Advances in the Assessment of Eye Damage and Irritation and Skin Sensitisation Potential of Pesticides Using Non-Animal Methods

PETA SCIENCE CONSORTIUM INTERNATIONAL e.V.
Advancing 21st Century Toxicology

HSE Health & Safety Executive

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Minimisation of Animal Testing

The Health and Safety Executive (HSE) has guidance in place to minimise animal testing:[1]

"…TESTING ON VERTEBRATE ANIMALS FOR THE PURPOSES OF THIS REGULATION SHALL BE UNDERTAKEN ONLY WHERE NO OTHER METHODS ARE AVAILABLE."

"…NO NEW STUDIES SHALL BE CONDUCTED IN VERTEBRATE ANIMALS WHERE VALIDATED ALTERNATIVE METHODS ARE AVAILABLE."

Relevant and reliable non-animal approaches are available for assessing the eye damage and irritation and skin sensitisation potential of pesticide products and active ingredients. The Organisation for Economic Co-operation and Development (OECD) has adopted test guidelines (TG) on defined approaches and various non-animal methods for assessing these endpoints.
The applicability of *in vitro/ex vivo* approaches to pesticide active ingredients and products are reviewed in this poster. Difficult-to-interpret HSE case studies are reviewed to demonstrate how OECD TGs can be implemented in complex circumstances.

Eye Damage and Irritation

Background

Data obtained exclusively from *in vitro/ex vivo* tests can be used to discriminate among GHS categories for eye damage and irritation (Category 1, Category 2, and No Category).[2,3]

These tests can completely replace the Draize rabbit test (OECD TG 405) and are as or more reflective of human biology and less variable than the *in vivo* test.[4,5]

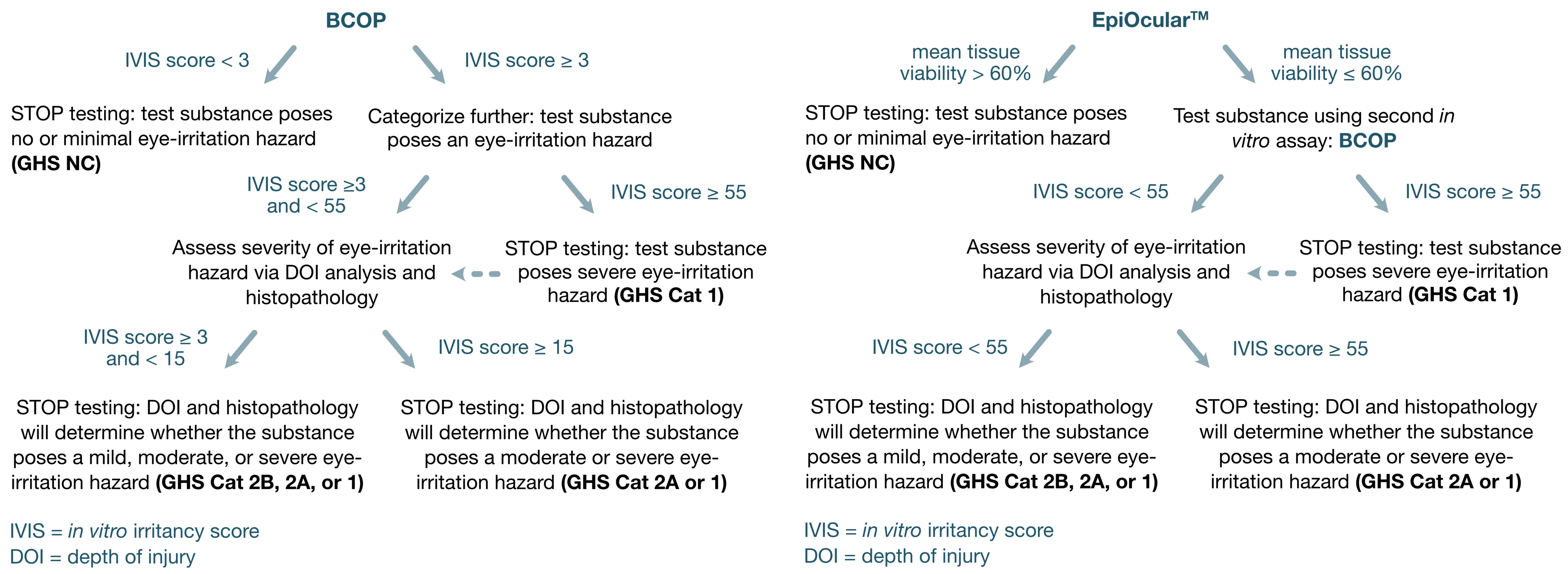

Figure 1. Defined approaches for assessing pesticide formulations (adapted from van der Zalm et al. 2023).[6] OECD TG 437: Bovine Corneal Opacity and Permeability (BCOP) test.[7] OECD 492: Reconstructed Human Cornea-Like Epithelium (RhCE) Test Method for Identifying Chemicals Not Requiring Classification and Labelling for Eye Irritation or Serious Eye Damage.[8]

HSE Case Study

The eye irritation potential of pesticide product, Product X, was assessed using the calculation method and BCOP.

Method	Outcome	Result
Calculation Method[9]	Generic concentration limit for eye damage Category 1 exceeded based on concentrations of relevant components	Category 1, according to Regulation (EC) 1272/2009 (GB/NI CLP)[9]
BCOP	Mean IVIS score = -0.9	No Category

Table 1. HSE case study of pesticide Product X with results and conclusions.

Conclusion: When interpreting Figure 2.1 in OECD Guidance Document (GD) 263 on Integrated Approaches to Testing and Assessment (IATA) for Serious Eye Damage and Eye Irritation,[3] Category 2 may be proposed as an average of the two results using a weight-of-evidence approach. However, both OECD GD 263 and OECD TG 437 state that BCOP is accepted as a standalone method for predicting GHS No Category or GHS Category 1. Thus, the BCOP result is sufficient to conclude No Category for Product X.
Furthermore, OECD TGs are covered by Mutual Acceptance of Data (MAD);[10] GDs are not, and the IATA requires expert judgment.

Skin Sensitisation

Background

The *in vivo* local lymph node assay (LLNA) has demonstrated limitations, including variability and a lack of human relevance.[11,12] Chemical and biological mechanisms linked with skin sensitisation are described in the Adverse Outcome Pathway (AOP) and *in chemico* and *in vitro* test methods based on these mechanisms address the first three key events (Figure 2).[13-16]

OECD TG 497: Defined Approaches on Skin Sensitisation[13]

- Integrates *in silico, in chemico,* and *in vitro* methods that can produce results as informative as, if not more informative than, the LLNA
- The integrated testing strategy (ITS) can discriminate among the three GHS sensitiser subcategories

Adverse Outcome Pathway

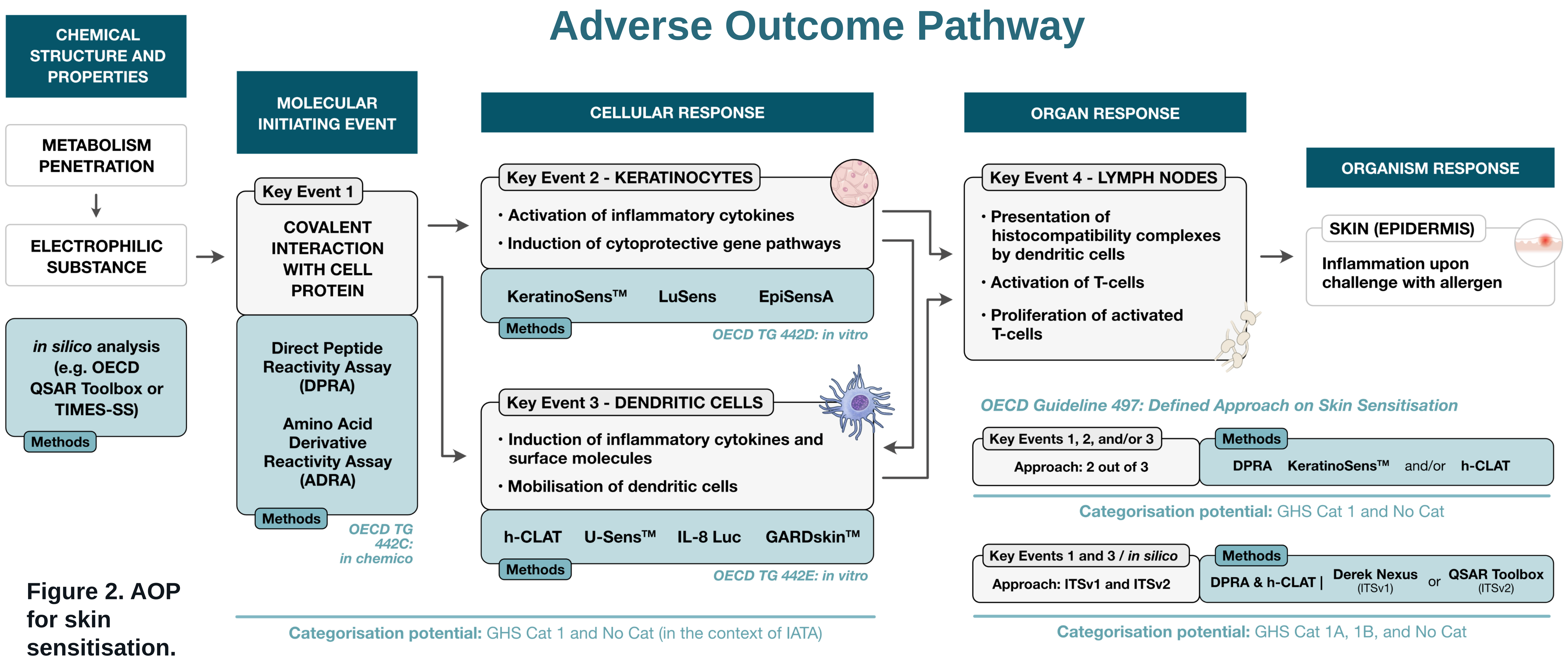

Figure 2. AOP for skin sensitisation.

HSE Case Study

The skin sensitisation potential of pesticide active ingredient, Chemical Y, was assessed using the OECD TG 497 2 out of 3 defined approach. Where 2 results are not concordant, a 3rd key event is investigated, and the conclusion is based on the two concordant results.

Method and Key Event	Outcome	Result	Explanation
DPRA KE 1	Negative	Inconclusive	Major deficiencies in the HPLC analysis of proficieny substances
LuSens KE 2	Negative	Negative	GLP and OECD-compliant
h-CLAT KE 3	Positive	Positive	Major deficienies in validity of postive control, but due to positive outcome, accepted as worst case
2 out of 3 Approach		**No conclusion**	**KE 1: Inconclusive KE 2: Negative KE 3: Positive**

Table 2. HSE case study of pesticide active ingredient Chemical Y using the 2 out of 3 defined approach.

Conclusion: Inconclusive DA predictions may be considered in a weight-of-evidence approach and/or within the context of an IATA with other information:[13]

- Clinical data
- Read across
- Existing *in vivo* data
- Other non-animal data, such as QSARs

References