Alternative Approaches to Eye Irritation Assessment of Agrochemicals and Plant Protection Products

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Pesticide Active ingredient (AI) and Plant Protection Products (PPP) animal testing

- Agrochemicals Active ingredients (AI) are one of the most rigorously tested group of chemicals on the market
  - ~18k animals across all disciplines (based on common guidelines) per AI
- Global reg agencies require acute toxicity assessment on every plant protection product (PPP) which compose a significant portion of testing
  - ~50 mammals for a single new product
  - Hundreds of new products each year across the globe are registered.

Endpoint	Study GL	Animal Use
Acute Oral Toxicity	OECD 423	3, Rat
Acute Dermal Toxicity	OECD 402	3, Rat
Acute Inhalation Toxicity	OECD 403	3-6, Rat
Primary Eye Irritation	**OECD 405**	**3, Rabbit**
Primary Skin Irritation	OECD 404	3, Rabbit
Skin Sensitization	OECD 429	35, Mouse
	Total	*50-53*

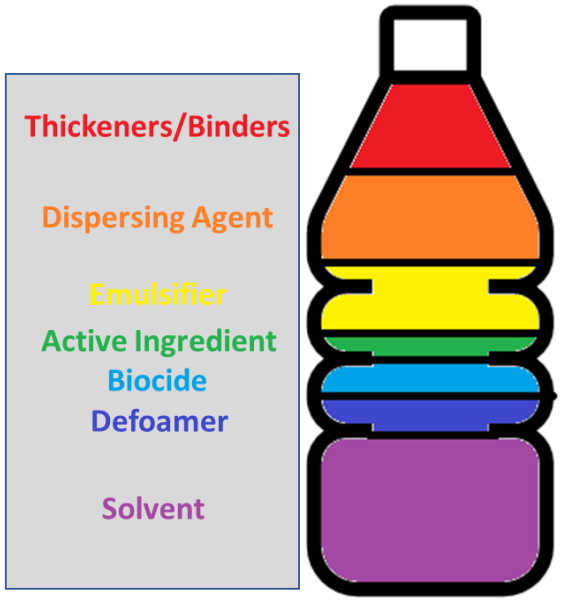
Necessary evaluation of PPP

The purpose of PPP acute tox testing is to evaluate hazards for the classification, labeling and worker safety

PPP may have differing acute toxicity potential (or compared to the AI) because of:

- Physical properties of PPP
- Routes of exposure
- Toxicity profile of co-formulants
- Possible additive effects among the formulants

Exposure by dust formation



Draize Eye Test

A <u>subjective</u> *in vivo* test with poor <u>reproducibility</u>

Never formally validated for its relevance to humans (Amy *et al.*, 2021)

- Presence of nictitating membrane in Rabbits (remove/trap)
- pH of Rabbit eye aqueous humor is more alkaline (8.2) than that of human (7.1-7.2)
- Rabbits are not efficient as humans in tear production

The EChA database was used for evaluation of substances with 2 or more Draize Tests

Compounds scoring a Category 1 (eye corrosive) had a 10.4% chance of having No Classification (No irritation potential) in a subsequent test and reproducibility was worse with Cat 2A or 2B

Previous test result	1	NC	Total
1	73.0%	**10.4%**	46
2A	4.2%	**59.4%**	138
2B	0.2%	**80.2%**	86
NC	1.1%	**93.9%**	400

(Luechtefeld et al., ALTEX 33(2), 2016)

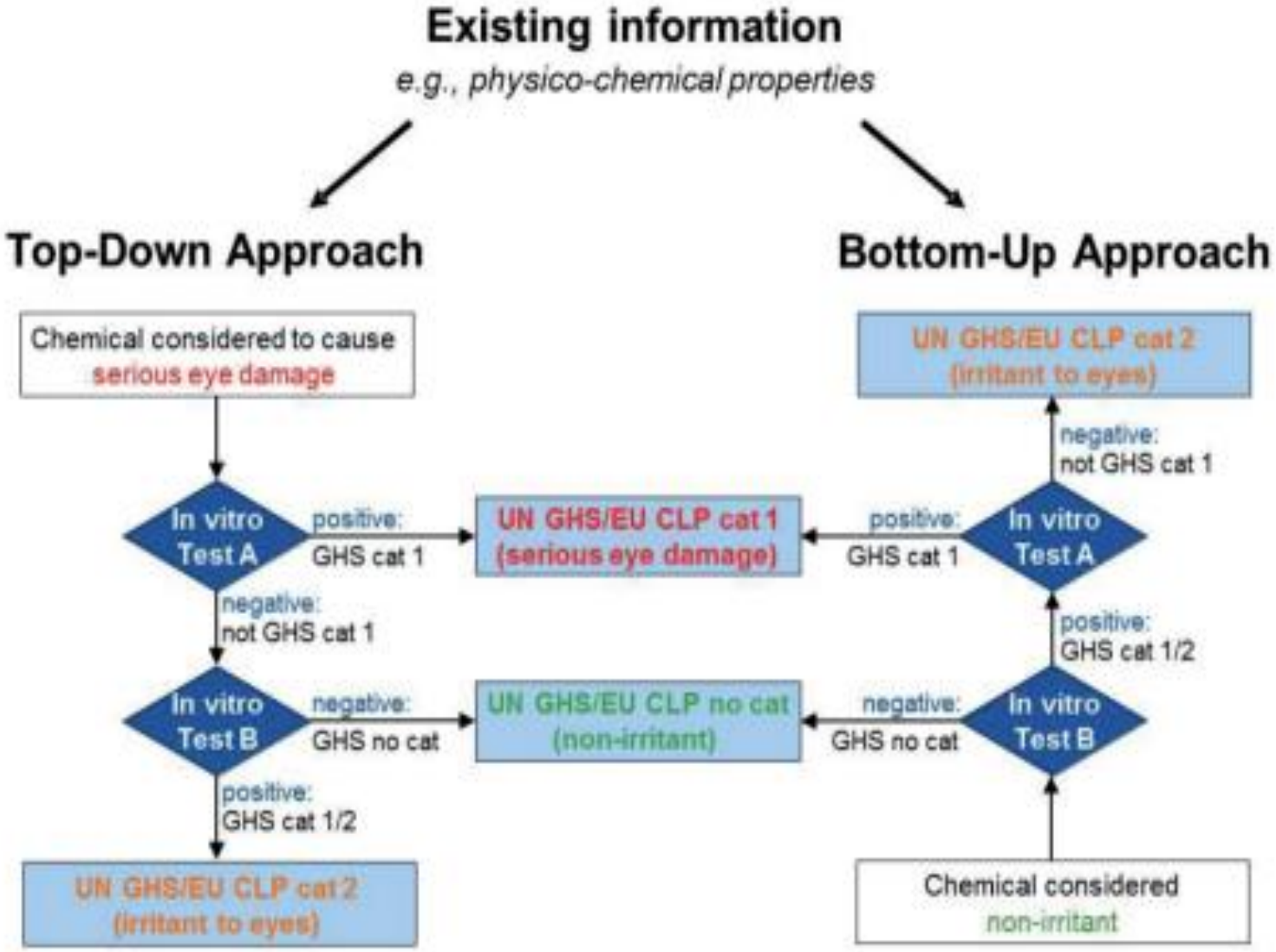
The Problem is compounded with agrichemical formulations which are complex mixtures that are often not severely irritating, but can have persistence of effects

Primary Eye Irritation Alternatives

- *In silico* predictions
  - OECD QSAR Toolbox, Derek Nexus, and OASIS Times can provide eye irritation potential assessment, but not routinely performed for RegTox assessments
  - Lower acceptability than *in vitro* assessments generally

- *In vitro* Assessments
  - OECD 492: Reconstructed human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage
  - OECD 437: Bovine Corneal Opacity and Permeability Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage
  - OECD 438: Isolated Chicken Eye Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage
- OECD guidelines and regional requirements necessitate evaluation of eye irritation potential before performing *in vivo* testing

In-Vitro testing strategy



Global Regulatory Acceptance

	In vivo tests	In vitro tests	Read Across from similar formulations with an in vivo test	GHS additivity
Australia/New Zealand	Accepted	**Potentially accepted**	Possible	Potentially Accepted
EU	Accepted (only option for some member states) UK: Not accepted (unless historic)	**Possible, but acceptability is discretionary** **UK: accepted**	Possible, but acceptability is discretionary UK: accepted	Possible, but acceptability is discretionary UK: accepted
USA	Main requirement	**Pilot program on going for future acceptability**	Possible	Pilot program on going for systemic toxicity
Canada	Accepted	**Pilot program on going for future acceptability**	Possible	Pilot program on going for systemic toxicity
Brazil	Accepted	**Not Accepted***	Possible	Not accepted
Other latin-america countries	Accepted	**Not accepted However discussion ongoing**	Possible, in some countries	Not accepted
Asian countries	Accepted	**Not accepted#**	Generally, not accepted	Not accepted

Corvaro, *et al*. 2017. A retrospective analysis of in vivo eye irritation, skin irritation and skin sensitization studies with agrochemical formulations: Setting the scene for development of alternative strategies. Regulatory Toxicology and Pharmacology 89: 131-147

* Brazil currently encourages *in vitro* alternatives

While many APAC countries have no clear guidance for the inclusion of *in vitro* tests, Japan, India encourage the use of alternatives

In vitro eye irritation & Corrosion Summary

Advantages	Disadvantages
• Routinely used as part of active and PPP acute toxicity assessments • Help to screen out obviously corrosive actives/formulations before they are ever used in rabbits • Used to conservative classify following consultation regulatory agencies • Good negative predictivity	• Validated in monoconstituent preparations • False positive / over predictive potential • Variable acceptance in many geographies

Project Overview - Collaboration with Crop Life Brazil on the Evaluation of In vitro eye irritation for plant protection products

Working Group had members from:

- BASF
- Bayer
- Corteva
- FMC
- IHARA
- Ourofino
- Sumitomo Chemical
- Syngenta

Goals

- To conduct a retrospective analysis of in vitro eye irritation assay results and GHS additivity calculation for pesticide formulations compared to in vivo GHS regulatory classifications.
- Propose testing strategies for evaluation of eye irritation potential of agrochemical formulations.

Data collection

- 8 Companies supplied data for a total of 187 formulations which fulfilled the following requirements
  - At least 1 in vivo test + at least 1 in vitro test
  - GHS additivity calculations were also provided for each formulation

GHS Category	Number of Formulations	%
1	21*	11.2
2	33	17.6
NC	**133**	**71.1**
Total	**187**	100

- Performance of each approach was evaluated for:
  - Concordance (with in vivo results) Sensitivity, Specificity, False positivity/negativity, and Negative/Positive predictivity

Catalano *et al.,* 2022. Reducing animal use for eye irritation testing of agrochemical in Brazil. SOT Poster

Results

EHCE - EPIOCULAR

Concordance	77%	62/81
Sensitivity	100%	16/16
Specificity	71%	46/65
False positive	29%	19/65
False negative	0%	0/16
Negative predictivity	**100%**	**46/46**
Positive predictivity	46%	16/35

ICE

Concordance	78%	43/55
Sensitivity	79%	15/19
Specificity	78%	28/36
False positive	22%	8/36
False negative	21%	4/19
Negative predictivity	**88%**	**28/32**
Positive predictivity	65%	15/23

BCOP

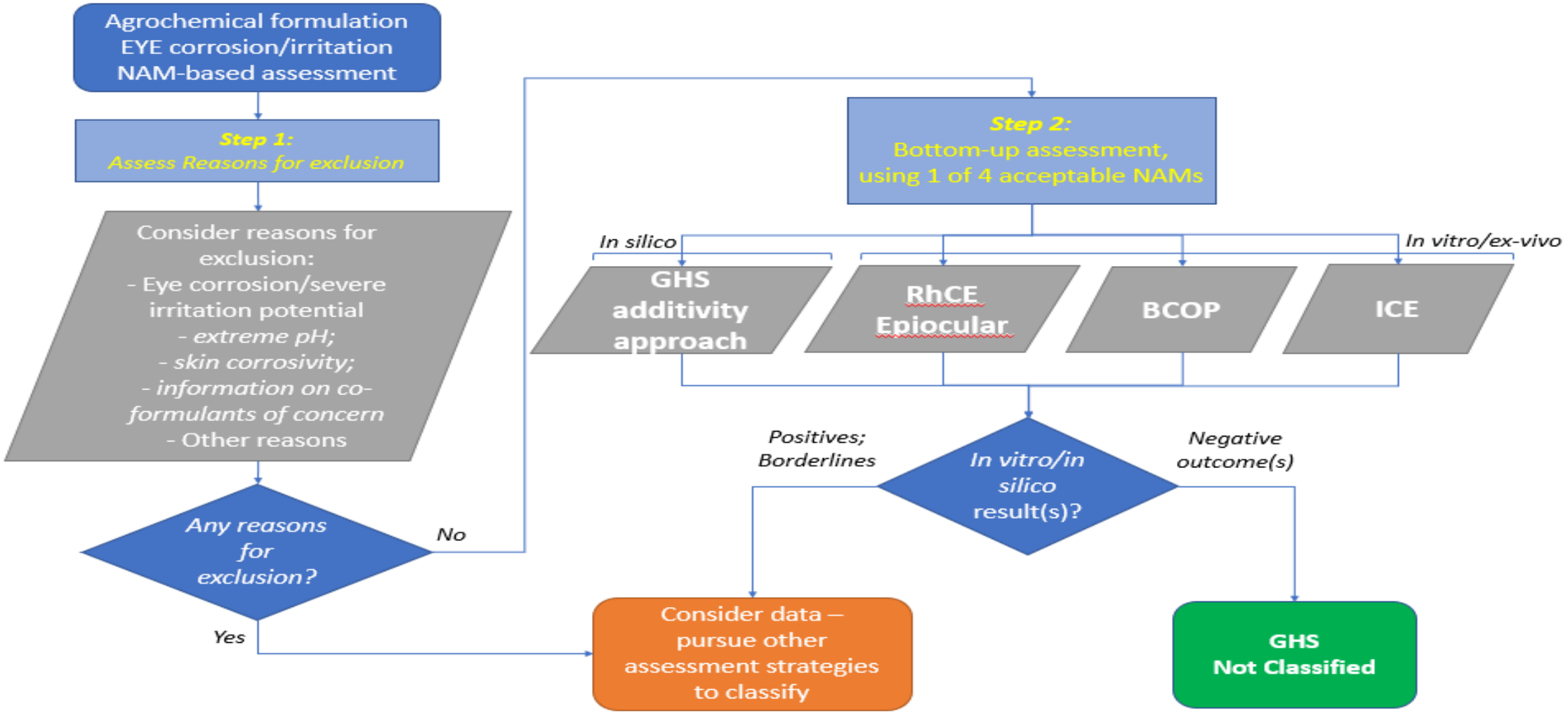
Concordance	49%	18/37
Sensitivity	92%	12/13
Specificity	25%	6/24
False positive	75%	18/24
False negative	8%	1/13
Negative predictivity	**86%**	**6/7**
Positive predictivity	40%	12/30

Calculation Method

Concordance	67%	125/187
Sensitivity	89%	48/54
Specificity	58%	77/133
False positive	42%	56/133
False negative	11%	6/54
Negative predictivity	**93%**	**77/83**
Positive predictivity	46%	48/104

Catalano *et al.,* 2022. Reducing animal use for eye irritation testing of agrochemical in Brazil. SOT Poster

Proposed decision flowchart for assessment of agrochemical formulation eye irritation/corrosion potential, using non-animal approaches



Catalano *et al.*, 2022. Reducing animal use for eye irritation testing of agrochemical in Brazil. SOT Poster

Take Aways

- The industry and the scientific community are currently in a **transitional phase** where Ag companies are running both *in vitro* and *in vivo* tests routinely per OECD Guidance
- The tendency towards over prediction affords greater confidence of human health protection from negative results
- High confidence that prediction of “not classified” from alternative method is a true “not classified” for all four approaches (negative predictivity range: 86% to 100%)
- Given the preponderance of agrochemical formulations that are “true negatives” (70%) such an approach could significantly reduce the use of animals for this endpoint (50-70% less rabbit tests)

References

OECD (2019), Test No. 492: Reconstructed human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris

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Thank you for your time!

Questions?