How to use new or revised *in vitro* test methods to address skin corrosion/irritation

(Revised in November 2017)

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). 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.

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.46 / OECD TG 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 the Integrated Approach to Testing and Assessment (IATA) Guidance Document and introduced the use of an alternative procedure to measure viability.

Test method EU B.40bis / OECD TG 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 subcategorisation 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 (colourants). The 2015 revision further includes a reference to the IATA Guidance Document and introduces the use of an alternative procedure to measure viability. The 2016 revision improves the capacity of these methods for the correct prediction of subcategory 1A.

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
Skin corrosion					
2015	TER	Validated and regulatory acceptance	B.40*/ TG 430	Cat. 1 or non corrosive	115
2016	EpiDerm ™ SCT	Validated and regulatory acceptance	B.40 bis*/ TG 431	Cat. 1, 1A, 1B/1C or non-corrosive	119
2016	EpiSkin ™	Validated and regulatory acceptance	B.40 bis*/ TG 431	Cat. 1, 1A, 1B and 1C or non-corrosive[1]	118
2016	SkinEthic ™ RHE	Validated and regulatory acceptance	B.40 bis*/ TG 431	Cat. 1, 1A, 1B/1C or non-corrosive	-
2016	epiCS®	Validated and regulatory acceptance	B.40bis*/ TG 431	Cat. 1, 1A, 1B/1C or non-corrosive	-
2015	Corrositex (*in vitro* membrane barrier test method)	Validated and regulatory acceptance	N.A. / TG 435	Cat. 1, 1A, 1B and 1C or non-corrosive	116
Skin irritation					
2015	EpiDerm ™ SIT	Validated and regulatory acceptance	B.46*/ TG 439	Cat. 1/Cat. 2 or NC	138
2015	EpiSkin ™	Validated and regulatory acceptance	B.46*/ TG 439	Cat. 1/Cat. 2 or NC	131

[1] The EpiSkin SOP allows for differentiating between the 3 subcategories 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.

* 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.

2015	SkinEthic ™ RHE	Validated and regulatory acceptance	B.46*/ TG 439	Cat. 1/Cat. 2 or NC	135
2015	LabCyte EPI-MODEL24 SIT	Validated and regulatory acceptance	B.46*/ TG 439	Cat. 1/Cat. 2 or NC	-

Test method EU B.40 / OECD TG 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 subcategorisation 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.

Test method OECD TG 435 – *In vitro* Membrane Barrier Test Method is an *in vitro* assay that allows subcategorisation 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.

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

Testing for skin corrosion/irritation must always start with *in vitro* test methods, in case 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; see Figure 1, "Top-down and bottom-up approaches", below.

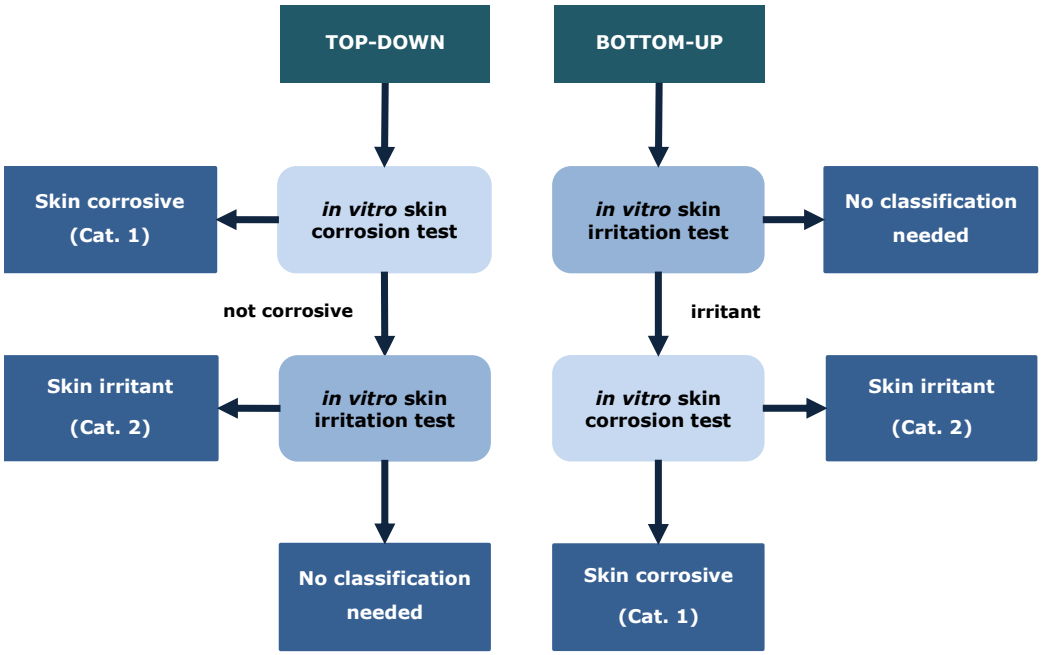
Certain steps need to take place before any testing (*in vitro* or *in vivo*) is conducted as described in the introductory paragraph to Annex VII, i.e. assessment of all available information which could be e.g. existing *in vitro*, *in vivo* and human data.

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).



<u>Figure 1:</u> Top-down and bottom-up approaches. A top-down approach should be applied when it is presumed that the substance is irritant or corrosive (based on existing information), and a bottom-up approach 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 a 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
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

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
https://echa.europa.eu/documents/10162/13632/information_requirements_r7a_en.pdf/e4a2a18f-a2bd-4a04-ac6d-0ea425b2567f

3) 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

4) 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.

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.

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

- While the validation database 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 subcategorisation of corrosive substances and mixtures into optional subcategory 1A, as well as into a combination of subcategories 1B and 1C.
- Does not allow discrimination between skin corrosive subcategories 1B and 1C in accordance with the CLP Regulation.
- Does not allow testing of gases and aerosols.

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

- Does not allow discrimination between corrosive subcategories, 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 TG 435:**

- Accepted to discriminate skin corrosive subcategories 1A, 1B and 1C from 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 TG 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 viability dye 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 standard operating procedures (SOPs) for the four validated test methods.
- Does not allow testing of gases and aerosols.
- While the database contained pure chemicals and mixtures of pure chemicals, experience with complex formulations is still limited.