National Institute of Environmental Health Sciences
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Defined Approaches for Predicting GHS and EPA Eye Irritation Classification of Agrochemicals

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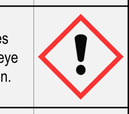
Introduction

- Regulators require that agrochemical labels indicate potential harmful effects caused by exposure.
- In vitro methods have been developed to assess eye irritation hazards and are accepted by some regulatory agencies. However, some regulators continue to require the Draize in vivo rabbit eye irritation test ("rabbit test"), which has documented limitations in reliability and relevance to humans [1].
- Defined approaches (DAs) use results from multiple methods in specific combinations and fixed data interpretation procedures to derive hazard predictions based on mechanisms of action of individual test methods.
- The Organisation for Economic Co-operation and Development (OECD) issued Test Guideline (TG) 467, which describes DAs for identifying chemicals with serious eye damage or eye irritation potential [2]. However, agrochemical formulations are outside the applicability domain of the DAs described in TG 467.
- Our goal was to develop DAs that leverage strengths of these methods to predict agrochemicals' eye irritation hazard potential according to the Globally Harmonized System of Classification and Labelling (GHS) and the U.S. Environmental Protection Agency (EPA) classification systems.

Materials and Methods

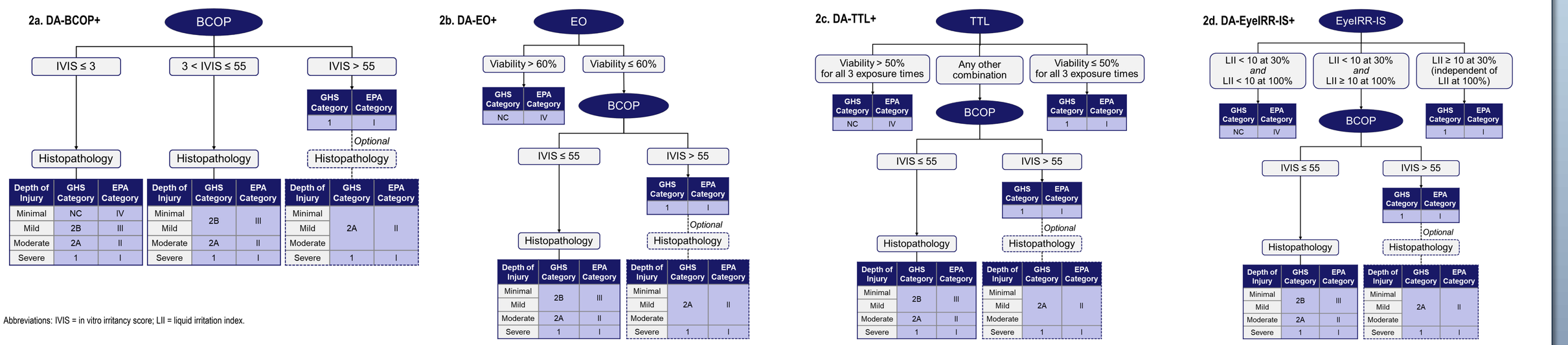
- We developed DAs based on prospective testing results of agrochemicals in a common set of in vitro methods [3,4].
  - All formulations (n=29) have historical rabbit test data, represent the most used agrochemical types (i.e., suspension concentrates, emulsifiable concentrates, soluble liquids, plus one microencapsulated emulsifiable concentrate), and span the full range of GHS and EPA hazard classifications.
- We included 4 test methods in the DAs based on their human relevance or status as an OECD TG or peer-reviewed method.
  - Bovine corneal opacity and permeability (BCOP; TG 437 [5]) assay (with histopathology)
  - EpiOcular™ Eye Irritation Test (EO; TG 492 [6])
  - SkinEthic time-to-toxicity for liquids (TTL; TG 492B [7])
  - EyeIRR-IS [8]
- Because these standalone test methods do not all include EPA classification criteria in their respective prediction models and given the similarity of the two classification systems (Figure 1), we considered the classification criteria of EPA Category I, II, III, and IV equal to GHS Category 1, 2A, 2B, and not classified (NC), respectively.
- Proposed DAs comprise BCOP with histopathology alone and combined with EO, TTL, or EyeIRR-IS:
  - DA-BCOP+ (Figure 2a)
  - DA-EO+ (Figure 2b)
  - DA-TTL+ (Figure 2c)
  - DA-EyeIRR-IS+ (Figure 2d)
- Given the limitations of the rabbit test, we calculated orthogonal concordance of GHS/EPA classifications predicted by the DAs and historical data (i.e., concordance was determined for each DA by comparison to a reference classification established based on the majority of predictions across all 5 approaches; see Table 1).
- We also evaluated the impact on hazard labeling and personal protective equipment (PPE) labeling associated with the GHS and EPA predictions, respectively.

Figure 1. 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.

Figure 2. Proposed DAs for Predicting GHS and EPA Eye Irritation/Corrosion Hazard Classification of Agrochemicals



Abbreviations: IVIS = in vitro irritancy score; LII = liquid irritation index.

Table 1. Results of GHS and EPA Orthogonal Concordance Analyses

	GHS						EPA					
Formulation Code	**DA-BCOP+**	**DA-EO+**	**DA-TTL+**	**DA-EyeIRR-IS+**	**Historical In Vivo**	**Majority GHS Prediction**	**DA-BCOP+**	**DA-EO+**	**DA-TTL+**	**DA-EyeIRR-IS+**	**Historical In Vivo**	**Majority EPA Prediction**
A	NC	NC	NC	NC	NC	**NC**	IV	IV	IV	IV	IV	**IV**
B	NC	NC	NC	NC	NC	**NC**	IV	IV	IV	IV	IV	**IV**
C	NC	NC	NC	NC	NC	**NC**	IV	IV	IV	IV	IV	**IV**
D	1	1	1	1	1	**1**	I	I	I	I	I	**I**
E	2B	2B	2B	1	1	**2B**	III	III	III	I	I	**III**
F	1	1	1	1	1	**1**	I	I	I	I	I	**I**
G	1	1	1	1	1	**1**	I	I	I	I	I	**I**
H	1	1	1	1	1	**1**	I	I	I	I	I	**I**
I	1	1	1	1	1	**1**	I	I	I	I	I	**I**
J	1	1	1	1	1	**1**	I	I	I	I	I	**I**
K	NC	2B	2B	2B	2A	**2B**	IV	III	III	III	II	**III**
L	NC	2B	2B	NC	NC	**NC**	IV	III	III	IV	III	**III**
M	NC	NC	NC	NC	NC	**NC**	IV	IV	IV	IV	IV	**IV**
N	NC	NC	NC	NC	NC	**NC**	IV	IV	IV	IV	IV	**IV**
O	NC	2B	2B	NC	NC	**NC**	IV	III	III	IV	IV	**IV**
P	NC	NC	NC	NC	NC	**NC**	IV	IV	IV	IV	IV	**IV**
Q	2A	2A	2A	2A	NC	**2A**	II	II	II	II	II	**II**
R	2A	2A	1	1	2A	**2A**	II	II	I	I	II	**II**
S	2B	2B	2B	2B	2B	**2B**	III	III	III	III	III	**III**
T	2B	NC	2B	NC	NC	**NC**	III	IV	III	IV	III	**III**
U	2A	2A	2A	1	2A	**2A**	II	II	II	I	II	**II**
V	1*	1*	1*	1*	2B	**1**	I*	I*	I*	I*	III	**I**
W	2B	2B	2B	2B	NC	**2B**	III	III	III	III	III	**III**
X	2A	2A	2A	1	2A	**2A**	II	II	II	I	II	**II**
Y	2B	2B	2B	2B	2A	**2B**	III	III	III	III	II	**III**
Z	2B	NC	NC	NC	NC	**NC**	III	IV	IV	IV	III	**IV**
AA	NC	2B	2B	2B	2A	**2B**	IV	III	III	III	II	**III**
AB	2A	2A	Not tested	Not tested	2B	**None**	II	II	Not tested	Not tested	III	**None**
AC	2B	2B	2B	NC	NC	**2B**	III	III	III	IV	III	**III**
Orthogonally concordant	**24/28; 86%**	**26/28; 93%**	**24/28; 86%**	**23/28; 82%**	**20/28; 71%**		**24/28; 86%**	**26/28; 93%**	**26/28; 93%**	**21/28; 75%**	**22/28; 79%**	
Orthogonally discordant	**4/28; 14%**	**2/28; 7%**	**4/28; 14%**	**5/28; 18%**	**8/28; 29%**		**4/28; 14%**	**2/28; 7%**	**2/28; 7%**	**7/28; 25%**	**6/28; 21%**	

*Denotes instances where the optional histopathological analysis would produce a less severe classification (i.e., GHS Category 2A/EPA Category II) due to "moderate" depth of injury findings.

Orthogonally concordant with majority prediction	Orthogonally discordant; hazard/PPE labeling overprotective relative to that of the majority prediction
Orthogonally discordant with majority prediction, but hazard/PPE labeling is maintained	Orthogonally discordant; hazard/PPE labeling underprotective relative to that of the majority prediction

Results and Conclusions

Results:

- For both classification systems, 97% (28/29) of formulations aligned across at least 3 of 5 approaches (considered a majority prediction).
  - GHS: Orthogonal concordance of the DA predictions ranged from 82-93%, compared with 71% for the historical in vivo data.
    - Relative to the majority GHS predictions, DA-BCOP+ and DA-EyeIRR-IS+ produced 2 and 1 discordant/underprotective results, respectively.
    - Predictions based on historical in vivo data produced 3 discordant/underprotective results.
  - EPA: Orthogonal concordance of the DA predictions ranged from 75-93%, compared with 79% for the historical in vivo data.
    - None of the DAs produced any discordant/underprotective results relative to the majority EPA predictions, but predictions based on historical in vivo data produced 1 discordant/underprotective result.

Conclusions:

- DA-BCOP+, DA-EO+, DA-TTL+, and DA-EyeIRR-IS+ are equally or more protective of human health than the in vivo rabbit eye test.
- These DAs are applicable to both the GHS and the EPA classification systems.
- These DAs present an opportunity to fully replace the use of the rabbit test for determining GHS and EPA hazard classification and labeling of agrochemical formulations in regulatory frameworks.

References and Acknowledgments

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