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.

Schematic depiction of the human eye

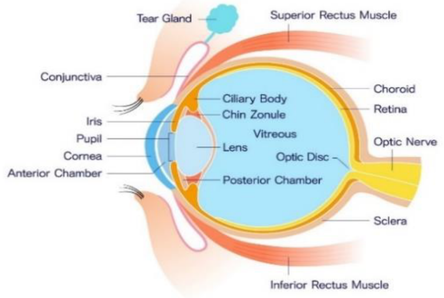


Image purchased from iStock

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 apical 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 in 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 nictitating membrane; humans do not
- rabbits have a larger conjunctival sac than humans
- the tissue structure, thickness, and biochemistry of human and rabbit cornea 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)

Human cornea section

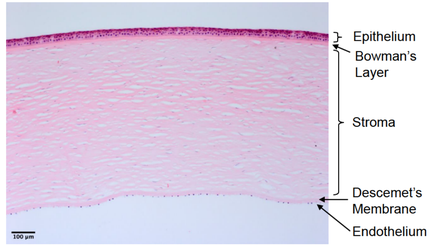


Image Courtesy of Hans E. Grossniklaus, MD

Rabbit cornea section

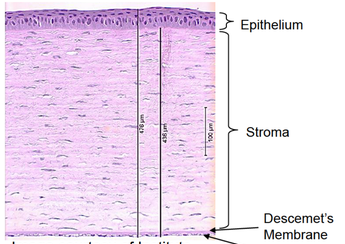
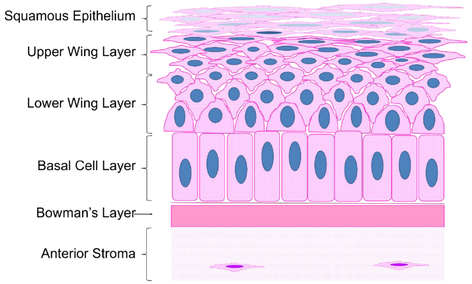


Image courtesy of Institute for In Vitro Sciences

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 or (**b**) full thickness cornea.

a)

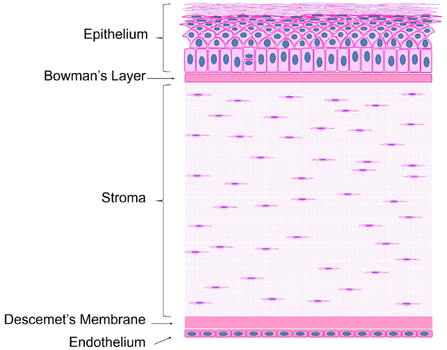


Superficial Conjunctival or Corneal Epithelium
3D Reconstructed Human Cornea-like Epithelial Tissue
EYEIRR-IS
Vitrigel-Eye Irritancy
Bovine Corneal Opacity and Permeability
Isolated Chicken Eye
Isolated Rabbit Eye
Porcine Cornea Opacity Reversibility Assay
Ex Vivo Eye Irritation Test (EVEIT)
Fluorescein Leakage
Short Time Exposure
Neutral Red Release
Cytosensor Microphysiometer
Ocular Irritection
OptiSafe

Wing Cell Layer of the Epithelium
3D Reconstructed Human Cornea-like Epithelial Tissue
EYEIRR-IS
Vitrigel-Eye Irritancy
Bovine Corneal Opacity and Permeability
Isolated Chicken Eye
Isolated Rabbit Eye
Porcine Cornea Opacity Reversibility Assay
Ex Vivo Eye Irritation Test (EVEIT)
Ocular Irritection
OptiSafe

Lower Wing Cell and Basal Cell Layers of the Epithelium
3D Reconstructed Human Cornea-like Epithelial Tissue
EYEIRR-IS
Bovine Corneal Opacity and Permeability
Porcine Cornea Opacity Reversibility Assay
Isolated Chicken Eye
Isolated Rabbit Eye
Ex Vivo Eye Irritation Test (EVEIT)
Ocular Irritection
OptiSafe

b)



Corneal Stroma
Bovine Corneal Opacity and Permeability
Isolated Chicken Eye
Isolated Rabbit Eye
Ex Vivo Eye Irritation Test (EVEIT)
Ocular Irritection
OptiSafe

Corneal Endothelium
Bovine Corneal Opacity and Permeability
Isolated Chicken Eye
Isolated Rabbit Eye
Ex Vivo Eye Irritation Test (EVEIT)

MECHANISM OF EYE IRRITATION

Depth of Injury Model

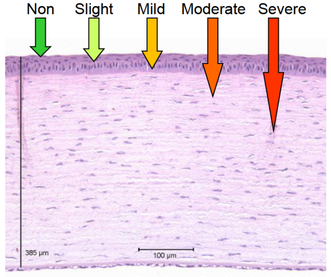


Image modified from Scott, et al., 2010

General potential events that may occur in the development of eye injury

MOLECULAR INITIATING EVENT
- binding to cell surface proteins
- binding to cytoplasmic proteins / enzymes
- surfactant intercalation and disruption of cell membrane lipid bilayer organization
- solvent dissolution of cell membrane proteins or lipids
- solvent precipitation of cell proteins
- alkaline saponification of membrane lipids
- acid precipitation of cell proteins, enzymes and nucleic acids
- oxidative damage to cell membrane lipids
- oxidative changes in cell proteins
- solvent dissolution of nucleic acids
- solvent dissolution of cytoplasmic components
- precipitation of nucleic acids
- binding to DNA and/or RNA

CELLULAR RESPONSE
- chemical antagonism of vital enzymes, nucleic acids
- cell stress responses
- breakdown of the tight junctions
- activation of matrix metalloproteases
- changes in cell surface markers and cell-to-cell and cell-to-basement membrane adhesion molecules, desmosomes / hemidesmosomes / anchoring proteins
- breakdown of cell membrane integrity
- release of chemokines and cytokines (e.g., IL-1α, TNFα)
- induction of secondary cytokines
- neural dendrites trigger TRPV1-type nociceptive response
- changes in cell metabolism/respiration
- changes in normal functional phenotype
- necrotic or apoptotic damage leading to cell death

ORGAN RESPONSE
- increased corneal or conjunctival permeability/loss of barrier function
- conjunctival hyperemia and discharge
- swelling of the conjunctival tissues / swelling of the eye lid tissues
- epithelial tissue swelling
- sloughing and loss of epithelial tissue layers
- corneal/stromal swelling and oedema, and swelling-related corneal opacity
- corneal opacity due to cellular/molecular denaturation/coagulation
- induction of wound healing response and basal cell regeneration/turnover
- inflammatory response and neutrophil migration
- induction of fibrosis, panus, and neovascularization
- loss of endothelium

ORGANISM RESPONSE
- increased corneal or conjunctival susceptibility to xenobiotics
- pain and nociceptive responses
- induction of lachrymation
- transient or permanent loss of visual acuity

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/ex vivo* method 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/ex vivo* methods should be considered applicable for use at this time.

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ILS Advancing Science, Improving Lives
IIVS Institute for In Vitro Sciences
NTP National Toxicology Program U.S. Department of Health and Human Services
European Commission Joint Research Centre
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