Serious eye damage / eye irritation

1. Which of the REACH information requirements may be met via testing?

Annex VII to the REACH Regulation includes a requirement for *in vitro* tests as a first step for addressing serious eye damage/eye irritation (Section 8.2.1). An overview of the available internationally validated *in vitro* methods is presented in Table 1. While those methods were adopted by UN GHS for classification purposes, the implementation under EU CLP Regulation is currently pending.

An *in vivo* eye irritation study shall only be considered at Annex VIII level (section 8.2) in case the *in vitro* serious eye damage/eye irritation test(s) are not applicable for the substance or the results obtained are not adequate for classification and risk assessment.

The test methods covered by this document may be used to meet the REACH information requirements as explained below. These test methods usually need to be used in combination (within a testing strategy), unless one test result is considered adequate to be used in a tiered approach for classification and risk assessment. More information on tiered approach for classification can be found in Guidance on Application of CLP Criteria (Figure 3.4) and Guidance on Information Requirements and Chemical Safety Assessment, Chapter R.7a: Endpoint specific guidance (Figure R.7.2–5).

The methods often have limitations and cannot be used for all kinds of substances. Therefore, the registrants and test facilities (end-users) of the test methods are advised to check the chapter "3. Use, scope, and limitations of the test methods" below and respective test guideline, before deciding on a new test/study. Finally, the end-users should consider the performance of the standalone test methods as highlighted for standalone methods or described in respective test guidelines for non-standalone test methods.

2. Overview of the non-animal methods

Serious eye damage is defined as tissue damage in the eye, or serious physical decay of vision, which is not fully reversible, whereas eye irritation means the production of changes in the eye, which are fully reversible.

Chemically induced serious eye damage/eye irritation, manifested *in vivo* mainly by corneal opacity, iritis, conjunctival redness and/or conjunctival chemosis, is the result of a cascade of events beginning with penetration of the chemical through the cornea and/or conjunctiva and production of damage to the cells. Cell damage can occur by several modes of action, including cell membrane lysis (e.g., by surfactants, organic solvents); coagulation of macromolecules (particularly proteins) (e.g., by surfactants, organic solvents, alkalis and acids); saponification of lipids (e.g., by alkalis); and alkylation or other covalent interactions with macromolecules (e.g., by bleaches, peroxides and alkylators). However, it has been shown that cytotoxicity plays an important, if not the primary, mechanistic role in determining the overall serious eye damage/eye irritation response of a chemical regardless of the physicochemical processes underlying tissue damage. Moreover, the serious eye damage/eye irritation potential of a chemical is principally determined by the extent of initial injury, which correlates with the extent of cell death and with the extent of the subsequent responses and eventual outcomes. The non-animal methods aim at addressing the mode of action(s) leading to the cell damage.

Currently, there are nine non-animal OECD test methods (Table 1) available for investigation of the eye hazard properties. The individual test methods have different

applicability domains and can be used for identification of different classification categories. Specific information about applicability domains of individual methods is available is available in respective OECD TG.

2.1 Standalone method and defined approaches

So far, only one of the OECD test methods (OECD 492B) enables complete identification of the eye hazard categories, i.e., for substances i) inducing serious eye damage (Cat. 1), ii) inducing eye irritation (Cat. 2), and iii) not requiring classification for eye irritation or serious eye damage (No Cat.). Additionally, one of the OECD test methods (OECD 467) describes defined approaches which also enable identification of all eye hazard categories without the need of expert judgment and weight of evidence approach.

2.2 Non-standalone methods

Other methods than the two mentioned above (i.e. OECD 492B and OECD 497) can be used to detect, directly or indirectly, some of the ocular effects evaluated in the rabbit ocular irritancy test method, and to some degree their severity, but they do not measure conjunctival and iridial injuries or the persistence/reversibility of effects. This means that based on those individual methods, it is not possible to predict that the substance is inducing eye irritation (CLP Cat. 2).

Still, those individual methods are useful to predict that a substance is either inducing serious eye damage (Cat.1), or not requiring classification for eye irritation (CLP No Cat.). To help the end-users to decide how to use the test methods that can only partially identify the ocular hazards and cannot be used as standalone test methods, there is an OECD guidance document (OECD GD 263) Integrated Approaches to Testing and Assessment (IATA) for Serious Eye Damage and Eye Irritation.

2.3 Integrated Approaches to Testing and Assessment

OECD guidance document (OECD GD 263) Integrated Approaches to Testing and Assessment (IATA) for Serious Eye Damage and Eye Irritation can provide insights on how to results of in vitro assays which cannot be used as standalone test method or combined in defined approaches.

Table 1: Overview of OECD adopted non-animal methods for serious eye damage/eye irritation.

Latest update	Test method	OECD Test Guideline	Classification according to CLP Regulation
2024	DA	OECD TG 467	Cat. 1, Cat. 2 or No Cat.
2024	RhCE-TTT	OECD TG 492B	Cat. 1, Cat. 2 or No Cat.
2023	BCOP	OECD TG 437	Cat. 1 or No Cat.
2023	ICE	OECD TG 438	Cat. 1 or No Cat.
2023	FL	OECD TG 460	Cat. 1
2023	STE	OECD TG 491	Cat. 1 or No Cat.
2024	RhCE	OECD TG 492	No Cat.
2021	EIT	OECD TG 494	No Cat.
2024	*In vitro* macromolecular test method	OECD TG 496	Ocular Irritection: Cat. 1 or No Cat. OptiSafe: No Cat.

3. Use, scope, and limitations of the test methods

The complete information on the use, scope and limitation is given in detail in respective test guideline and the latest version should be used.

3.1 Reconstructed Human Cornea-like Epithelium Time-To-Toxicity (RhCE-TTT) test (OECD 492B)

RhCE-TTT Test Method for Eye Hazard Identification is the only standalone test method which can be currently used to identify substances requiring classification in any of the ocular hazard classification categories. The test method is recommended as a full replacement to the *in vivo* Draize acute eye irritation test. It directly measures cytotoxicity resulting from penetration of the substance through the corneal epithelium and production of cell and tissue damage following substance exposure.

Note on performance:

The performance of the currently only validated test method (SkinEthic™ Human Corneal Epithelium (HCE) Time-to-Toxicity (TTT)) is reported in OECD TG 492B as in the tables below:

The weighed **performance** of the SkinEthic™ HCE TTT showing correct, under- and over-predictions per UN GHC category **for liquids and solids together**:

UN GHS categories	**SkinEthic HCE TTT – Predicted categories (n/N%)**		
	Cat. 1 (n)	**Cat. 2 (n)**	**No Cat. (n)**
Cat. 1 (N=50)	79.2% (39.60)	20.8% (10.40)	0% (0.00)
Cat. 2 (N=44)	18.3% (8.06)	69.2% (30.46)	12.5% (5.48)
No Cat. (N=57)	1.8% (1.00)	23.3% (13.33)	74.9% (42.67)

Performance of the SkinEthic™ HCE TTT showing correct, under- and over- predictions per UN GHC category for **liquids (TTL protocol)**:

UN GHS categories	**SkinEthic HCE TTL – Predicted categories**		
	Cat. 1	**Cat. 2**	**No Cat.**
Cat. 1 (N=21)	85.4%	14.6%	0.0%
Cat. 2 (N=25)	20.2%	79.8%	0.0%
No Cat. (N=24)	0.0%	20.8%	79.2%

Performance of the SkinEthic™ HCE TTT showing correct, under- and over- predictions per UN GHC category for **solids (TTS protocol)**:

UN GHS categories	**SkinEthic HCE TTS – Predicted categories**		
	Cat. 1	**Cat. 2**	**No Cat.**
Cat. 1 (N=29)	74.7%	25.3%	0.0%
Cat. 2 (N=19)	15.8%	55.3%	28.9%
No Cat. (N=33)	3.0%	25.3%	71.7%

We recommend to the end-users of the test method to consider uncertainties related to the performance metrics of the currently only validated test method in the OECD TG 492B. The performance metrics shows that e.g., for liquids, about 15% of UN GHS Cat. 1 liquids were underpredicted as Cat. 2, whereas for solids, it was about 25% (1 in 4). Also, for solids, only slightly above half of UN GHS Cat. 2 can be correctly identified, whereas nearly 30% of UN GHS Cat. 2 were underpredicted, and about 28% of UN GHS solids not requiring classification were overpredicted.

3.2 Defined Approaches (DA) method (OECD 497)

OECD 467 - Defined Approaches for Serious Eye Damage and Eye Irritation describes defined approaches how to combine data obtained from certain *in vitro* methods and data on physicochemical properties. The eye hazard identification is based on defined data interpretation procedure, i.e., procedure which is applied to the results.

The guideline currently contains two defined approaches: i) DAL-1 which combines physicochemical properties and OECD 437 (BCOP-LLBO) *in vitro* data from for neat non-surfactant liquids, and ii) DAL-2 which combines *in vitro* data from OECD 437 and OECD 491 (BCOP-LLBO and STE) for non-surfactant neat liquids, liquids and solids dissolved in water to identify eye hazard classification categories.

Note on performance:

There is a remaining uncertainty in correct hazard identification based the DAs. The uncertainty should be considered by the end-user of the defined approach. Whilst there are

no target values defined to assess the predictivity of defined approaches for eye hazard identification to distinguish between the three UN GHS categories, Cosmetics Europe proposed target values with justification based on the uncertainty of the Draize eye test by considering the within- and between-test variability. The performance metrics currently applied for predictivity assessment of defined approaches is reported in the table below.

Performance metrics for assessment of the predictivity of a DA for eye hazard identification (OECD Supporting Document 354, see References)

UN GHS categories	**Defined Approach**		
	Cat. 1	**Cat. 2**	**No Cat.**
Cat. 1	≥75%	≤25%	≤5%
Cat. 2	≤30%	≥50%	≤35%
No Cat.	≤5%	≤30%	≥70%

In practice, this means that the performance metrics and performance of DAS would allow for correct labelling and risk management of only 50% of UN GHS Cat. 2 chemicals. Also, up to 1 of 4 UN GHS Cat. 1 substances causing serious eye damage can be underpredicted as only Cat. 2 based on the current SD performance metrics.

The performance metrics also allows for 35% overprediction of UN GHS non-Cat. 1. substances as Cat. 1. It means that more than one third of UN GHS non-Cat. 1 substances would be labelled and risk-managed as Cat. 1 if the regulators relied on a DA satisfying the current performance metrics. The regulatory implications for 30% of UN GHS No Cat. substances overpredicted as DA Cat. 2. are analogical.

3.3 Bovine Corneal Opacity and Permeability (BCOP) test (OECD TG 437)

- *in vitro (ex vivo)* assay that may be used to predict chemicals (substances or mixtures) as either (i) causing serious eye damage (Cat. 1 of CLP), or (ii) not requiring classification for eye irritation or serious eye damage according to CLP

- The results of the BCOP can be used in combination if Cat. 1 or No Cat. is not possible to as following:

- Coupled with laser-light based opacitometer (LLBO), BCOP can be used in DA (OECD 467) in combination with other test results or information on physicochemical properties.

- May result in false positive predictions for alcohols and ketones and false negative predictions for solids.

- Does not allow testing of gases and aerosols.

3.4 Isolated Chicken Eye (ICE) test (OECD TG 438)

- *in vitro (ex vivo)* assay that may be used to predict chemicals (substances or mixtures) as either (i) causing "serious eye damage" (Cat. 1 of CLP), or (ii) not requiring classification for eye irritation or serious eye damage according to the CLP (No Cat.).

- The revised Test Guideline includes information on applicability domain of the ICE test method to the testing of surfactants, alcohols and solids.

- While this test method is not considered valid as a complete replacement for the *in vivo* rabbit eye test, it may be useful as part of a tiered testing strategy for classification and labelling.

3.5 Fluorescein leakage (FL) test (OECD TG 460)

- *In vitro* assay that may be used for predicting water-soluble substances as causing "serious eye damage" (Cat. 1 of CLP).
- While this test method is not considered valid as a complete replacement for the *in vivo* rabbit eye test, it may be useful as part of a tiered testing strategy for classification and labelling, e.g., in top-down approach (IATA, GD 263).

3.6 Short Time Exposure (STE) test (OECD TG 491)

- Cytotoxicity-based *in vitro* assay that may be used to predict chemicals (substances or mixtures) as either (i) causing "serious eye damage" (Cat. 1 of CLP), or (ii) not requiring classification for eye irritation or serious eye damage according to the CLP (No Cat.).
- The method is suitable for chemicals that are dissolved or uniformly suspended for at least 5 minutes in physiological saline, 5% dimethylsulfoxide in saline, or mineral oil.
- Can be used in DAs in combination with other test results or information on physicochemical properties.

3.7 Reconstructed human Cornea-like Epithelium (RhCE) test (OECD TG 492)

- *in vitro* assay that may be used to predict chemicals not requiring classification and labelling for eye irritation or serious eye damage (No Cat.).
- May be useful in tiered testing strategy for classification and labelling, eg., in bottom-up approach (IATA, OECD GD 263).
- Covers multiple me-too methods.
- The methods are applicable to substances and mixtures, and to solids, liquids, semi-solids and waxes. The liquids may be aqueous or non-aqueous; solids may be soluble or insoluble in water.
- The current Test Guideline does not allow testing of gases and aerosols.

3.8 Eye Irritancy Test (EIT) test (OECD TG 494)

- *In vitro* test method
- Identification of test chemicals not requiring classification and labelling for eye irritation or serious eye damage (CLP No Cat.).
- May be useful in tiered testing strategy for classification and labelling, eg., in bottom-up approach (IATA, OECD GD 263).
- Test chemical preparations of both solids and liquids showing acidity (pH ≤ 5) and rapid phase separation are not in the applicability domain.
- When the absolute difference of the absorbance values of the 2.5% weight/volume (w/v) test chemical preparation at 0 and 3 minutes is greater than 0.1, the chemical should not be tested.

3.9 *In vitro* macromolecular test method (OECD TG 496)

- *In vitro* (acellular biochemical) assay
- Currently two test methods: Ocular Irritection (OI) and OptiSafe Eye Irritation Test

(OS)

- Applicable to substances and mixtures
- OI can be used to predict Cat.1 and No Cat., OS can be used to predict No Cat. substance and mixtures only
- Substances that interfere with the test system: Intensely coloured, precipitation causing, high concentrations of surfactants, highly volatile substances.

4. Animal test method only as a last resort

Certain steps need to take place before any testing (*in vitro* or *in vivo*) is conducted, as described in the introductory paragraph to Annex VII of REACH, i.e., assessment of all available information, which could be, e.g., information from skin corrosion studies.

Testing for serious eye damage/eye irritation must always start with non-animal test methods when new testing is required. *In vivo* testing is only needed if non-animal methods are not suitable for the substance or if results of the non-animal methods are not adequate for classification and risk assessment.

If results of the first non-animal method allow a decision on the classification and labelling, a second test does not need to be conducted.

Acute Eye Irritation/Corrosion Test Method (*in vivo* Draize test, OECD 405) provides information on health hazard likely to arise from exposure to test substance (liquids, solids and aerosols) by application to the eye. The Test Guideline specifies that the albino rabbit is the preferred species for *in vivo* testing. The test substance is applied in a single dose into the conjunctival sac of one eye of each animal. The other eye, which remains untreated, serves as a control. The initial test uses a single animal; the dose level depends on the nature of the test substance. A confirmatory test should be made if a corrosive effect is not observed in the initial test, the irritant or negative response should be confirmed using up to two additional animals. At any time, if animals show continuous signs of pain and/or distress at any stage of the test, they should be humanely killed.

Registrants must make sure that the substance falls under the scope and applicability domain of the specific *in vitro* tests performed, and that there are no chemical- specific limitations to use those tests (see the first two links below). For most substances, the use of adopted OECD or EU *in vitro* test guidelines for serious eye damage/eye irritation purposes will provide results of regulatory acceptance under REACH.

Reference to the relevant sources of information

1) Link to the OECD site
http://www.oecd.org/env/ehs/testing/oecdguidelinesforthetestingofchemicals.htm

2) Practical Guide "How to use alternatives to animal testing to fulfil your information requirements for REACH registration"
https://echa.europa.eu/documents/10162/13655/practical_guide_how_to_use_alternatives_en.pdf/148b30c7-c186-463c-a898-522a888a4404

This website provides practical information and tools in relation to help using existing information and non-test methods as a first step to meeting the REACH information requirements.

3) Guidance on information requirements and chemical safety assessment (ECHA Guidance R7a), Chapter R.7.2 Skin and eye irritation/corrosion and respiratory irritation
http://echa.europa.eu/documents/10162/13632/information_requirements_r7a_en.pdf

4) Guidance Document on an Integrated Approach on Testing and Assessment (IATA) for Serious Eye Damage and Eye Irritation
http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2017)15&doclanguage=en

5) Webinar on "Use *in vitro* data to fulfil REACH information requirements" held on 22 September 2016
https://echa.europa.eu/-/use-of-alternative-methods-to-animal-testing-in-your-reach-registration

6) EURL ECVAM – validation and regulatory acceptance
https://eurl-ecvam.jrc.ec.europa.eu/validation-regulatory-acceptance

This website provides information on the validation and regulatory acceptance status of alternative methods including information on the validation studies.

7) OECD Supporting Document 354
https://one.oecd.org/document/env/cbc/mono(2022)10/en/pd

8) Tracking system for alternative test methods review, validation and approval in the context of EU regulations on chemicals (TSAR)
http://tsar.jrc.ec.europa.eu/

This website provides information on the validation and adoption status of an alternative test, whether the test method is a replacement and in which context the method should be used.