How to use new or revised *in vitro* test methods to address Serious eye damage/Eye irritation

(Revised in February 2018)

Which of the REACH information requirements may be met with the test(s)

The REACH Regulation includes a requirement for *in vitro* tests for serious eye damage/eye irritation (Annex VII, section 8.2.1). An overview of the available internationally validated *in vitro* methods is presented in Table 1.

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 (see section 1 c. below, in the chapter "How to use these *in vitro* methods").

The test methods covered in 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 for classification and risk assessment. The methods often have limitations and cannot be used for all kinds of substances. Therefore, registrants and test houses are advised to check the chapter "**Specific scope and limitations of the *in vitro* tests"** below, before deciding on a new test/study.

The *in vitro* test methods can be summarised as follows:

Test method EU B.47 / OECD TG 437 – The Bovine Corneal Opacity and Permeability Test Method (BCOP) is an *in vitro* assay that may be used to identify 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. The revised Test Guideline includes clarifications on the applicability of the BCOP test method to the testing of alcohols, ketones and solids.

Test method EU B.48 / OECD TG 438 – Isolated Chicken Eye Test Method (ICE) is an *in vitro* assay that may be used to identify 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. The revised Test Guideline includes information on applicability of the ICE test method to the testing of surfactants, alcohols and solids.

Test method OECD TG 460 – Fluorescein Leakage Test Method (FL) is an *in vitro* assay that may be used for identifying water soluble substance 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 is recommended for use as part of a tiered testing strategy for regulatory classification and labelling.

Test method OECD TG 491 – Short Time Exposure Test Method (STE) is a cytotoxicity-based *in vitro assay* that may be used to identify 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. 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.

Table 1: Test methods to be used within the testing strategy.

Latest update	Test method	Validation status, regulatory acceptance	EU test method / OECD Test Guideline	Classification according to CLP Regulation	EURL ECVAM DB-ALM protocol number
Serious eye damage/eye irritation					
2017	BCOP	Validated and regulatory acceptance	B.47* / OECD TG 437	Cat. 1 or NC	98, 124
2017	ICE	Validated and regulatory acceptance	B.48* / OECD TG 438	Cat. 1 or NC	80
2017	FL	Validated and regulatory acceptance	N.A. / OECD TG 460	Cat. 1	71
2017	STE	Validated and regulatory acceptance	N.A. / OECD TG 491	Cat. 1 or NC	N.A.
2017	RhCE	Validated and regulatory acceptance	N.A. / OECD TG 492	NC	N.A.
N.A.	CM [1]	Validated and considered to be scientifically valid	N.A. / OECD draft TG available and being considered for adoption	Cat. 1 or NC	130

Note: The latest version of the test guideline should always be used independent of whether it is published by the EU or OECD.

Test method OECD TG 492 – Reconstructed human Cornea-like Epithelium Test Method (RhCE) is an *in vitro* assay that may be used to identify chemicals not

[1] The CM test method was validated by EURL ECVAM and considered to be scientifically valid (https://eurl-ecvam.jrc.ec.europa.eu/validation-regulatory-acceptance/topical-toxicity/eye-irritation; section 1.2) and was also reviewed by ICCVAM (http://ntp.niehs.nih.gov/?objectid=807EF83B-92CC-9A6C-3FFE8725DF1F9F5D); a draft OECD Test Guideline is available at: http://www.oecd.org/env/ehs/testing/section4healtheffects.htm.

* The EU method is outdated and does not reflect the latest update of the corresponding OECD method. Any new test should be performed following the updated OECD TG.

requiring classification and labelling for eye irritation or serious eye damage. The *in vitro* test methods currently covered by this Test Guideline are the EpiOcular™ Eye Irritation Test (EIT) and SkinEthic™ Human Corneal Epithelium (HCE) Eye Irritation Test (EIT). 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.

Test method Cytosensor Microphysiometer (CM) (draft OECD TG under discussion) is suitable for identifying chemicals causing "serious eye damage" (Cat. 1 of CLP) for the chemical applicability domain of water soluble chemicals and also for identifying non-irritants from all other classes of water-soluble surfactants, and water-soluble surfactant-containing mixtures.

The *in vivo* test method (only as a last resort)

Test method EU B.5 / OECD TG 405 – (*in vivo*) Acute Eye Irritation/Corrosion Test Method 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.

Status of the validation by EURL ECVAM or other bodies, when necessary

All of these tests were validated before adoption by the OECD and EU.

How to use these *in vitro* methods

Usually a single *in vitro* eye irritation test cannot fully replace the OECD TG 405 *in vivo* acute eye irritation/corrosion test (also known as the Draize eye irritation test) across the full range of irritation, and for different chemical classes. However, combinations of alternative test methods may be able to replace the Draize eye test.

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

If results of the first *in vitro* test allow a decision on the classification, a second test does not need to be conducted.

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, i.e. assessment of all available information, which could be e.g. information from skin corrosion studies. If

a conclusion on the classification cannot be made based on existing information, the next steps are:

a. In the case of a positive and definitive result from BCOP, ICE, FL or STE, the substance can be classified as inducing "**serious eye damage**" (Cat. 1 of CLP), and no further *in vivo* test is necessary.

b. In addition, BCOP, ICE, STE or RhCE tests can also provide information on whether the substance does **not require any classification** for serious eye damage/eye irritation. If the tests show that no classification is needed, no further *in vivo* testing is necessary.

c. At Annex VIII, if neither of these conclusions can be made, other *in vitro* study(ies) for this endpoint shall be considered. If the *in vitro* studies are not suitable for the substance, or the results are not adequate for classification and risk assessment, a further test conducted *in vivo* to assess the eye irritation potential shall be considered, i.e. none of the *in vitro* methods described above can be used for the direct identification of eye irritants (Cat. 2 of CLP).

Testing strategies such as the top-down or bottom-up[2] approaches are acknowledged to provide a means of incorporating existing information, QSAR predictions, and *in vitro* test results.

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.

Link to the OECD site

http://www.oecd.org/env/ehs/testing/oecdguidelinesforthetestingofchemicals.htm

Link to the EC Test Methods Regulation

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32008R0440:en:NOT

[2] The top-down approach should be used when available information suggests that the substance may cause serious eye damage. The bottom-up approach, on the other hand, should be followed only when available information suggests that the substance may not be irritant to the eye.

Reference to the relevant guidances

1) 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.

2) 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

3) 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

4) 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

5) 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.

6) 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.

Specific scope and limitations of the test guidelines

For example, limitations on chemical categories covered, if any, and limitation on classification and labelling are addressed below.

All methods are able to measure, 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 iridal injuries or the persistence/reversibility of effects.

Bovine Corneal Opacity Test Method (BCOP), OECD TG 437:

- Recommended to identify chemicals inducing serious eye damage, i.e. chemicals to be classified as CLP Cat. 1, without further testing, and also recommended to identify chemicals that do not require classification for eye irritation or serious eye damage.
- If the results of testing the substance is not classified as Cat. 1 under CLP or not classified, more testing is required.
- May result in false positive predictions for alcohols and ketones and false negative predictions for solids.
- Does not allow for an assessment of the potential for systemic toxicity associated with ocular exposure.
- Does not allow testing of gases and aerosols.

Isolated Chicken Eye Test Method (ICE), OECD TG 438:

- Recommended to identify chemicals inducing serious eye damage, i.e. chemicals to be classified as CLP Cat. 1, without further testing, and also recommended to identify chemicals that do not require classification for eye irritation or serious eye damage.
- If the results of testing the substance is not classified as Cat. 1 under CLP or not classified, more testing is required.
- May result in false positive predictions for alcohols and false negative predictions for solids and surfactants.
- Does not allow for an assessment of the potential for systemic toxicity associated with ocular exposure.
- Does not allow testing of gases and aerosols.

Fluorescein Leakage Test Method (FL), OECD TG 460:

- Recommended to identify chemicals inducing serious eye damage, i.e. chemicals to be classified as CLP Cat. 1, without further testing.
- Not recommended for the identification of chemicals which should be classified as mild/moderate irritants or of chemicals which should not be classified for ocular irritation (substances and mixtures).
- Only applicable to water-soluble chemicals (substances and mixtures) and/or where the toxic effect is not affected by dilution.
- Applicability domain does not include strong acids and bases, cell fixatives and highly volatile chemicals.
- If the results of testing the substance is not classified as Cat. 1 under CLP, more testing is required.

Short Term Exposure Test Method (STE), OECD TG 491:

- Recommended to identify chemicals inducing serious eye damage, i.e. chemicals to be classified as CLP Cat. 1, without further testing, and also recommended to identify chemicals that do not require classification for eye irritation or serious eye damage.
- Not recommended for the identification of chemicals which should be classified as mild/moderate irritants.
- Applicable to chemicals that are dissolved or uniformly suspended for at least 5 minutes in physiological saline, 5 % dimethyl sulfoxide (DMSO) in saline, or mineral oil.
- The use of mineral oil in this method is possible because of the short-time exposure. Therefore, the STE test method is suitable for predicting the eye hazard potential of water-insoluble test chemicals (e.g. long-chain fatty alcohols or ketones), provided that they are miscible in at least one of the three above proposed solvents.
- Not suitable for test chemicals that are insoluble or cannot be uniformly suspended for at least 5 minutes in physiological saline, 5 % DMSO in saline, or mineral oil.

Reconstructed human Cornea-like Epithelium Test Methods (RhCE), OECD 492:

- Recommended to identify chemicals (substances and mixtures) not requiring classification and labelling for eye irritation or serious eye damage.
- Not recommended for the identification of chemicals which should be classified for eye irritation or serious eye damage.
- 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.
- Not applicable for testing of gases and aerosols.

Cytosensor Microphysiometer Test Method (CM, draft OECD TG under discussion):

The limitations can be addressed when the TG has been approved by OECD.