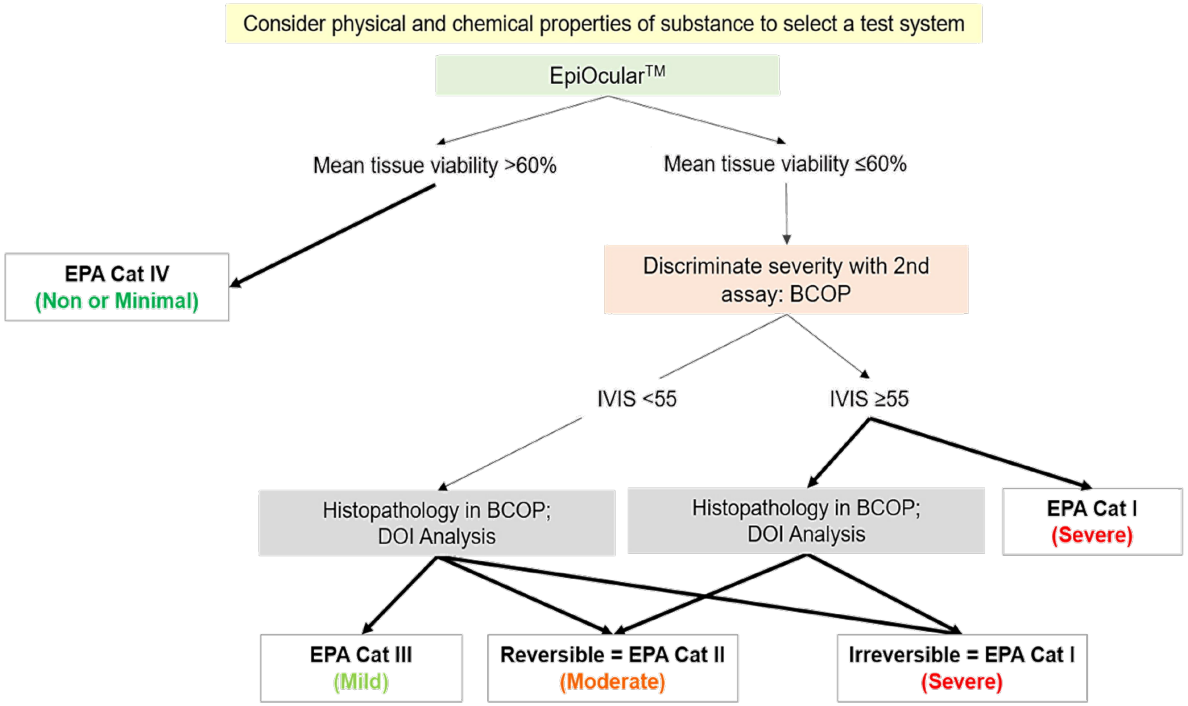
Defined approaches to classify agrochemical formulations into EPA hazard categories developed using EpiOcular™ reconstructed human corneal epithelium and bovine corneal opacity and permeability assays

Anna J. van der Zalm[a], Amber B. Daniel[b], Hans A. Raabe[c], Neepa Choksi[b*], Tara Flint Silva[d], Julie Breeden-Alemi[d], Lindsay O'Dell[e], Nicole C. Kleinstreuer[f], Anna B. Lowit[e], David G. Allen[b] and Amy J. Clippinger[a]





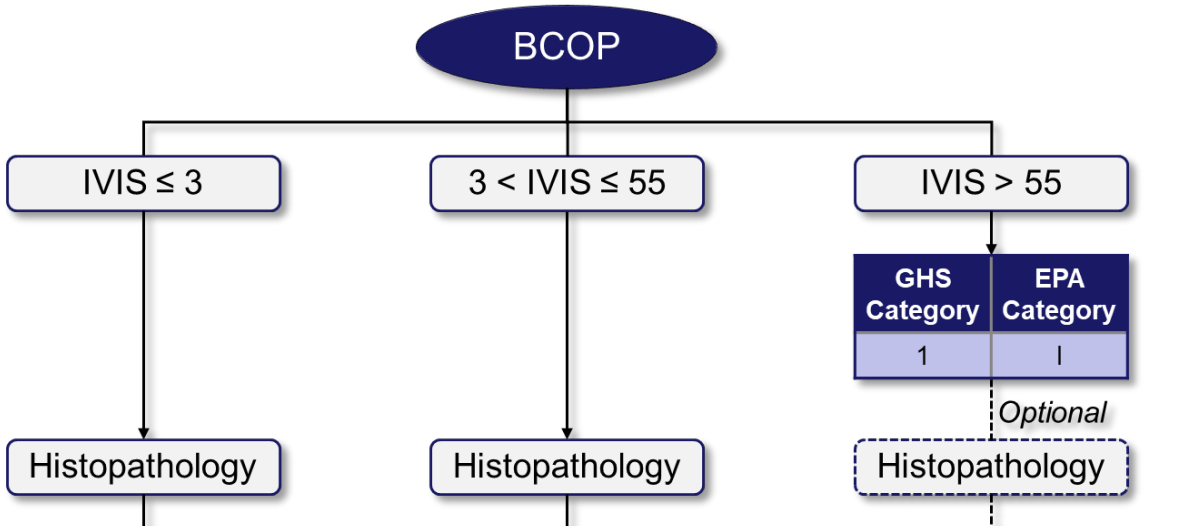
GHS and EPA Labeling Requirements

	GHS				EPA			
	Cat.*	Signal Word	Hazard Statement	Pictogram	Cat.*	Signal Word	Hazard Statement	PPE Labeling
Corrosive	1	DANGER	Causes severe eye damage.		I	DANGER	Corrosive. Causes irreversible eye damage.	Appropriate protective eyewear
Moderate Irritant	2A	WARNING	Causes severe eye irritation.		II	WARNING	Causes substantial but temporary eye injury.	Appropriate protective eyewear
Mild Irritant	2B	WARNING	Causes eye irritation.	None required	III	CAUTION	Causes moderate eye irritation.	None required†
Non-corrosive/ Minimal Irritant	NC	No hazard labeling required			IV	No hazard or PPE labeling required†		

*Based on in vivo results and associated decision criteria that are distinct for each system. †Registrant may choose to include, if appropriate.
Abbreviations: Cat. = category; NC = not classified; PPE = personal protective equipment.

DAs for **GHS and EPA** Classification of Agrochemicals:

DA-BCOP+

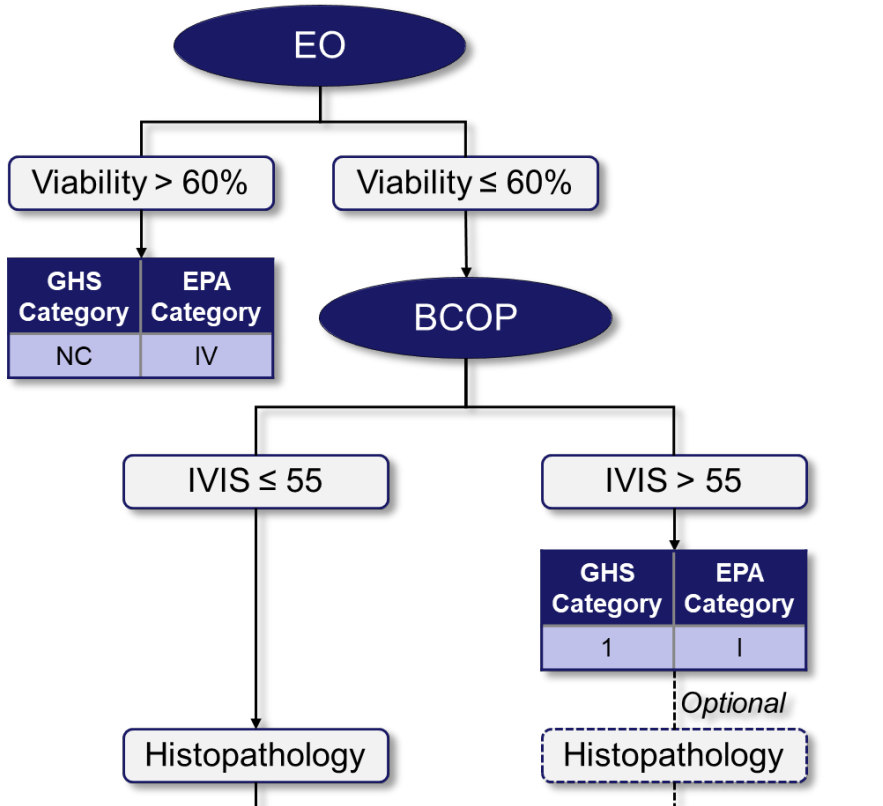


Depth of Injury	GHS Category	EPA Category
Minimal	NC	IV
Mild	2B	III
Moderate	2A	II
Severe	1	I

Depth of Injury	GHS Category	EPA Category
Minimal	2B	III
Mild		
Moderate	2A	II
Severe	1	I

Depth of Injury	GHS Category	EPA Category
Minimal	2A	II
Mild		
Moderate		
Severe	1	I

DA-EO+

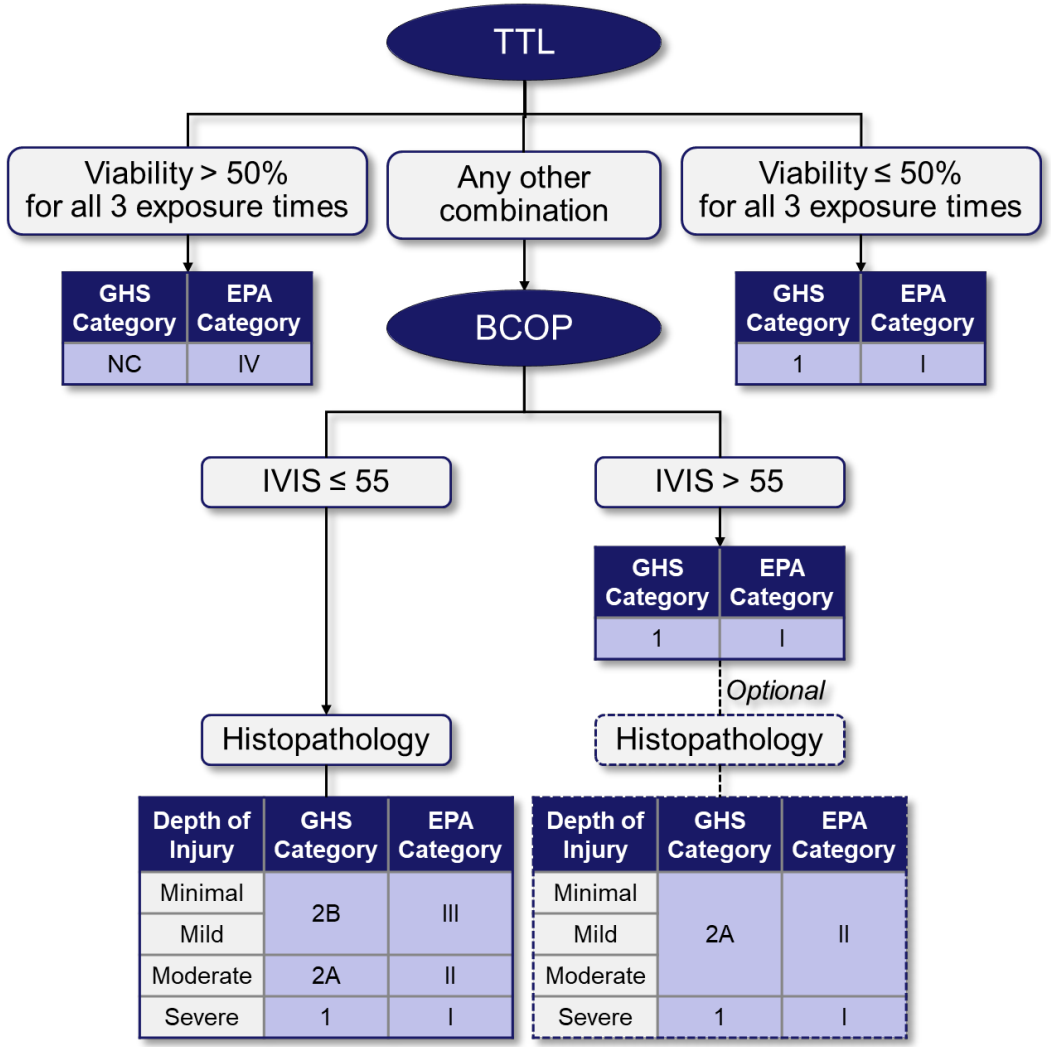


Depth of Injury	GHS Category	EPA Category
Minimal	2B	III
Mild		
Moderate	2A	II
Severe	1	I

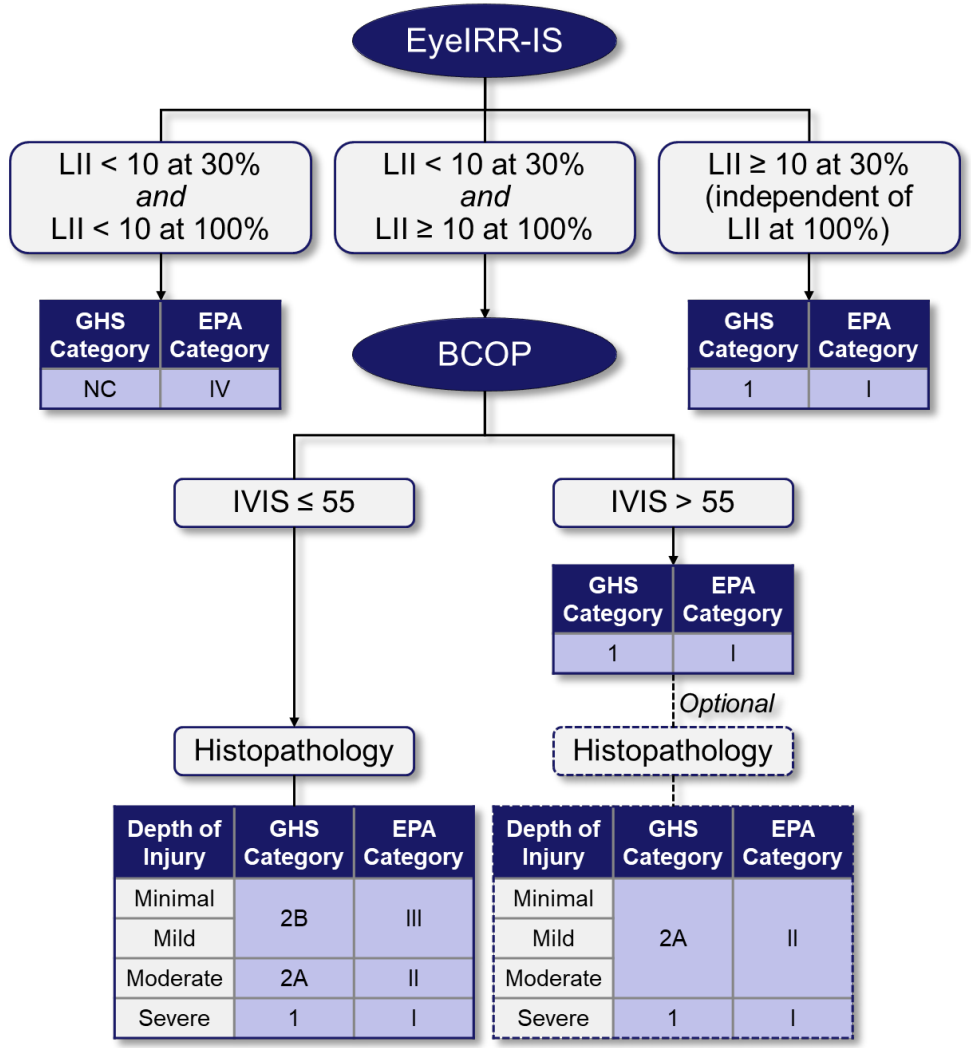
Depth of Injury	GHS Category	EPA Category
Minimal	2A	II
Mild		
Moderate		
Severe	1	I

Abbreviations: EPA: US Environmental Protection Agency; GHS: UN Globally Harmonized System; IVIS: in vitro irritancy score; LII: liquid irritation index; NC: not classified

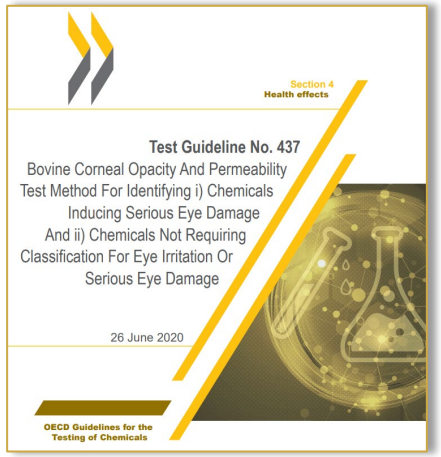
DA-TTL+



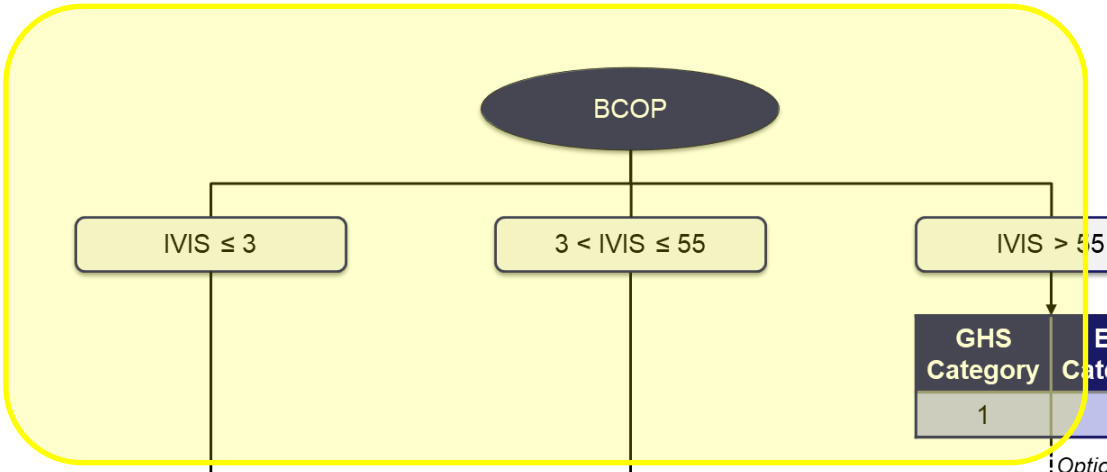
DA-EyeIRR-IS+



Abbreviations: EPA: US Environmental Protection Agency; GHS: UN Globally Harmonized System; IVIS: in vitro irritancy score; LII: liquid irritation index; NC: not classified



OP-KIT Opacitometer In Vitro Irritation Score (IVIS)	GHS Classification
IVIS ≤ 3	NC
3 < IVIS	No stand-alone prediction can be made
IVIS > 55	1



Depth of Injury	GHS Category	EPA Category
Minimal	NC	IV
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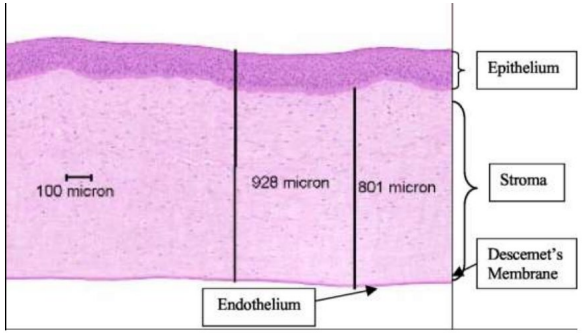
Depth of Injury	GHS Category	EPA Category
Minimal	2B	III
Mild		
Moderate	2A	II
Severe	1	I

Depth of Injury	GHS Category	EPA Category
Minimal	2A	II
Mild		
Moderate		
Severe	1	I

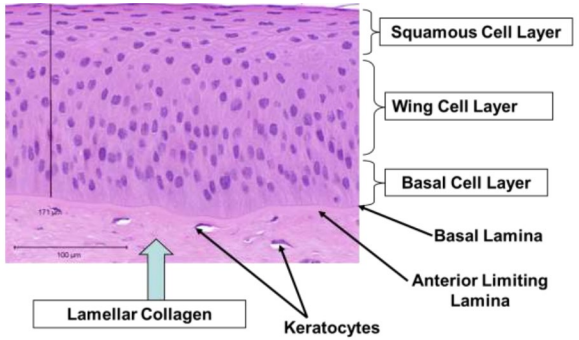
Adapted from Redden et al. 2009:

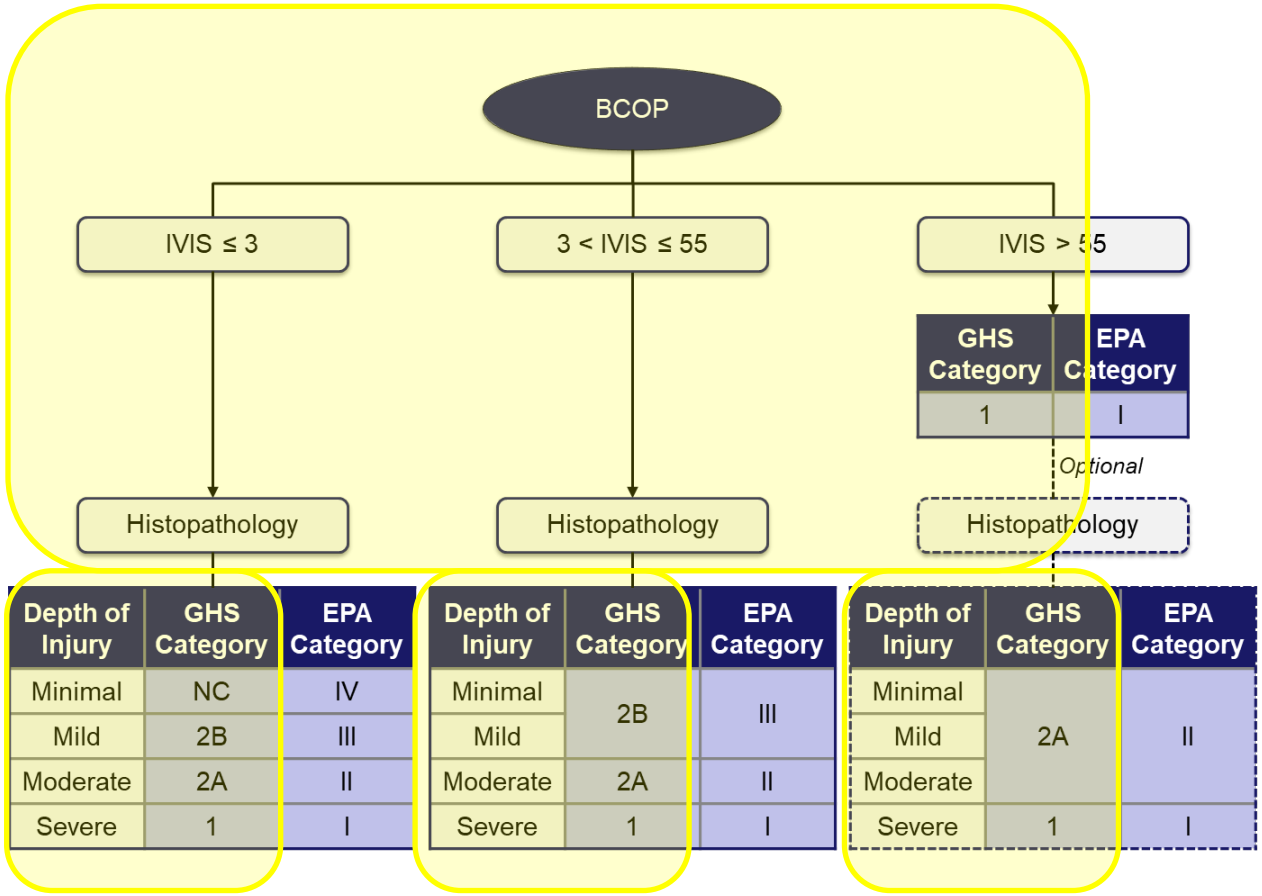
Histopathological Findings		GHS Classification
Damage or loss limited to the surface squamous cell layer in the epithelium; wing cell and basal cell layers intact	"Minimal"	NC
Damage or loss extends to the wing cell layers in the epithelium; basal cell layer and basal lamina intact	"Mild"	2B
Damage involves all layers of the epithelium and may cause keratocyte damage to the upper third to half of the stroma	"Moderate"	2A
Keratocyte damage extends into the lower half of the stroma and may include damage to the endothelium	"Severe"	1

Full thickness (4x)



Epithelium (20x)





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Moderate	2A	II
Severe	1	I

Depth of Injury	GHS Category	EPA Category
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Depth of Injury	GHS Category	EPA Category
Minimal	2A	II
Mild		
Moderate		
Severe	1	I

IIVS Abridged Guidelines for BCOP Histopathology, 2016

Data Analysis

- Given the limitations and low reliability of the in vivo test, it was not appropriate to assess performance of the DAs based on direct concordance with the rabbit test data.
- Instead, we conducted orthogonal concordance analyses. For each formulation, we:
  - Used in vitro test data to apply the DAs.
  - Orthogonally compared the GHS and EPA classifications predicted by the DAs and by the historical rabbit test data against each other.
  - Evaluated orthogonal concordance based on agreement across the five approaches.
    - Orthogonally concordant if the prediction aligned with the prediction of at least two other approaches (i.e., at least 3 of 5 approaches achieved the same “majority prediction”).
    - Orthogonally discordant if the prediction misaligned with the majority prediction.
      - Also evaluated whether orthogonal discordance affected hazard labeling (GHS) or PPE labeling (EPA).

Main Results: GHS

- Majority prediction determined for **97%** of formulations.
- Orthogonal concordance of DAs **82-93%** (vs. 71% historical in vivo).
- Hazard labeling: All DAs produced fewer underprotective predictions than historical in vivo.

Formulation Code	DA-BCOP+	DA-EO+	DA-TTL+	DA-EyeIRR-IS+	Historical In Vivo	Majority Prediction
A	NC	NC	NC	NC	NC	**NC**
B	NC	NC	NC	NC	NC	**NC**
C	NC	NC	NC	NC	NC	**NC**
D	1	1	1	1	1	**1**
E	2B	2B	2B	1	1	**2B**
F	1	1	1	1	1	**1**
G	1	1	1	1	1	**1**
H	1	1	1	1	1	**1**
I	1	1	1	1	1	**1**
J	1	1	1	1	1	**1**
K	NC	2B	2B	2B	2A	**2B**
L	NC	2B	2B	NC	NC	**NC**
M	NC	NC	NC	NC	NC	**NC**
N	NC	NC	NC	NC	NC	**NC**
O	NC	2B	2B	NC	NC	**NC**
P	NC	NC	NC	NC	NC	**NC**
Q	2A[a]	2A	2A	2A	NC	**2A**
R	2A	2A	1	1	2A	**2A**
S	2B[a]	2B	2B	2B	2B	**2B**
T	2B[a]	NC	2B	NC	NC	**NC**
U	2A	2A	2A	1	2A	**2A**
V	1[b]	1[b]	1[b]	1[b]	2B	**1**
W	2B	2B	2B	2B	NC	**2B**
X	2A	2A	2A	1	2A	**2A**
Y	2B[a]	2B	2B	2B	2A	**2B**
Z	2B	NC	NC	NC	NC	**NC**
AA	NC	2B	2B	2B	2A	**2B**
AB	2A	2A	Not tested	Not tested	2B	**None**
AC	2B	2B	2B	NC	NC	**2B**
Orthogonally concordant	**24/28; 86%**	**26/28; 93%**	**24/28; 86%**	**23/28; 82%**	**20/28; 71%**	
Orthogonally discordant	**4/28; 14%**	**2/28; 7%**	**4/28; 14%**	**5/28; 18%**	**8/28; 29%**	
Hazard labeling maintained[c]	0	0	1	4	5	
Hazard labeling overprotective[c]	2	2	3	0	0	
Hazard labeling underprotective[c]	2	0	0	1	3	

Orthogonally concordant prediction

Orthogonally discordant prediction; hazard labeling maintained

Orthogonally discordant prediction; hazard labeling overprotective

Orthogonally discordant prediction; hazard labeling underprotective

[a]IVIS < 3, but histopathology DoI analysis led to a more severe classification.
[b]Optional histopathology DoI analysis would lead to a less severe classification (i.e., GHS Cat. II).
[c]Relative to that of the majority prediction.

Main Results: EPA

- Majority prediction determined for **97%** of formulations.
- Orthogonal concordance of DAs **75-93%** (vs. 79% historical in vivo).
- PPE labeling: All DAs produced fewer underprotective predictions than historical in vivo.

Orthogonally concordant prediction

Orthogonally discordant prediction; PPE labeling maintained

Orthogonally discordant prediction; PPE labeling overprotective

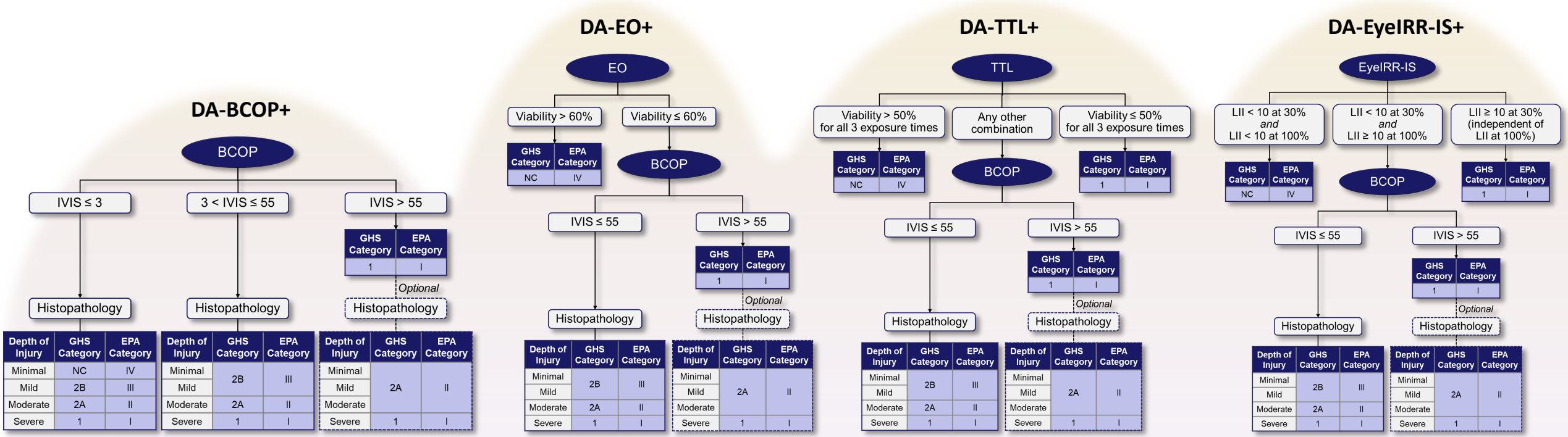
Orthogonally discordant prediction; PPE labeling underprotective

Formulation Code	DA-BCOP+	DA-EO+	DA-TTL+	DA-EyeIRR-IS+	Historical In Vivo	Majority Prediction
A	IV	IV	IV	IV	IV	**IV**
B	IV	IV	IV	IV	IV	**IV**
C	IV	IV	IV	IV	IV	**IV**
D	I	I	I	I	I	**I**
E	III	III	III	I	I	**III**
F	I	I	I	I	I	**I**
G	I	I	I	I	I	**I**
H	I	I	I	I	I	**I**
I	I	I	I	I	I	**I**
J	I	I	I	I	I	**I**
K	IV	III	III	III	II	**III**
L	IV	III	III	IV	III	**III**
M	IV	IV	IV	IV	IV	**IV**
N	IV	IV	IV	IV	IV	**IV**
O	IV	III	III	IV	IV	**IV**
P	IV	IV	IV	IV	IV	**IV**
Q	II[a]	II	II	II	II	**II**
R	II	II	I	I	II	**II**
S	III[a]	III	III	III	III	**III**
T	III[a]	IV	III	IV	III	**III**
U	II	II	II	I	II	**II**
V	I[b]	I[b]	I[b]	I[b]	III	**I**
W	III	III	III	III	III	**III**
X	II	II	II	I	II	**II**
Y	III[a]	III	III	III	II	**III**
Z	III	IV	IV	IV	III	**IV**
AA	IV	III	III	III	II	**III**
AB	II	II	Not tested	Not tested	III	**None**
AC	III	III	III	IV	III	**III**
Orthogonally concordant	**24/28; 86%**	**26/28; 93%**	**26/28; 93%**	**21/28; 75%**	**22/28; 79%**	
Orthogonally discordant	**4/28; 14%**	**2/28; 7%**	**2/28; 7%**	**7/28; 25%**	**6/28; 21%**	
PPE labeling maintained[c]	4	2	2	5	1	
PPE labeling overprotective[c]	0	0	0	2	4	
PPE labeling underprotective[c]	0	0	0	0	1	

[a]IVIS < 3, but histopathology DoI analysis led to a more severe classification.
[b]Optional histopathology DoI analysis would lead to a less severe classification (i.e., EPA Cat. II).
[c]Relative to that of the majority prediction.

Summary/Conclusions

- These DAs are **equally or more protective** of human health than the in vivo test.
- These DAs present an opportunity to **fully replace** the use of the in vivo test for determining GHS and EPA hazard classification and labeling of agrochemical formulations.



Abbreviations: EPA: US Environmental Protection Agency; GHS: UN Globally Harmonized System; IVIS: in vitro irritancy score; LII: liquid irritation index; NC: not classified

National Institute of Environmental Health Sciences
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Acknowledgments

Amber.Daniel@inotiv.com

Co-authors

- Anna van der Zalm, PSCI
- Hans Raabe, IIVS
- Amy Clippinger, PSCI
- Neepa Choksi, formerly of Inotiv-NICEATM
- Emily Reinke, Inotiv-NICEATM
- Dave Allen, formerly of Inotiv-NICEATM
- Nicole Kleinstreuer, NICEATM

Testing Labs

- EpiSkin
- IIVS
- ImmunoSearch
- Lebrun Labs
- MatTek

Agrochemical Companies

- BASF
- Bayer
- Corteva
- Syngenta

Other Collaborators/Contributors

- Julie Breeden-Alemi, EPA/OPP
- Tara Flint, EPA/OPP
- Lindsay O'Dell, EPA/OPP
- Monique Perron, EPA/OPP
- Anna Lowit, EPA/OPPT
- Elizabeth Farley-Dawson, Inotiv-NICEATM
- Kim To, formerly of Inotiv-NICEATM