How to use new or revised *in vitro* test methods to address Serious eye damage/Eye irritation - A testing and assessment strategy

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) for substances manufactured or imported in quantities of one tonne or more per year. 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”). It is also possible to adapt from the standard information requirements of REACH Annexes VII and VIII, if conditions specified under specific rules of adaptation (column 2) or general rules of adaptation (Annex XI) are met.

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 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.
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 requiring classification and labelling for eye irritation or serious eye damage. The only *in vitro* test method currently covered by this Test Guideline is the EpiOcular™ Eye Irritation Test (EIT). The method is 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.

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

<table>
<thead>
<tr>
<th>Latest update</th>
<th>Test method</th>
<th>Validation status, regulatory acceptance</th>
<th>EU Test Method/OECD test guideline</th>
<th>Classification according to CLP Regulation</th>
<th>EURL ECVAM DB-ALM protocol Nr.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Serious eye damage / eye irritation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2013</td>
<td>BCOP</td>
<td>Validated and regulatory acceptance</td>
<td>B.47 / OECD TG 437</td>
<td>Cat 1 or NC</td>
<td>98, 124</td>
</tr>
<tr>
<td>2013</td>
<td>ICE</td>
<td>Validated and regulatory acceptance</td>
<td>B.48 / OECD TG 438</td>
<td>Cat 1 or NC</td>
<td>80</td>
</tr>
<tr>
<td>2012</td>
<td>FL</td>
<td>Validated and regulatory acceptance</td>
<td>N.A. / OECD TG 460</td>
<td>Cat 1</td>
<td>71</td>
</tr>
<tr>
<td>2015</td>
<td>STE</td>
<td>Validated and regulatory acceptance</td>
<td>N.A. / OECD TG 491</td>
<td>Cat 1 or NC</td>
<td>N.A.</td>
</tr>
<tr>
<td>2015</td>
<td>RhCE</td>
<td>Validated and regulatory acceptance</td>
<td>N.A. / OECD TG 492</td>
<td>NC</td>
<td>N.A.</td>
</tr>
<tr>
<td>N.A.</td>
<td>CM¹</td>
<td>Validated and considered to be scientifically valid</td>
<td>N.A. / OECD draft TG available and being considered for adoption</td>
<td>Cat 1 or NC</td>
<td>130</td>
</tr>
</tbody>
</table>

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

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)

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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](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-3FFEB725DF1F9F5D](http://ntp.niehs.nih.gov/?objectid=807EF83B-92CC-9A6C-3FFEB725DF1F9F5D)). A draft OECD Test Guideline is available at: [http://www.oecd.org/env/ehs/testing/section4healtheffects.htm](http://www.oecd.org/env/ehs/testing/section4healtheffects.htm).
by application to the eye. It is recommended in the test guideline that other information is assessed before deciding whether the in vivo test is necessary. 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

It is generally accepted that no single in vitro eye irritation test can 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 several alternative test methods may be able to replace the Draize eye test.

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:

1) An in vitro study for eye irritation should be performed:

   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 no 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.

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\(^2\) 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.
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 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:


REFERENCE TO THE RELEVANT GUIDANCES


This web page provides you with practical information and tools to help using existing information and non-test methods as a first step to meeting the REACH information requirements.


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 alternative test, whether the test method is a replacement and in which context the method should be used.


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

SPECIFIC SCOPE AND LIMITATIONS OF EACH OF THE IN VITRO TEST

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 TG437:

- 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 Method (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.