How to use new or revised *in vitro* test methods to address Skin corrosion/irritation - A testing and assessment strategy

WHICH OF THE REACH INFORMATION REQUIREMENTS MAY BE MET WITH THE TEST(S)

Annex VII of the REACH Regulation includes a requirement for *in vitro* tests for skin corrosion (section 8.1.1) and for skin irritation (8.1.2) 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* skin irritation study shall only be considered at Annex VIII level (section 8.1) in case the *in vitro* skin corrosion and irritation tests are not applicable for the substance or the results obtained are not adequate for classification and risk assessment. Limitations of the application of the *in vitro* tests are specified below. It is also possible to adapt the standard information requirements of REACH Annexes VII and VIII, if the conditions specified under the 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.46 / OECD 439 - *in vitro* skin irritation: Reconstructed Human Epidermis Test Method (RHE) is an *in vitro* assay that allows distinction between irritants (CLP Cat 1/Cat 2) and substances not classified. Note, in case information is only available from this test method and the outcome is positive i.e. Cat 1/Cat 2, an *in vitro* skin corrosion study is needed to assess if the substance is corrosive or irritant. The revised test guideline (OECD 2013) includes a new “me-too” test method. The 2015 revision further included a reference to Integrated Approach to Testing and Assessment (IATA) guidance document and introduced the use of an alternative procedure to measure viability.

Test method B.40bis / OECD 431 - *in vitro* skin corrosion: Reconstructed Human Epidermis Test Method (RHE) is an *in vitro* assay that allows distinction between corrosives (CLP Cat 1) and non-corrosives. The revised test guideline (OECD 2013) includes sub-categorisation of corrosives i.e. Cat 1A and Cat 1B/C (of CLP). No distinction between categories 1B and 1C can be made. In addition the revised test guideline (2014) contains instructions how to address chemicals that directly reduce the viability dye (MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) or interferes with the optical density measurements (colorants). The 2015 revision further includes a reference to IATA guidance document and introduces the use of an alternative procedure to measure viability.

B.40 / OECD 430 - Transcutaneous Electrical Resistance Test Method (TER) is an *in vitro* assay that allows distinction between corrosives (CLP Cat 1) and non-corrosives. The test method does not allow sub-categorisation of corrosives. The test method uses epidermal skin discs obtained from rats and measures loss of stratum corneum integrity and barrier function as a reduction of transcutaneous electrical resistance. The revised test guideline (OECD 2013) now contains performance standards for the assessment of similar and modified TER-based test methods. The 2015 revision includes a reference to the IATA guidance document.
OECD 435 - *In vitro Membrane Barrier Test Method* is an *in vitro* assay that allows sub-categorisation of corrosives. The test method provides information if the substance is a corrosive (CLP Cat 1) or non-corrosive. It is suitable for liquids and solids (acids, bases, and halides). Some substance types e.g. agro- and petrochemicals and industrial cleaners may provide incorrect results. The 2015 revision includes a reference to the IATA guidance document and the updated list of proficiency substances.

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 Methods/OECD test guideline</th>
<th>Classification according to CLP Regulation</th>
<th>EUR L ECVAM DB-ALM protocol Nr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin corrosion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2015</td>
<td>TER</td>
<td>Validated and regulatory acceptance</td>
<td>B.40/TG 430</td>
<td>Cat. 1 or non-corrosive</td>
<td>115</td>
</tr>
<tr>
<td>2015</td>
<td>EpiDerm™ SCT</td>
<td>Validated and regulatory acceptance</td>
<td>B.40 bis/TG 431</td>
<td>Cat. 1, 1A, 1B/1C or non-corrosive</td>
<td>119</td>
</tr>
<tr>
<td>2015</td>
<td>EpiSkin™</td>
<td>Validated and regulatory acceptance</td>
<td>B.40 bis/TG 431</td>
<td>Cat. 1, 1A, 1B and 1C or non-corrosive</td>
<td>118</td>
</tr>
<tr>
<td>2015</td>
<td>SkinEthic™ RHE</td>
<td>Validated and regulatory acceptance</td>
<td>B.40 bis/TG 431</td>
<td>Cat. 1, 1A, 1B/1C or non-corrosive</td>
<td>-</td>
</tr>
<tr>
<td>2015</td>
<td>epiCS®</td>
<td>Validated and regulatory acceptance</td>
<td>B.40 bis/TG 431</td>
<td>Cat. 1, 1A, 1B/1C or non-corrosive</td>
<td>-</td>
</tr>
<tr>
<td>2015</td>
<td>Corrositex (in vitro membrane barrier test method)</td>
<td>Validated and regulatory acceptance</td>
<td>N.A./TG 435</td>
<td>Cat. 1, 1A, 1B and 1C or non-corrosive</td>
<td>116</td>
</tr>
<tr>
<td>Skin irritation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2015</td>
<td>EpiDerm™ SIT</td>
<td>Validated and regulatory acceptance</td>
<td>B.46/TG 439</td>
<td>Cat. 1/Cat. 2 or NC</td>
<td>138</td>
</tr>
<tr>
<td>2015</td>
<td>EpiSkin™</td>
<td>Validated and regulatory acceptance</td>
<td>B.46/TG 439</td>
<td>Cat. 1/Cat. 2 or NC</td>
<td>131</td>
</tr>
<tr>
<td>2015</td>
<td>SkinEthic™ RHE</td>
<td>Validated and regulatory acceptance</td>
<td>B.46/TG 439</td>
<td>Cat. 1/Cat. 2 or NC</td>
<td>135</td>
</tr>
<tr>
<td>2015</td>
<td>LabCyte EPI-MODEL24 SIT</td>
<td>Validated and regulatory acceptance</td>
<td>B.46/TG 439</td>
<td>Cat. 1/Cat. 2 or NC</td>
<td>-</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.

1 The EpiSkin SOP allows for differentiating between the 3 sub-categories and OECD Guidance Document 203 suggests the use of this method to distinguish 1B from 1C before *in vivo* testing is considered. However, OECD TG 431 currently only permits the use of EpiSkin to distinguish 1A from 1B/1C.
**STATUS OF THE VALIDATION BY EURL ECVAM**

These test methods have been validated before adoption by the OECD and EU.

**HOW TO USE THESE IN VITRO METHODS**

For irritation and corrosion it is currently accepted that no single *in vitro* test method can fully replace the *in vivo* test for skin irritation. However, these *in vitro* methods may be used as partial replacement studies within a tiered testing strategy or as a stand-alone replacement test depending on the outcome of the study.

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. existing *in vitro*, *in vivo* and human data.

If a conclusion on the classification cannot be made based on existing information, the following test(s) needs to be performed:

1) Skin corrosion, *in vitro*

2) Skin irritation, *in vitro*

Testing strategies such as the top-down and bottom-up approaches may be applied, based on presumed properties (see Figure 1). In case only one *in vitro* test is needed to conclude on the skin corrosion/irritation potential, an adaptation statement shall be submitted for the second test (both *in vitro* corrosion and irritation tests are standard information requirements).

Figure 1. Top-down and bottom-up approaches. Top-down should be applied, when it is presumed that the substance is irritant or corrosive (based on existing information), and bottom-up, when that is not the case. The rationale of this approach is that in case the first *in vitro* test confirms the presumption, then in many cases, conclusion on classification can be made and further testing is not necessary.
After these steps, no new *in vivo* test is necessary (for any tonnage level) unless

- the substance does not fall under the scope and applicability domain of the specific *in vitro* tests performed, and there are chemical-specific limitations to use those tests (see below), or
- the Registrant cannot use the results of *in vitro* tests performed for classification and risk assessment.

For most substances, the use of adopted OECD or EU *in vitro* test guidelines for skin irritation/corrosion purposes will provide results that will have regulatory acceptance under REACH.

**LINK TO THE OECD SITE**


**LINK TO THE EC TEST METHODS REGULATION**


**REFERENCE TO THE RELEVANT GUIDANCES**

1) Information toolkit


   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, pp. 165 – 219,


3) Webinar on "How to use *in vitro* data to fulfil REACH information requirements held on 29 February 2012

   » [http://echa.europa.eu/view-article/-/journal_content/7def3c04-4b2b-4cfd-86d0-5ce36797faa8](http://echa.europa.eu/view-article/-/journal_content/7def3c04-4b2b-4cfd-86d0-5ce36797faa8)

4) Tracking system for alternative test methods review, validation and approval in the context of EU regulations on chemicals (TSAR)


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

5) EURL ECVAM - validation and regulatory acceptance


   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 THE *IN VITRO* TESTS

**In vitro skin corrosion: Reconstructed Human Epidermis Test Method (RHE), OECD 431:**

- While the validation data base contained pure chemicals and mixtures of pure chemicals, the experience with complex formulations is still limited. However, there is data indicating at least a high specificity for the classification of corrosive vs. non-corrosive complex formulations (Kolle et al., 2013, Applicability of *in vitro* tests for skin irritation and corrosion to regulatory classification schemes: Substantiating test strategies with data from routine studies, Regul Toxicol Pharmacol. 2012 Dec;64(3):402-14).
- Allows the identification of non-corrosive and corrosive substances and mixtures.
- Supports the sub-categorization of corrosive substances and mixtures into optional Sub-category 1A, as well as into a combination of sub-categories 1B and 1C.
- Does not allow discrimination between skin corrosive sub-categories 1B and 1C in accordance with the CLP.
- Does not allow testing of gases and aerosols.

**Electrical Resistance Test Method (TER), OECD 430:**

- Does not allow discrimination between corrosive sub-categories i.e. 1A, 1B and 1C, discriminates only skin corrosives (Cat. 1) from non-corrosives.
- Does not allow testing of gases and aerosols.

**In vitro Membrane Barrier Test Method, OECD 435:**

- Accepted to discriminate skin corrosive sub-categories 1A, 1B and 1C versus non-corrosives.
- Limited applicability domain of only acids, bases and acid derivatives.
- Not suitable for testing materials, which do not cause detectable changes in the chemical detection system (4.5 < pH < 8.5).
- Does not allow testing of gases and aerosols.

**In vitro Skin Irritation: Reconstructed Human Epidermis Test Method (RHE), OECD 439:**

- Discriminates skin corrosives/irritants (Cat. 1/Cat. 2) from chemicals not classified for skin irritation (no Cat.) under CLP. Should not be used to classify chemicals to the optional UN GHS Cat. 3 (mild irritants).
- If a test chemical acts directly on the MTT (e.g. is a direct MTT-reducer), is naturally coloured, or becomes coloured during tissue treatment, additional controls should be used to detect and correct for any test chemical interference with the viability measurement technique. Detailed descriptions of how to correct direct MTT reduction and interferences by colouring agents is available in the SOPs for the four validated test methods.
- Does not allow testing of gases and aerosols.
- While the data base contained pure chemicals and mixtures of pure chemicals, experience with complex formulations is still limited.