Three-Phase Testing of Agrochemical Formulations: Developing a Defined Approach for Eye Irritation Potential

A.B. Daniel[1], A.J. van der Zalm[2], A.J. Clippinger[2], N. Kleinstreuer[3], D.G. Allen[1]

[1]Inotiv, RTP, NC, United States; [2]PETA Science Consortium International e.V., Stuttgart, Germany; [3]NIH/NIEHS/DTT/PTB/NICEATM, RTP, NC, United States

Regulators require that agrochemical manufacturers provide information about the harmful effects potentially caused by their products and active ingredients. The Draize rabbit test has historically been conducted to assess eye irritation potential of agrochemical formulations. A major reason for the continued reliance on the in vivo rabbit eye test is that the accuracy of data from new methods have typically been determined solely through direct comparison to results from the in vivo rabbit eye test, despite its demonstrated lack of reproducibility and relevance to humans. Ultimately, our objective is to develop defined approaches that leverage strengths of non-animal methods to predict the complete spectrum of eye irritancy potential. To achieve this, agrochemical formulations that met specific inclusion criteria were selected for testing in a three-phase study using a common set of non-animal test methods. Criteria included availability of historical rabbit data or ocular irritancy classification information, representation of common agrochemical formulation types, and a range of U.S. Environmental Protection Agency (EPA) and United Nations Globally Harmonised System of Classification and Labelling of Chemicals (GHS) hazard classifications. In Phase 1, six formulations classified as non-irritating (EPA Category IV; GHS Not Classified [NC]) or severely irritating (EPA Category I; GHS Category 1) were evaluated in seven in vitro or ex vivo eye irritation test method protocols as a proof-of-principle to determine which methods might be useful in the defined approach. Ten additional formulations representing the full range of classifications (EPA Categories I, II, III, and IV; GHS Categories 1, 2A, 2B, and NC) were tested in Phase 2. Based on an assessment of the results and considering the relevance of each method to humans, four in vitro or ex vivo assays were selected to proceed with Phase 3 testing of an additional twelve formulations classified as moderately (EPA Category II; GHS Category 2A) or mildly irritating (EPA Category III, GHS Category 2B). Additionally, a subset of thirteen formulations were tested in a fifth method. A total of 29 formulations have now been tested in as many as five methods: Bovine Corneal Opacity and Permeability (with histopathology; Organisation for Economic Co-operation and Development Test Guideline [OECD TG] 437), EpiOcular (OECD TG 492), SkinEthic Time-to-Toxicity (OECD TG 492B), In Vitro Depth of Injury, and EyeIRR-IS. Data generated in this study were used to analyze alignment across these five non-animal methods and the rabbit test. Consensus predictions for each formulation were determined based on majority alignment among individual assay results. Consensus predictions were achieved for 26 and 27 of 29 formulations for EPA and GHS classification systems, respectively. Data were insufficient to determine consensus predictions for the remaining formulations. These data form the basis of ongoing work to develop a defined approach for assessing eye irritation potential of agrochemical formulations. This project was funded in whole or in part with federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C.

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