

# Predicting GHS Hazard Classification for Eye Irritation/Corrosion Potential of Agrochemical Formulations Using Defined Approaches

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## Introduction

- Regulators require that agrochemicals be labeled to indicate potential harmful effects caused by ingestion, inhalation, or contact with skin or eyes.
- In vitro methods have been developed to assess eye irritation hazards and are accepted by some regulatory agencies. However, many regulators continue to require the Draize in vivo rabbit eye irritation test ("rabbit test"), in part due to the view that in vitro results should align with rabbit test results.
- Discordance between some in vitro methods and the rabbit test is particularly common for substances identified as mild or moderate irritants by the rabbit test, which is also the range of irritancy where the rabbit test generates reproducible results less than 50% of the time (Luechtefeld et al. 2016).
- Defined approaches (DAs) use results from multiple methods in specific combinations and a fixed data interpretation procedure to derive a hazard prediction by applying the specific 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 (OECD 2022a). However, the applicability of the DAs described in TG 467 are limited to non-surfactant neat liquids, and liquids and solids dissolved in water.
- The goal of this study was to assess the applicability of in vitro methods to agrochemical formulations and develop DAs that leverage strengths of these methods to predict eye irritation hazard potential.

## Materials and Methods

### Prospective Testing:

- Prospective testing was conducted in three phases to test the eye irritation potential of agrochemical formulations using a common set of in vitro methods. At the conclusion of each testing phase, results were assessed to determine which methods should advance, and to select additional formulations and methods to test in subsequent phases.
  - Test methods were included based on their relevance to mechanisms of human eye irritation (Clippinger et al. 2021).
  - Formulations were selected for prospective testing based on availability of historical rabbit test data and to represent common agrochemical formulation types and to span the full range of United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS) hazard classifications.
- | GHS Classification | Effects                        | Personal Protective Equipment |
|--------------------|--------------------------------|-------------------------------|
| Category 1         | Corrosive                      | Eye protection                |
| Category 2A        | Moderate irritant              | Eye protection                |
| Category 2B        | Mild irritant                  | Eye protection                |
| Not Classified     | Non-corrosive/minimal irritant | None noted                    |
- At the conclusion of prospective testing, 29 formulations were tested in up to five methods:
    - Bovine corneal opacity and permeability (BCOP; TG 437 [OECD 2023a]), including histopathology
    - The EpiOcular™ Eye Irritation Test (EO; TG 492 [OECD 2023b])
    - SkinEthic time-to-toxicity for liquids (TTL; TG 492B [OECD 2022b])
    - In vitro depth of injury (IVDol)
    - EyeIrr-IS

### Defined Approaches:

- A preliminary analysis of alignment across these five in vitro methods and historical rabbit test data produced consensus predictions for each formulation, based on majority alignment across individual assay results.
- Four methods were then selected for inclusion in DAs based on their human relevance or status as an OECD TG or validated, peer-reviewed method. These were developed into four DAs to predict the full spectrum of GHS classifications.
- DAs were based on BCOP with histopathology alone and EO, TTL, or EyeIrr-IS combined with BCOP with histopathology:
  - DA-BCOP+ (Figure 1a)
  - DA-EO+ (Figure 1b)
  - DA-TTL+ (Figure 1c)
  - DA-EyeIrr-IS+ (Figure 1d)
- For each formulation, GHS classifications predicted by the DAs and the historical rabbit test data were assessed for concordance with the consensus prediction (Table 1).
- Personal protective equipment (PPE) labeling associated with each prediction was also compared to that based on the consensus prediction.

Figure 1. Defined Approaches

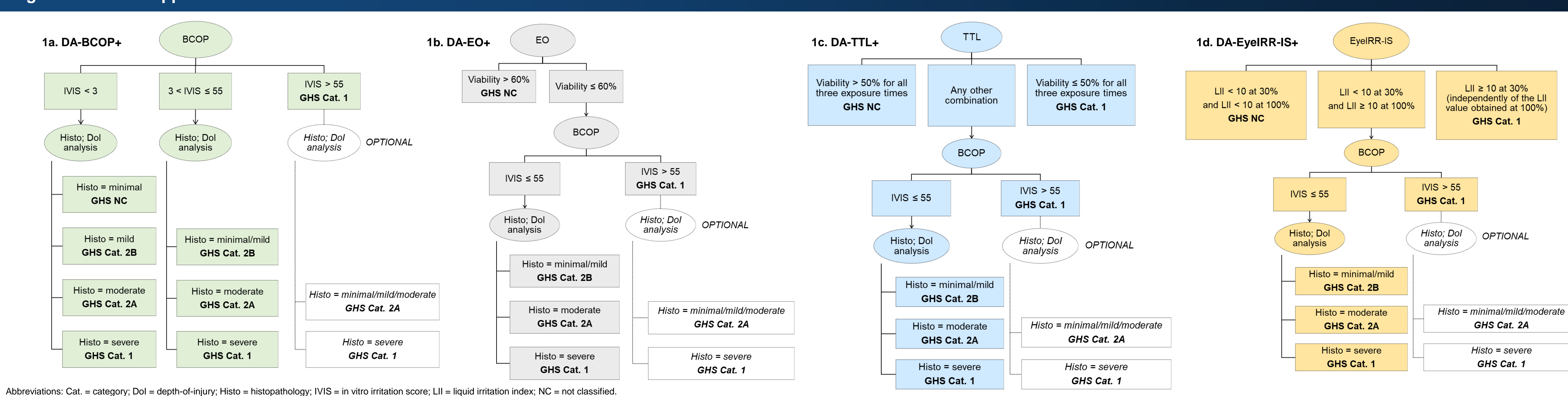


Table 1. GHS Classifications and PPE Labeling Predictions Based on DAs and Historical Rabbit Test Data

Formulation Code	Formulation Type	Consensus Prediction <sup>#</sup>	DA-BCOP+	DA-EO+	DA-TTL+	DA-EyeIrr-IS+	Historical In Vivo
A	EC/ME	NC	NC	NC	NC	NC	NC
B	SC	NC	NC	NC	NC	NC	NC
C	SC	NC	NC	NC	NC	NC	NC
D	EC	1	1	1	1	1	1
E	EC	1	2B	2B	2B	1	1
F	SL	1	1	1	1	1	1
G	EC	1	1	1	1	1	1
H	SL	1	1	1	1	1	1
I	SL	1	1	1	1	1	1
J	EC	1	1	1	1	1	1
K	SL	2A	NC	2B	2B	2B	2A
L	EC	NC	NC	2B	2B	NC	NC
M	SL	NC	NC	NC	NC	NC	NC
N	SC	NC	NC	NC	NC	NC	NC
O	SL	NC	NC	2B	2B	NC	NC
P	SC	NC	NC	NC	NC	NC	NC
Q	SL	2A	2A*	2A	2A	2A	NC
R	SL	1	2A	2A	1	1	2A
S	SL	2B	2B*	2B	2B	2B	2B
T	SC	NC	2B*	NC	2B	NC	NC
U	EC	2A	2A	2A	2A	1	2A
V	SL	1	1†	1†	1†	1†	2B
W	SL	2B	2B	2B	2B	2B	NC
X	EC	2A	2A	2A	2A	1	2A
Y	EC	2B	2B*	2B	2B	2B	2A
Z	EC	NC	2B	NC	NC	NC	NC
AA	EC	2A	NC	2B	2B	2B	2A
AB	EC	Inconclusive	2A	2A	Not tested - insufficient donated volume	2B	2B
AC	EC	Inconclusive	2B	2B	2B	NC	NC

Note: All DAs offer the option to conduct a histopathological depth-of-injury analysis (white fields in Figure 1) to downgrade a GHS Category 1 classification to Category 2A. This option is not reflected in these results.

<sup>#</sup>Consensus prediction is based on majority agreement among classifications from the five in vitro methods (including IVDol) and the historical rabbit test data (data not shown).

\*IVIS < 3 but histopathology analysis led to a more severe classification.

†Optional histopathology analysis would lead to a less severe classification (GHS Category 2A).

Abbreviations: EC = emulsifiable concentrate; ME = microencapsulated; SC = suspension concentrate; SL = soluble liquid.

Green	Concordant with consensus
Light Green	Underpredicted relative to consensus, but same PPE labeling
Yellow	Overpredicted relative to consensus, but same PPE labeling
Dark Green	Overpredicted relative to consensus; PPE (overprotective)
Red	Underpredicted relative to consensus; no PPE (underprotective)

## Results

- Consensus predictions were achieved for 27 of 29 formulations.
- Relative to the consensus predictions, 93% of classifications predicted by the historical rabbit test data were either concordant or would not affect PPE labeling.
  - The remaining 7% were underpredicted and associated in vivo-based PPE labeling was underprotective of potential eye irritation.
- Classifications predicted by DA-BCOP were similar with 7% (2/27) underpredicted and underprotective.
- For DA-EyeIrr-IS+, 100% of predicted classifications were concordant or would not affect PPE labeling.
- For DA-EO+ and DA-TTL+, 93% and 89%, respectively, were either concordant or would not affect PPE labeling.
  - The remaining 7% and 11%, respectively, were overpredicted and associated PPE labeling was overprotective.

## References

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## Conclusions

- This study employed a strategy to determine a consensus prediction for agrochemical formulations by using historical rabbit test data and data from reliable in vitro methods as equal components.
- Discordance between predictions based on historical rabbit test data and consensus predictions suggests that the rabbit test may not be a suitable reference method for deriving eye irritation hazard classification information for agrochemical products, and that alternative methods such as DAs can be used instead.
- All four proposed DAs may have high utility for predicting the GHS classification of agrochemical formulations, as the PPE labeling associated with the predictions are as or more protective of human health compared with the rabbit test.

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