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 best 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 acral outcomes in the rabbit eye
- provides limited mechanistic information
- may not elucidate modes of action that occur in humans
- uses a longer exposure time than anticipated by humans due to accidental exposures
- was never validated for its relevance to humans
- has significant intra- and inter-study variability

There are numerous differences between rabbit and human eyes, including:
- rabbits have a noctilucent 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 (7.4-7.8)

CONCLUSIONS

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:

a) more closely model potential exposures in humans and allow for precise dosing
b) model (human) corneal tissue barrier functions and penetration kinetics
c) include relevant cell types within each of the tissue layers

d) provide quantitative results

e) reproducible within and between laboratories

f) 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 protect human health, data from the in vitro/vivo models should be considered applicable for use at this time.

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Schematic depiction of the human eye

Human cornea section

Rabbit cornea section

MECHANISM OF EYE IRRITATION

Depth of Injury Model

General potential events that may occur in the eye injury

Depth of Injury Model

MOLECULAR INITIATING EVENT
- chemical events of cell enzymes, nucleic acids
- cell stress responses
- breakdown of the light junctions
- activation of matrix metalloproteinases
- changes in cell surface markers and cell-cell and cell-tissue interactions
- adhesion molecules, desmosomes / hemidesmosomes / anchoring proteins
- changes in cell morphology and cytokines (e.g., IFN, TNF)
- reduction of secondary cytokines
- cell death / apoptosis
- oxidative stress
- nuclear damage / DNA damage

CELLULAR RESPONSE
- increased synthesis or degradation of cytokines and other mediators
- activation of antioxidant enzymes
- cell cycle arrest
- cell detachment
- cell membrane lysis
- cell death

ORGAN RESPONSE
- increased secretory or excretory function
- epithelial cell death
- tissue necrosis
- inflammation

ORGANISM RESPONSE
- increased secretory or excretory function
- epithelial cell death
- tissue necrosis

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 or (b) full thickness cornea.

IN VITRO/EX VIVO ASSAYS