METHODS FOR PREDICTING SERIOUS EYE DAMAGE AND IRRITATION

METHOD	PRINCIPLE OF THE TEST	APPLICABILITY DOMAIN	GHS CATEGORISATION
DEFINED APPROACHES			
OECD Test Guideline 467: Defined Approaches for Serious Eye Damage and Eye Irritation, comprising two defined approaches for liquids (DAL1 and DAL2), a defined approach for solids (DAS), and a defined approach for surfactants (DASF)	DAL1 combines information from three sources: (1) physiochemical properties of the test substance, (2) results from testing in OECD TG 437 BCOP assay using the laser light based opacitometer (LLBO), and (3) results from testing in OECD TG 492, either the EpiOcular Eye Irritation Test (EIT) or the SkinEthic Human Corneal Epithelium EIT. DAL2 combines information from two sources: (1) results from testing in OECD TG 437 BCOP assay using the LLBO and (2) OECD TG 491 Short Time Exposure (STE) assay. DAS combines information from two *in vitro* sources: (1) results from testing in OECD TG 437 BCOP assay using the LLBO and (2) results from testing in OECD TG 492 (using the SkinEthic™ Human Corneal Epithelium EIT). DASF combines information from two *in vitro* sources: (1) results from testing in a technically adapted version of the STE method (from OECD TG 491) and (2) the EpiOcular™ EIT or the SkinEthic™ Human Corneal Epithelium EIT (from OECD TG 492).	DAL1 is applicable to neat non-surfactant liquids, DAL2 is applicable to neat and diluted non-surfactant liquids, DAS is applicable to neat solids, and DASF is applicable to surfactants. For all approaches, the applicability domain of each test guideline used within the defined approach should also be considered.	The approaches can be used for the identification of substances causing serious eye damage (GHS Cat 1), causing eye irritation (GHS Cat 2), and not requiring classification for eye irritation or serious eye damage (GHS No Cat). Each approach can be used as a full replacement for the Draize rabbit eye irritation test.
RECONSTRUCTED THREE-DIMENSIONAL HUMAN TISSUE ASSAYS			
OECD Test Guideline 492: Reconstructed Human Cornea-like Epithelium Test Method for Identifying Chemicals Not Requiring Classification and Labelling for Eye Irritation or Serious Eye Damage (e.g. EpiOcular™, SkinEthic™, LabCyte, and MCTT HCE™)	The test substance is applied to reconstructed tissue from human cells, which have been cultured to form a stratified, highly differentiated squamous epithelium that is morphologically similar to that found in the human cornea. Cell viability (MTT or WST-8 assay) is used to predict toxicity.	This assay is applicable to substances and mixtures and to solids, liquids, semi-solids, and waxes.	This assay can be used for the identification of substances not requiring classification for eye irritation or serious eye damage (GHS No Cat).
OECD Test Guideline 492B: Reconstructed Human Cornea-like Epithelium Test Method for Eye Hazard Identification (SkinEthic™)	The test substance is applied to reconstructed tissue from human cells (as in OECD TG 492). Depending on whether the test substance is a solid or a liquid, cell viability is assessed at two or three exposure times, respectively.	This assay is applicable to substances and mixtures and to solids, liquids, semi-solids, and waxes.	This assay can be used for the identification of substances causing serious eye damage (GHS Cat 1), causing eye irritation (GHS Cat 2), and not requiring classification for eye irritation or serious eye damage (GHS No Cat). This method can be used as a full replacement for the Draize rabbit eye irritation test.
OECD Test Guideline 494: Vitrigel-Eye Irritancy Test Method for Identifying Chemicals Not Requiring Classification and Labelling for Eye Irritation or Serious Eye Damage	Human corneal epithelium models fabricated in a collagen vitrigel membrane are exposed to a test substance. Damage to the barrier function of the models is assessed by analysing time-dependent changes in transepithelial electrical resistance values.	This assay is applicable to substances and mixtures with a pH > 5 (based on 2.5% weight/volume preparation). It is not applicable to solids.	This assay can be used for the identification of substances not requiring classification for eye irritation or serious eye damage (GHS No Cat).

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ORGANOTYPIC *EX VIVO* ASSAYS			
OECD Test Guideline 437: Bovine Corneal Opacity and Permeability (BCOP) Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage	The test substance is applied directly to cows' eyes obtained as by-products from abattoirs. Corneal opacity (measured quantitatively as the amount of light transmission through the cornea) and permeability (measured quantitatively as the amount of sodium fluorescein dye that passes across the full thickness of the cornea) are measured. Optional histopathology can be conducted for additional information.	This assay is applicable to solids, liquids (including semi-solids, creams, and waxes), and mixtures.	This assay can be used for the identification of substances causing serious eye damage (GHS Cat 1) and substances not requiring classification for eye irritation or serious eye damage (GHS No Cat).
OECD Test Guideline 438: Isolated Chicken Eye (ICE) Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage	The test substance is applied directly to chickens' eyes obtained as by-products from abattoirs. Corneal swelling, opacity, and fluorescein retention are assessed.	This assay is applicable to solids (which may be soluble or insoluble in water), liquids, emulsions, and gels.	This assay can be used for the identification of substances causing serious eye damage (GHS Cat 1) and substances not requiring classification for eye irritation or serious eye damage (GHS No Cat).
CYTOTOXICITY AND CELL FUNCTION BASED *IN VITRO* ASSAYS			
OECD Test Guideline 460: Fluorescein Leakage (FL) Test Method for Identifying Ocular Corrosives and Severe Irritants	Epithelial monolayer Madin-Darby canine kidney cells are cultured on permeable inserts. The test chemical is applied for one minute and then removed. Next, the non-toxic, highly fluorescent sodium fluorescein dye is added, and the amount of dye that passes through the cell layer is measured spectrofluorometrically and used to predict toxicity.	This assay is applicable to water-soluble chemicals or mixtures. There are limitations for coloured or highly viscous substances. (However, predictivity is improved by increasing the number of wash steps.) It is not applicable to strong acids and bases, cell fixatives, or highly volatile substances.	This assay can be used for the identification of substances causing serious eye damage (GHS Cat 1).
OECD Test Guideline 491: Short Time Exposure (STE) *In Vitro* Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage	This test measures cell viability (via the MTT assay) of Statens Seruminstitut Rabbit Cornea (SIRC) corneal epithelial cells in 96 well plates. As compounds are generally cleared from human eyes in one to two minutes and from rabbit eyes in three to four minutes, this test requires a five-minute exposure.	This assay is applicable to test chemicals that are soluble in saline, DMSO, or mineral oil.	This assay can be used for the identification of substances causing serious eye damage (GHS Cat 1) and substances not requiring classification for eye irritation or serious eye damage (GHS No Cat).
MACROMOLECULAR MATRIX ASSAYS			
OECD Test Guideline 496: *In Vitro* Macromolecular Test Method for Identifying Chemicals Inducing Serious Eye Damage and Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage	The test substance is applied directly to an *in chemico* macromolecular matrix model composed of lipids, proteins, glycoproteins, carbohydrates, and low molecular weight substances that model the cellular structure of the human corneal epithelium. An increase in optical density is used to predict the ocular hazard effects of chemicals.	This assay is applicable to solids (which may be soluble or insoluble in water) and liquids (which may be viscous or non-viscous) whose 10% solution/dispersion has a pH in the range 4 ≤ pH ≤ 9. There are some limitations for intensely coloured chemicals, chemicals that cause salting-out precipitation, high concentrations of some surfactants, and highly volatile chemicals. It is also applicable to mixtures.	This assay can be used for the identification of substances causing serious eye damage (GHS Cat 1) and/or substances not requiring classification for eye irritation or serious eye damage (GHS No Cat).

For more information on the above methods, see Clippinger AJ et al. Human-relevant approaches to assess eye corrosion/irritation potential of agrochemical formulations. *Cutan Ocul Toxicol.* 2021;40(2):145-167.
For more information on tiered testing strategies for serious eye damage and eye irritation, see the following:

- OECD. 2019. Guidance document on integrated approaches to testing and assessment (IATA) for serious eye damage and eye irritation. No 263. Series on Testing and Assessment.
- US Environmental Protection Agency. 2015. Use of an alternate testing framework for classification of eye irritation potential of EPA pesticide products.
- European Chemicals Agency. 2017. Guidance on information requirements and chemical safety assessment. Chapter R.7a: Endpoint specific guidance. Version 6.0. See R.7.2.8–R.7.2.11.

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IN SILICO TOOLS FOR PREDICTING SERIOUS EYE DAMAGE AND IRRITATION

In silico tools are used for assessing the potential of chemicals to cause irritation or serious eye damage. Eye effects are often driven by immediate local cytotoxicity and physicochemical reactivity, and *in silico* strategies focus on predicting these fundamental properties to categorise hazards. The use of physicochemical properties (e.g., pH, water solubility, vapour pressure, octanol-water partition coefficient, and surface tension) to predict toxicity is increasingly being incorporated into regulatory frameworks (e.g., US EPA) or Defined Approaches (e.g., OECD TG 467). For instance, chemicals with extreme pH (pH ≤ 2 or ≥ 11.5) are classified as corrosive (UN GHS Category 1) by default. Conversely, specific combinations of high lipid solubility (LogP), low water solubility, and low vapor pressure can be used to predict a lack of eye irritation (No Category). These physicochemical properties can be predicted by *in silico* tools. In addition, **statistical** QSAR models correlate molecular descriptors with existing data to predict irritation potential, while **expert rule-based** models predict eye hazards based on the presence of molecular features known to induce irritation or corrosion.

In silico tools are integral components of Integrated Approaches to Testing and Assessment (IATA) and Defined Approaches. For example, OECD Test Guideline 467 on Defined Approaches for Serious Eye Damage and Irritation includes DAL-1, which integrates physicochemical predictions (water solubility or vapour pressure, octanol-water partition coefficient, and surface tension) with *in vitro* data (from a reconstructed human Cornea-like Epithelium (RhCE)-based model and the bovine corneal opacity and permeability (BCOP) test methods) to distinguish between severe irritants (Category 1), irritants (Category 2), and non-irritants (No Category) for neat, non-surfactant liquids. This formalised integration allows computational predictions to contribute directly to regulatory classification.

A non-exhaustive list of *in silico* tools for predicting eye irritation and corrosion is provided below. Please contact Kyle Martin at kmartin@thepsci.eu to include additional resources on this list or with any questions.

TOOL	DEVELOPER	METHOD/APPROACH	AVAILABILITY
CASE Ultra	MultiCASE Inc.	Statistical	Commercial
Derek Nexus	Lhasa Limited	Expert rule-based	Commercial
iSafeRat	KREATiS	Statistical	Commercial
OECD QSAR Toolbox	OECD/ Laboratory of Mathematical Chemistry	Statistical (read-across/QSAR)	Open-source
Toxtree	IDEAconsult Ltd./ European Commission	Expert rule-based	Open-source
VEGA HUB	Istituto di Ricerche Farmacologiche Mario Negri	Consensus/hybrid	Open-source

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