**Human-Relevant Approaches to Assess Eye Corrosion/Irritation Potential of Agrochemical Formulations**

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**INTRODUCTION**

Multiple in vitro and ex vivo eye irritation and corrosion test methods are available as internationally harmonized test guidelines for regulatory use. Despite their demonstrated usefulness to a broad range of substances, they are not routinely used for testing agrochemical formulations due to a lack of concordance with results from the rabbit eye test. The inherent variability of the rabbit test, anatomical differences between rabbit and human eyes, and differences in modeling exaggerated exposures in rabbit eyes relative to actual human exposure contribute to this lack of concordance. Ultimately, the regulatory purpose for these tests is protection of human health; therefore, there is a need for testing approaches based on human biology.

Consideration of the mechanisms of eye irritation and the strengths and limitations of all available methods show that the in vitro/ex vivo methods are as or more reflective of human biology and less variable than the currently used rabbit test. Combining structural and functional information about a test substance with results from human-relevant methods will ensure the protection of humans following accidental eye exposure to agrochemicals.

**RABBIT DRAIZE TEST**

The U.S. Environmental Protection Agency Office of Pesticide Programs receives data on agrochemicals from more than 200 rabbit eye irritation tests each year. The rabbit Draize test:
- evaluates qualitative and/or quantitative ocular parameters
- provides limited mechanistic information
- may not elucidate mechanisms of action that occur in humans
- uses a longer exposure time than anticipated in humans due to accidental exposures
- was never validated for its relevance to humans
- has significant intra- and inter-subject variability

There are numerous differences between rabbit and human eyes, including:
- rabbits have a notochord membrane, humans do not
- rabbits have a larger conjunctival sac than humans
- the tissue structure, thickness, and biochemistry of human and rabbit corneas differ
- rabbits produce less tears than humans
- the pH of a rabbit eye aqueous humor is more alkaline (8.2) than that of a human eye (7.1-7.2)

**IN VITRO/EX VIVO ASSAYS**

Schematic of a human corneal section showing which in vitro/ex vivo assays are appropriate for evaluating specific layers, with models relevant to the (a) corneal epithelium and (b) full thickness cornea.

**MECHANISM OF EYE IRRITATION**

**DEMOGRAPHY**

- **depth of injury model**
  - Non
  - Slight
  - Moderate
  - Severe

**MOLECULAR INITIATING EVENT**

- leading to cell surface proteins
- binding to complement proteins / enzymes
- surface interaction and desorption of cell membrane lead blayer organization
- solvent desorption of cell membrane proteins or lipids
- solvent precipitation of cell proteins
- selective depletion of membrane lipids
- acid precipitation of cell proteins, enzymes and nucleic acids
- acid precipitation of components of the extracellular matrix
- oxidative changes in cell proteins
- solvent desorption of nucleic acids
- solvent desorption of components of the extracellular matrix
- precipitation of nucleic acids
- binding to DNA and/or RNA

**CELLULAR RESPONSE**

- chemical additives of vital enzymes, nutrients, acids
- cell stress responses
- breakdown of the light function
- activation of matrix metalloproteinases
- changes in cell surface markers and cell-cell and cell-to-matrix membrane adhesive molecules, desmosomes / hemidesmosomes / anchoring proteins
- release of interleukins and cytokines (e.g., IL-1β, TNF-α)
- induction of secondary cytokines
- cellular density trigger (CDT) type non-cytopathic necrosis
- changes in cell metabolism / regeneration
- changes in normal cell phenotype
- exotoxins or endotoxins damage leading to cell death

**ORGANIC RESPONSE**

- increased serum or epidemiological barrier function
- toxic effects on plasma and drainage
- swelling of the conjunctival tissues / swelling of the conjunctival tissues
- epithelial tissue swelling
- epithelial and non-epithelial tissue layers
- corneal and conjunctival swelling and edema, and swelling related corneal opacity
- corneal opacity due to stromal/membranous destabilization/edema
- evocation of wound healing response and anti-inflammatory response and neuropathy
- induction of sight, pain, and neovascularization
- loss of endothelium

**CONECLUSIONS**

The in vitro and ex vivo models described herein are more human relevant and robust than the rabbit test because they include one or more of the following properties:

- more closely model potential exposures in humans and allow for precise dosing
- model human corneal tissue barrier functions and penetration kinetics
- include relevant cell types within each of the tissue layers
- provide quantitative results
- are reproducible within and between laboratories
- discriminate a range of cytotoxic responses within each layer

- The scientific validity of an in vitro/vivo model should be assessed by understanding its relevance to human biology and mechanisms of eye irritation.
- Considering the variability of the currently used rabbit test and an understanding of human biology and mechanisms of eye irritation, to best protect human health, data from the in vitro/vivo methods should be considered for use at this time.

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Image modified from Scott, et al., 2010

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**Cornea Stromal**

- Corneal Corneal Opacity and Permeability
- Isolated Chicken Eye
- Isolated Rat Eye
- Ex Vivo Eye Irritation Test (EVIT)
- Ocular Irritation
- OptiSafe

**Cornea Endothelium**

- Corneal Opacity and Permeability
- Isolated Chicken Eye
- Isolated Rat Eye
- Ex Vivo Eye Irritation Test (EVIT)