Defined Approaches for EPA Categorization Assessing Eye Irritation Potential of Agrochemical Formulations

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ABSTRACT

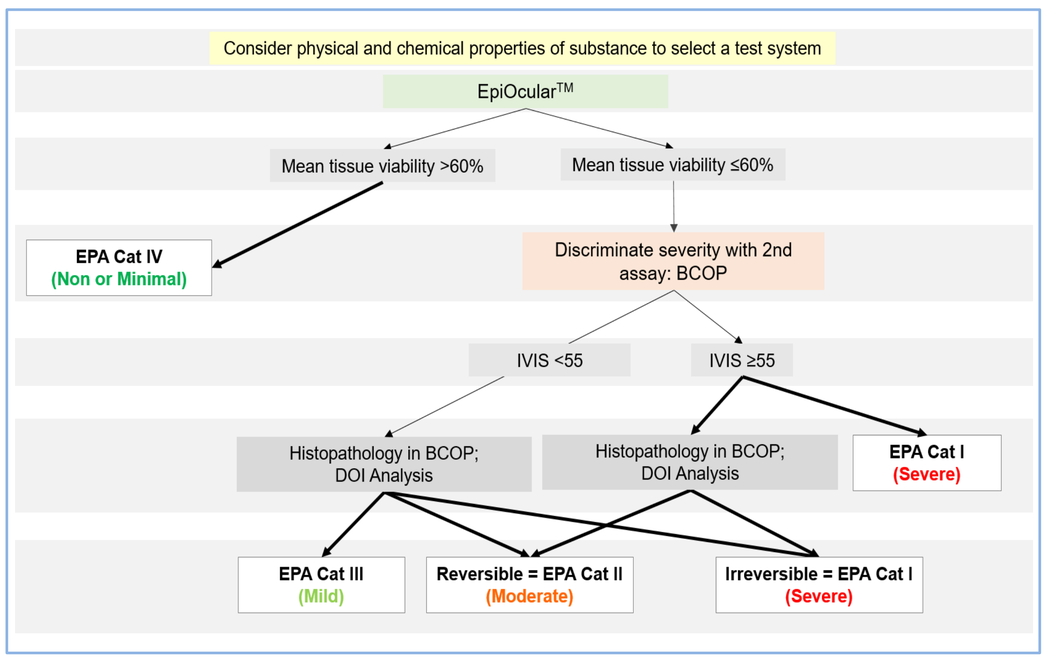
Many sectors have seen complete replacement of the *in vivo* rabbit eye irritation test with reproducible and relevant *in vitro* and *ex vivo* methods to assess the eye irritation potential of chemicals. However, the *in vivo* rabbit eye irritation test remains the standard test used for agrochemical formulations in some countries. Therefore, two defined approaches (DAs) for assessing conventional agrochemical formulations were developed, using EpiOcular™ (Organisation for Economic Co-operation and Development (OECD) test guideline (TG) 492; EO) and the Bovine Corneal Opacity and Permeability (OECD TG 437; BCOP) test with histopathology.

Presented here are the results from testing 29 agrochemical formulations, which were evaluated to determine their eye irritation potential against the United States Environmental Protection Agency's (EPA) pesticide classification and labeling system, and assessed using orthogonal validation, rather than direct concordance analysis with the historical *in vivo* rabbit data. Scientific confidence was established by evaluating the methods and testing results using an established framework that considers fitness for purpose, human biological relevance, technical characterization, data integrity and transparency, and independent review. The *in vitro* and *ex vivo* methods used in the DAs were demonstrated to be as or more fit for purpose, reliable and relevant than the *in vivo* rabbit eye irritation test. Overall, there is high scientific confidence in the use of these DAs for assessing the eye irritation potential of agrochemical formulations.

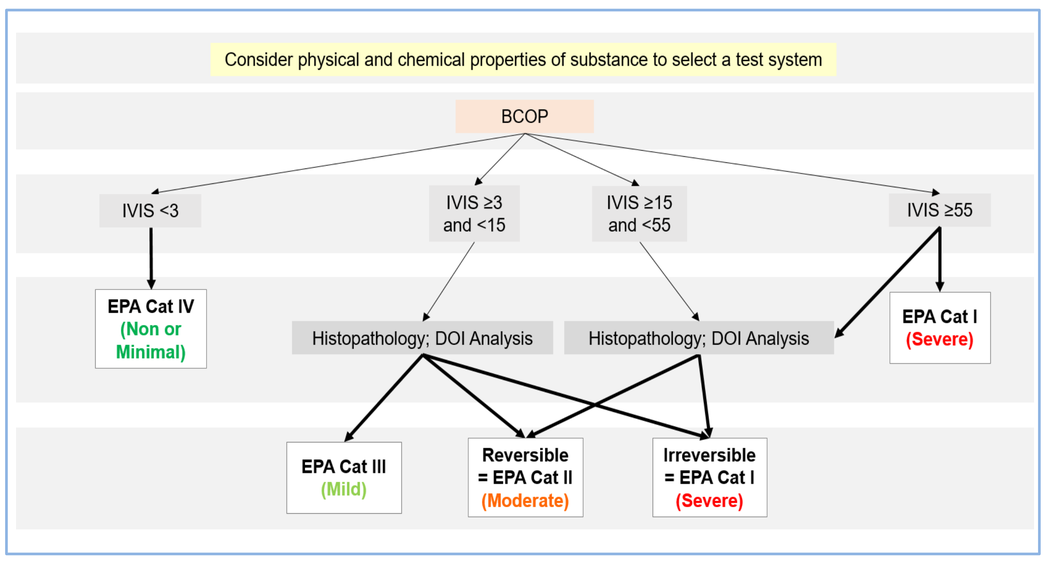
DEFINED APPROACHES (DA-EO+BCOP and DA-BCOP)

Schematics showing (a) the classification flowchart for DA-EO+BCOP for EPA hazard classification of eye irritation of agrochemical formulations using the EpiOcular™ and BCOP assays, for formulations predicted to be non- or minimally irritating, and (b) the classification flowchart for DA-BCOP for EPA hazard classification of eye irritation of agrochemical formulations using the BCOP assay, for formulations predicted to be irritating. Acronyms: IVIS – *in vitro* irritancy score; DOI – Depth of Injury.

a)



b)



DISCUSSION

Alignment across the three approaches was good, with alignment between the predictions of at least two approaches for 26/29 formulations (89.6%). Observed discrepancies may be explained by the following common themes:

- *In vivo* rabbit test lacks reproducibility, in particular for the mild and moderate irritant categories. Scoring of apical effects is subjective.
- Historical *in vivo* rabbit category predictions being driven by an effect observed in a single rabbit.
- Blinded study design → authors unable to consider the formulation's physical and chemical properties. Knowledge of properties would inform whether DA-EO+BCOP or DA-BCOP would be most reliable.
- Cut-off values for Category IV in EpiOcular™ within DA-EO+BCOP and in BCOP within DA-BCOP are conservative, but slight differences in results for the two assays can lead to EPA Category III prediction for DA-EO+BCOP and EPA Category IV prediction for DA-BCOP.
- Species differences between cell types may affect outcomes. DA-EO+BCOP use human cells or cow tissues, DA-BCOP uses cow tissues, and the historical *in vivo* rabbit test uses rabbits. For assessing human health effects, the reliance should be on human tissue models, though mechanistically, full thickness corneal models (i.e. BCOP) can assess more severe irritancy potential.

RESULTS

Table showing the EPA categories predicted with DA-EO+BCOP, DA-BCOP, and historical *in vivo* rabbit data. Green = alignment between two or more of the predicted categories. Orange = a misalignment of one category prediction, resulting in no change to risk management requirements for personal protective equipment (PPE). Red = a misalignment of one or more category prediction, resulting in a change to risk management requirements for PPE. ‡ = that histopathology conducted for the BCOP method suggests a mild to moderate eye irritation response, corresponding to either an EPA Category II or III. When this occurs, EPA Category II (the more conservative option) is predicted. Formulation types: SC = suspension concentrate, EC = emulsifiable concentrate, EC/ME = microencapsulated EC formulation, SL = soluble liquid.

Formulation code	Formulation type	Predicted EPA classification using DA-EO+BCOP	Predicted EPA classification using DA-BCOP	Predicted EPA classification from historical *in vivo* data
D	EC	I	I	I
G	EC	I	I	I
J	EC	I	I	I
F	SL	I	I	I
H	SL	I	I	I
I	SL	I	I	I
V	SL	I	I	III
U	EC	II	II	II
X	EC	II‡	II‡	II
R	SL	II	II	II
AB	EC	II	II	III
K	SL	III	IV	II
Q	SL	II‡	IV	II
AC	EC	III	III	III
W	SL	III	III	III
E	EC	III	III	I
L	EC	III	IV	III
S	SL	III	IV	III
O	SL	III	IV	IV
Y	EC	III	IV	II
AA	EC	III	IV	II
A	EC/ME	IV	IV	IV
B	SC	IV	IV	IV
C	SC	IV	IV	IV
N	SC	IV	IV	IV
P	SC	IV	IV	IV
M	SL	IV	IV	IV
T	SC	IV	IV	III
Z	EC	IV	III	III

WEIGHT OF EVIDENCE ASSESSMENT

- DA-BCOP and DA-EO+BCOP were developed to expand the applicability of certain biologically relevant *in vitro* and *ex vivo* methods to classify agrochemical formulations into EPA toxicity categories for eye irritation, and to provide information to protect public health.
- EpiOcular™, BCOP, and the rabbit test have been characterized with respect to their relevance to human ocular anatomy, exposure, and mechanisms of eye irritation. EpiOcular™ and BCOP assays were demonstrated to be as, or more, reliable and relevant to human eye irritation than the *in vivo* rabbit eye irritation test. (More details in Clippinger et al 2021.)
- EpiOcular™ and BCOP assays are approved as OECD test guidelines, and therefore have been extensively studied and transparently described.

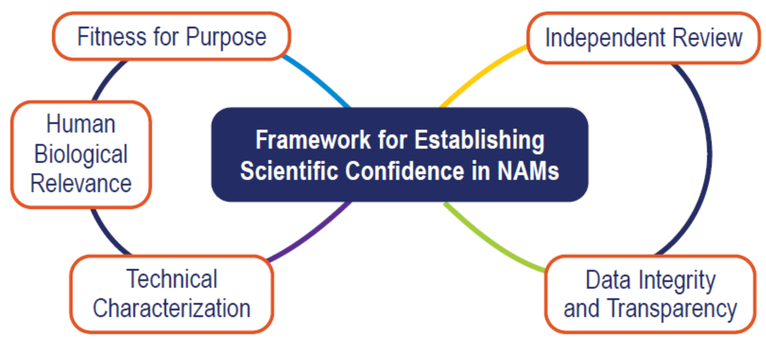


Figure from van der Zalm et al. 2022

CONCLUSION

DA-EO+BCOP and DA-BCOP were evaluated using an established scientific confidence framework and demonstrated to be as or more fit for purpose and human-relevant than the *in vivo* rabbit test. Further, the DAs are built on methods that have been demonstrated to be reproducible, and were internationally evaluated, transparently described, and independently reviewed. Thus, we can conclude that the DAs are equal to or better than the *in vivo* rabbit test for predicting the effects of agrochemical formulation exposure to humans.

Disclaimer: The views expressed in this poster are those of the authors and do not necessarily represent the views or policies of their respective employers or their stakeholders. Raw data are available on request. Publication of the data presented here is submitted to Cutaneous and Ocular Toxicology.

References: Clippinger AJ, Raabe HA, Allen DG, et al. Human-relevant approaches to assess eye corrosion/irritation potential of agrochemical formulations. Cutan Ocul Toxicol. 2021;40(2):145-167; van der Zalm AJ, Barroso J, Browne P, et al. A framework for establishing scientific confidence in new approach methodologies. Arch Toxicol. 2022;10.1007/s00204-022-03365-4. Others available on request and in forthcoming publication.

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