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Committee of Experts on the Transport of Dangerous Goods and on the Globally Harmonized System of Classification and Labelling of Chemicals

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Sub-Committee of Experts on the Transport of Dangerous Goods

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Item 10 of the provisional agenda
Issues relating to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS)

Sub-Committee of Experts on the Globally Harmonized System of Classification and Labelling of Chemicals

Twenty-first session
Geneva, 27–29 June 2011
Item 4 (c) of the provisional agenda
Implementation of the GHS: Cooperation with other bodies or international organizations

Update on work of the informal joint correspondence group on corrosivity criteria

Transmitted by the expert from the United Kingdom on behalf of the informal joint correspondence group

Introduction

1. At the 20th session of the GHS Sub-committee, it was agreed that a joint TDG-GHS informal correspondence group should be established to consider further the harmonisation of corrosivity criteria in the transport Model Regulations and the GHS.

2. At the same session, a set of terms of reference for the work of the above group were agreed. These were as follows:

(a) Verify the definition of "skin destruction" as mentioned in the Model Regulation on the transport of dangerous goods complemented with reference to the Organisation for Economic Co-operation and development (OECD) test guidelines. If the definition is not aligned with paragraph 3.2.2.4.1 in Chapter 3.2 of the GHS, propose appropriate improvements.

(b) Identify and analyse the discrepancies between assignment to subcategories 1A, 1B and 1C, based on in vitro and in vivo testing and alternative approaches (bridging principles, mixtures calculations, pH…)

(c) Identify differences in assignment to categories in lists provided by different regulations and guidance documents for a few representative common substances. Analyse the underlying data and origin of these differences and use these results for the work under paragraphs a, b and d.

(d) Check the way OECD guidelines are referenced and their relevance.

(e) Report findings and make recommendations that meet the need of all sectors with the aim of achieving consistent classification outcomes for skin corrosivity.

3. It was also agreed that:

(a) The GHS expert from the United Kingdom should co-ordinate the work of the joint TDG-GHS informal correspondence group during the next (2011-2012) biennium;

(b) The work should start by compiling information about available databases, OECD guidelines, etc. and that a first report be provided at the next sessions of both sub-committees in June 2011.

(c) Subject to the approval of the TDG and GHS sub-committees, an agenda for a face-to-face meeting in December 2011 be proposed.

4. Accordingly, this informal document summarises the work carried out within this informal group to date.

Activities to date

5. The expert from the United Kingdom invited expressions of interest and initial information and views as to how to proceed from experts in the work of the group. A list of participants who have expressed an interest is given in **Annex I**.

6. In responding to the initial invitation a number of experts have also provided further information and comments relevant to the 5 workstreams above. These are produced in **Annexes II-VI**. **Annex II** contains a summary of the initial information gathered; **Annex III** contains tables compiled by the expert from the United Kingdom comparing the packing group and corrosivity classifications taken from Annex VI of the CLP Regulation of substances listed in the Dangerous Goods List; **Annex IV** contains a list submitted by the expert from the Netherlands of corrosivity classifications from different sources for a number of substances, together with explanatory notes; **Annex V** contains additional comments received from other members of the informal group; and **Annex VI** contains the results of a contract study contributed by the expert from Switzerland titled “Application of alternative methods in the regulatory assessment of chemical safety related to human skin corrosion and irritation – current status and future prospects”.

7. At this early stage it would be inappropriate to draw general conclusions but some initial observations are also offered in **Annex II** based on the information provided.

Next steps

8. Specific next steps within each of the 5 work streams are suggested in Annex II under each work stream.

9. In line with the agreed plan of work for the joint informal group, and subject to the agreement of both subcommittees, the expert from the United Kingdom proposes to organise a joint TDG-GHS face-to-face meeting of the joint informal group in December 2011. The specific agenda will depend on activities between now and then but will be circulated in advance to both sub-committees in due course.

Action requested of the TDG and GHS sub-committees

10. The TDG and GHS sub-committees are invited to:

(a) Provide any comments on the initial information and observations set out in Annex II;

(b) Comment and provide suggestions on the proposed next steps given for each work stream in the Annex.

11. The expert from the United Kingdom warmly invites any further experts from either TDG or GHS sub-committees who wish to be involved in this work to contact Mr Robin Foster (robin.foster@hse.gsi.gov.uk) and/or Mr Pierre Cruse (pierre.cruse@hse.gsi.gov.uk).

Annexes

Annex I

List of participants

Name	*Country/Organisation*
Friedrich Kirchnawy	Austria
Laurence Musset	OECD
Thomas Gebel	Germany
Shane Kelley	US (DOT)
Deana Holmes	US (OSHA)
Lennox John	AISE
Karola Grodzki	European Commission
Jeff Hart	UK
Arne Bale	UK
Nils Henrik Agerup	Norway
Christine Bjørge	Norway
Stine Husa	Norway
Patrick van Lancker	Belgium
Michael Bogaert	Belgium
Joke Herremans	Netherlands
Sjofn Gunnarsdottir	Netherlands
Paul Huurdeman	Netherlands
Dieter Heitkamp	CEFIC
Marie-Noëlle Blaude	Belgium
Markus Hofmann	Switzerland
Juan Luis Valverde Villarreal	Spain
Rosa Martínez Arrieta	Spain
Lía Calleja-Barcena	Spain
Duong Van Long	Vietnam
Tran Quang Thuy	Vietnam
Tran Thi Hoai Thu	Vietnam
Thomasina Barron	Ireland
Caroline Walsh	Ireland
Lennart Dock	Sweden

Annex II

Summary of information collected and preliminary observations

A. Work stream (a)

Verify the definition of "skin destruction" as mentioned in the Model Regulations on the transport of dangerous goods complemented with reference to the Organisation for Economic Co-operation and development (OECD) test guidelines. If the definition is not aligned with paragraph 3.2.2.4.1 in Chapter 3.2 of the GHS, propose appropriate improvements.

Comments and observations:

1. The definitions of 'corrosion' in the Transport Model Regulations and the GHS are as follows:

UN Model Regulations (16th Rev ed, 2.8.1, p.159):

Class 8 substances (corrosive substances) are substances which, by chemical action, will cause severe damage when in contact with living tissue, or, in the case of leakage, will materially damage, or even destroy, other goods or the means of transport.

GHS

Skin corrosion is the production of irreversible damage to the skin; namely, visible necrosis through the epidermis and into the dermis, following the application of a test substance for up to 4 hours. Corrosive reactions are typified by ulcers, bleeding, bloody scabs, and, by the end observation at 14 days, by discolouration due to blanching of the skin, complete areas of alopecia, and scars. Histopathology should be considered to evaluate questionable lesions.

2. In addition, the transport Model Regulations define the criteria for assigning substances to packing groups I, II and III. Paragraph 2.8.2.4 of the UN Model Regulations, 16th revised edition states that in assigning the packing group 'account shall be taken of human experience in instances of accidental exposure. In the absence of human experience the grouping shall be based on data obtained from experiments in accordance with OECD Test Guideline 404 or 435.' The same paragraph also states that substances determined not to be corrosive in accordance with OECD Test Guideline 430 or 431 may be considered not to be corrosive for the purposes of the Model Regulations without further testing. Paragraph 2.8.2.5 of the Model Regulations then writes out the criteria correlating full thickness destruction of intact skin with exposure and observation periods. For example, Packing Group I is assigned to "substances that cause full thickness destruction of intact skin tissue within an observation period up to 60 minutes starting after the exposure time of three minutes or less". Similar wording is used in the criteria for assigning Packing Groups II and III. Packing Group III is also assigned to substances which are not corrosive to skin but are corrosive to metals according to given criteria.

3. The expert from the OECD has provided the following additional information relating to the definition of skin corrosion used in OECD test guidelines.

4. Four OECD test guidelines relate to the assessment of skin corrosion:

(a) TG 404: Acute Dermal Irritation/Corrosion (updated in 2002)

(b) TG 430: In vitro Skin Corrosion: Transcutaneous Electrical Resistance Test (TER)(2004). *(N.B. An update of this Test Guideline is included in the Test Guidelines work plan.)*

(c) TG 431: In vitro skin Corrosion: Human Skin Model Test (2004). *(N.B. An update of this Test Guideline is included in the Test Guidelines work plan.)*

(d) TG 435: In vitro Membrane Barrier Test Method for Skin Corrosion (2006)[1]

5. Each of these test guidelines include definitions of skin corrosion. The definition is the same in TG 404, TG 430 and TG 431, but refers in TG 404 to "dermal corrosion" and in TG 430 and TG 431 to "skin corrosion *in vivo*":

(a) TG OECD 404, p. 8: **Dermal corrosion** is the production of irreversible damage of the skin; namely, visible necrosis through the epidermis and into the dermis, following the application of a test substance for up to four hours. Corrosive reactions are typified by ulcers, bleeding, bloody scabs, and, by the end of observation at 14 days, by discoloration due to blanching of the skin, complete areas of alopecia, and scars. Histopathology should be considered to evaluate questionable lesions;

(b) TG OECD 430, p.12 and TG OECD 431, p.8: **Skin corrosion *in vivo*** is the production of irreversible damage of the skin; namely, visible necrosis through the epidermis and into the dermis, following the application of a test substance for up to four hours. Corrosive reactions are typified by ulcers, bleeding, bloody scabs, and, by the end of observation at 14 days, by discoloration due to blanching of the skin, complete areas of alopecia, and scars. Histopathology should be considered to evaluate questionable lesions.

6. TG 435 includes a shortened definition which refers directly to GHS instead of giving the extended definition found in GHS, as in the previous test guidelines:

> TG 435, p.11 Skin corrosion**:** The production of irreversible damage to the skin, manifested as visible necrosis through the epidermis and into the dermis, following the application of a test material (1) [reference to GHS].

7. The expert from OECD has also drawn attention to the document, "Detailed Review Document on Classification systems for Skin Irritation/Corrosion in OECD Member Countries" (OECD, 1999, http://www.oecd.org/document/30/0,3746,en_2649_34377_1916638_1_1_1_1,00.html), which includes details of the definitions of corrosivity used in OECD Member Countries prior to the advent of the GHS (see Table 2 in the referenced document).

8. It worth noting in this context that Annex VI of the European Union Dangerous Substances Directive 67/548/EEC, which sets out the European Union pre-GHS system for classification and labelling of substances, also uses the term 'full thickness destruction of skin tissue' in characterizing skin corrosion. For example, section 3.2.5 of Annex VI of Directive 67/548/EEC (p.13) states[2]:

[1] These Test Guidelines are on the OECD public website at: http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788

[2] Available at: http://ec.europa.eu/environment/chemicals/dansub/pdfs/annex6_en.pdf

"- a substance or a preparation is considered to be corrosive if, when it is applied to healthy intact animal skin, it produces full thickness destruction of skin tissue in at least one animal [during the test for skin irritation cited in Annex V or an equivalent method]."

9. On the basis of the above, the following preliminary observations can be made:

(a) The definition of skin corrosion in the GHS is in line with the definition of corrosivity used in OECD test guidelines 404, 430, 431 and 435.

(b) The definition of corrosivity given in the Model Regulations differs from those in the above OECD test guidelines.

(c) The criteria given in the Model regulations for determining packing groups on the basis of (skin) corrosivity refer to 'full thickness destruction of intact skin tissue'. This term is not used in the four OECD test guidelines for skin corrosion (though was previously used in Annex VI of the European Union Dangerous Substances Directive 67/548/EEC)

(d) The Model Regulations contains reference to OECD test guidelines 404, 435, 430 and 431 and assigns test guidelines 404 and 435 for determining Packing Groups.

10. **Proposed next step**: determine to what extent there is a discrepancy between the criteria used for determining corrosivity in the Model Regulations and the criteria used in the GHS in the context of the definition as used by the OECD and explore possibilities for appropriate improvements, in particular as regards the use of the terms 'full thickness destruction' versus 'irreversible damage' etc.

B. Work stream (b)

Identify and analyse the discrepancies between assignment to subcategories 1A, 1B and 1C, based on in vitro and in vivo testing and alternative approaches (bridging principles, mixtures calculations, pH…)

Comments and observations:

11. Our comments at this stage are restricted to some observations regarding the capacity of OECD test methods to make an assignment to subcategories 1A, 1B and 1C based on in vitro and in vivo testing.

12. Classification for corrosivity can be carried out on the basis of pH and non test-based methods, e.g. with reference to the tiered testing strategy in TG 404, and *in vivo* testing of substances reasonably believed to be corrosive is discouraged across a number of regulatory jurisdictions. However if testing is required, of the four test methods outlined above, TG 404 gives the only *in vivo* test method for skin corrosion, based on the Draize test performed on albino rabbits. TG 430 is an in vitro test based on transcutaneous electrical resistance for rat skin. TG 431 assesses decrease in cell viability following application of a test material to a three-dimensional skin model. TG 435 is a further in vitro test which detects membrane damage after a test substance is applied to an artificial membrane barrier.

13. Of the *in vitro* methods, only TG 435 allows sub-classification of corrosive substances into GHS subcategories 1A, 1B and 1C or transport Packing Groups I, II and III. However TG 435 has a limited applicability domain, meaning that not all chemical classes can be tested. To determine whether the test substance is detectable by the Chemical Detection System to be used in the test, the test substance must be subject to an initial compatibility test.

14. Subclassification for corrosivity according to TG 435 is done based on exposure and observation times as set out in GHS Table 3.2.1. TG 430 and TG 431 allows only identification of substances and mixtures as corrosive (without subdivision), and of non-corrosive when supported by a weight-of-evidence determination. TG 431 may provide an indication of the distinction between more severe and less severe corrosives, however it does not allow breakdown into GHS subcategories.

15. TG 404 does not explicitly refer to the GHS subcategories or transport packing groups. Once it has been determined that animal testing is required, the approach taken is as follows: "Up to three test patches are applied sequentially to the animal. The first patch is removed after three minutes. If no serious skin reaction is observed, a second patch is applied at a different site and removed after one hour. If the observations at this stage indicate that exposure can humanely be allowed to extend to four hours, a third patch is applied and removed after four hours, and the response is graded". If no corrosive effect is observed observations continue for up to 14 days, or until a corrosive effect occurs.

16. GHS chapter 3.2 uses the above exposure and observation times to determine subcategorisation of hazard in Table 3.2.1:

Category 1: Corrosive	**Corrosive subcategories**	**Corrosive in ≥ 1 of 3 animals**	
(applies to authorities not using subcategories)	(only applies to some authorities)	**Exposure**	**Observation**
Corrosive	1A	≤ 3 min	≤ 1h
	1B	> 3 min ≤ 1 h	≤ 14 days
	1C	> 1h ≤ 4h	≤ 14 days

17. However, it was noted in correspondence by the expert from AISE that TG 404 does not explicitly use the testing protocol above to grade hazard, rather the implication seems to be that the gradual increase in exposure periods is to ensure humane treatment of the animal.

18. In the transport regulations, paragraph 2.8.2.4 of the UN Model Regulations, 16th revised edition states that in assigning the packing group 'account shall be taken of human experience in instances of accidental exposure. In the absence of human experience the grouping shall be based on data obtained from experiments in accordance with OECD Test Guideline 404 or 435'. Where testing is used as the basis for classification, packing groups I, II and III are assigned based on the same exposure and observation periods as those in GHS Table 3.2.1. However, the same comment would apply as made in relation to GHS chapter 3.2, that TG 404 does not itself provide a correlation between exposure/observation period and packing group (which, in the transport regulations, are intended to reflect degree of hazard, see para 2.8.2.1).

19. **Proposed next step**: examine the relationship between classification based on *in vivo* and *in vitro* tests according to OECD test guidelines and the use of the alternative methods referred to (extreme pH, bridging principles etc.). The effect of the (editorial) changes being proposed to GHS chapters 3.2 and 3.3 will also need to be considered in this context.

C. Work stream (c)

Identify differences in assignment to categories in lists provided by different regulations and guidance documents for a few representative common substances. Analyse the

underlying data an origin of these differences and use these results for the work under paragraphs a, b and d.

Comments and observations

20. A useful resource for this work is document UN/SCETDG/37/INF.12-UN/SCEGHS/19/INF.7 prepared by the Secretariat, comparing classifications for transport packing groups given in the Dangerous Goods List and the GHS classifications of corresponding chemicals set out in Annex VI of European Regulation EC No 1272/2008 on classification, labelling and packaging of substances and mixtures (CLP Regulation). The CLP list gives GHS classifications of around 8000 substances agreed by experts within the European Union.

21. The CLP list has been used for present purposes because it provides indicative GHS classifications for a large range of substances. However, it should be noted that the substances in the list were classified according to the previous European Union classification system, and the GHS classifications were derived from these older classifications using a translation table, given in Annex VII to the CLP Regulation.[3] Because the substances in the CLP list were not classified directly according to the GHS criteria they should not be considered definitive and should in due course be compared with those in other official national, regional or sectoral lists. This document will refer to the GHS classifications in the CLP list as 'CLP classifications'.

22. The expert from the Netherlands has also provided a list of corrosivity classifications for a number of substances, covering the DGL of the Model Regulations (16th edition), Annex VI of CLP (0 and 1st ATP), the CLP Inventory (March 2011), and the GESAMP Composite list EHS 47/9 (30 July 2010). This is included at **Annex IV** together with supporting explanatory notes.

23. With reference to the table in Annex IV, the following general observations can be made:

(a) There are different classifications and/or specific concentration limits assigned to the same substance listed in the DGL of the UN Model Regulations, Annex VI of the CLP Regulation and the global GESAMP[4] composite list.

(b) Different classifications have been notified for the same substance to the CLP inventory of industry self-classifications.

24. Based on document UN/SCETDG/37/INF.12-UN/SCEGHS/19/INF.7, the expert from the United Kingdom has in addition collated the following information, which is given in Tables 1 and 2 in **Annex III**:

(a) a list of substances in the DGL which are assigned Packing Group I on the basis of their classification as corrosive (Class 8), together with the corresponding CLP classification;

(b) a list of substances in the DGL whose CLP classification is corrosive category 1A, together with the corresponding classification given in the Dangerous Goods List.

[3] Unofficial non-final version available as a word file at http://ec.europa.eu/enterprise/sectors/chemicals/files/ghs/w_annex_vii_en.doc

[4] GESAMP is the Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection that advises the UN system on the scientific aspects of marine environmental protection and is sponsored among others by IMO, IAEA and UNEP

25. With reference to the tables in Annex III, the following further observations can be made:

(a) Excluding generic and N.O.S. entries, 29 substances are assigned to class 8 (as primary hazard) and to Packing Group I in the DGL, of which 18 have classifications given in Annex VI to CLP. Of these 18 substances, CLP assigns to 12 a classification as corrosive to skin category 1A, whereas 5 are classified as corrosive category 1B. In addition, one DGL entry (1828 SULPHUR CHLORIDES) has two CLP entries (disulphur dichloride/ sulphur monochloride; sulphur dichloride), one classified as corrosive 1A and one as 1B. Entries where the CLP and DGL classifications differ are highlighted in yellow in Table 1.

(b) Also excluding generic and N.O.S entries, 61 of the substances in the DGL are assigned the CLP classification corrosive to skin 1A in Annex VI to CLP. These substances are assigned various packing groups in the DGL: some are assigned PGI, some PGII, some PGIII and some are not assigned a PG. Also, some of the substances have a primary classification as corrosive, some a secondary classification, and some are not classified as corrosive at all. This makes it difficult to see clear relationships, although over half (39) can be identified as having a 'lower' classification in the DGL than in CLP, either because PGII or PGIII is assigned, or because the substance is not given a corrosivity classification in the DGL at all[5]. In the table, entries where PG II is assigned to a corrosive 1A substance are highlighted in orange; where PGIII is assigned this is highlighted in pink; and where no corrosive classification is assigned at all in the DGL, the entry is highlighted in red.

(c) In many of the above cases, the transport packing group can be used to deduce the GHS classification to which the DGL entry corresponds, using the correlation PGI = 1A; PGII = 1B; PGIII = 1C. However, there are several cases where Class 8 is given as a subsidiary risk in the DGL, therefore it cannot be determined to which GHS subcategory the transport risk translates (since the corrosivity classification does not directly determine packing group).

(d) Of the substances in the DGL which have CLP classifications as corrosive category 1A, 7 are not classified for transport as corrosive at all, but are assigned a packing group on the basis of other risks. These are marked in red in Table 2.

26. The overall picture as regards the comparison of CLP classifications and TDG classifications is therefore complex. Many substances that have a CLP classification as corrosive 1A are also assigned packing group I for transport, though slightly more have a 'lower' classification for transport (PGII or III). However a few substances also have a 'higher' corrosivity classification for transport than for CLP, and in some cases a direct comparison cannot be made since in the DGL, corrosion is only a subsidiary risk and cannot be identified with a specific packing group. Moreover, because most CLP classifications were derived from a translation table rather than direct classification according to GHS criteria, the relationship between the DGL and directly derived GHS classifications may be different.

[5] In fact the situation is more complicated, since some CLP corrosive 1A substances are assigned no packing group at all, and some are given PGI, but have corrosion as a subsidiary risk. It is not possible in these cases to determine whether or not the DGL gives a 'lower' classification than CLP.

27. On the basis of the above the expert from the United Kingdom suggests that initial work could start on workstream (c) by selecting a small number of representative substances from the above tables for which the classifications in the Dangerous Goods List and Annex VI of the CLP Regulation are different. The expert from the United Kingdom welcomes suggestions for suitable substances, however an initial suggestion would be to further investigate the available information relevant to the classification of the following common substances, which present various relationships between the transport and CLP classification:

(a) Sodium hydroxide and potassium hydroxide (both transport Class 8, PG II; GHS (CLP) corrosive cat 1A for C ≥ 5%)

(b) Sulphuric acid (Transport Class 8, PG II; CLP corrosive cat 1A for C ≥ 15%)

(c) Morpholine (transport PG I, Class 8, subsidiary risk Class 3; CLP Skin corr. 1B)

(d) Phosphorous acid (Transport Class 8, PG III; CLP skin corr. 1A)

(e) Zinc chloride (Transport Class 8, PGIII; CLP skin corr. 1B)

(f) Iodine (Transport Class 8, subsidiary risk 6.1, PGIII; CLP no skin corrosivity classification)

28. **Proposed next step:** Investigate available information relevant to classification of the above substances (or others as appropriate) and compare with the classification as listed for transport and CLP and in other official classification systems.

D. Work stream (d)

Check the way OECD guidelines are referenced and their relevance.

Comments and observations

29. In addition to the comments on the definitions and test methods made above (paras 1-10 and 11-18), which are also pertinent here, it is worth summarising the way in which the Model Regulations and the GHS reference the OECD test guidelines.

30. The Model Regulations reference the OECD test guidelines in paragraph 2.8.2.4:

"In assigning the packing group to a substance in accordance with 2.8.2.2, account shall be taken of human experience in instances of accidental exposure. In the absence of human experience the grouping shall be based on data obtained from experiments in accordance with OECD Test Guideline 404 [reference] or 435 [reference]. A substance which is determined not to be corrosive in accordance with OECD Test Guideline 430 [reference] or 431 [reference] may be considered not to be corrosive to skin for the purposes of these Regulations without further testing" (Model Regulations, p.159)

31. The GHS refers to OECD test methods for corrosion (as opposed to irritation) only as a note to Figure 3.2.1: Tiered testing and evaluation of skin corrosion and irritation potential (GHS, 3rd Rev ed., p. 122). Step 5 in this tiered testing strategy is, 'Valid and accepted *in vitro* skin corrosion test (d)' and note (d) states that '*Examples of internationally accepted validated in vitro test methods for skin corrosion are OECD Test Guidelines 430 and 431*'.

32. However, it should be borne in mind that the above reference may be amended or deleted in light of the ongoing review of GHS chapters 3.2 and 3.3 (see, for example, working document ST/SG/AC.10/C.4/2011/1 (Germany)).

33. **Proposed next step:** none at this stage.

E. Work stream (e)

Report findings and make recommendations that meet the need of all sectors with the aim of achieving consistent classification outcomes for skin corrosivity.

Comments and observations

34. None at this stage.

Annex III

Comparison tables between Dangerous Goods List and Annex VI of Regulation 1272/2008 on classification, labelling and packaging of substances and mixtures (CLP Regulation)

Table 1:
List of substances in Dangerous Goods List (excluding generic/N.O.S. entries) for which Packing Group I is assigned as a result of a corrosivity classification (primary hazard), together with corresponding GHS-CLP classification

Table 2:
List of substances in Dangerous Goods List (excluding generic/N.O.S. entries) together with corresponding GHS-CLP classification, for which a CLP-GHS classification as skin corrosive 1A is assigned

NOTE: *The tables are based on those in UN/SCETDG/37/INF.12-UN/SCEGHS/19/INF.7 (secretariat), however certain annotations in italics have been added by the expert from the United Kingdom.*

Table 1: List of substances in Dangerous Goods List (excluding generic/N.O.S. entries) for which PGI is assigned as a result of a corrosivity classification (primary hazard), together with corresponding GHS-CLP classification

UN Model Reg. Rev.16						CLP regulation												Classif. TDG - GHS	Classif. CLP -GHS
										Classification		Labelling							
UN No	Proper shipping name/additional data FP = flash point BP = Boiling point	Class or Div.	Sub. risk	PG	SP	Index No	Int. Chem. ID	EC No	CAS No	Haz Class +Cat	Haz Stat	Pict, SW	Haz stat	Suppl. Haz. St.	Specific Conc. Limits, M-factors	Notes	ATP inserted/ ATP Updated	P= Marine pollutant PP= Severe marine pollutant	* highest minimum classif
1052	HYDROGEN FLUORIDE, ANHYDROUS	8	6.1	I		009-002-00-6	hydrogen fluoride	231-634-8	7664-39-3	Acute Tox. 2 * Acute Tox. 1 Acute Tox. 2 * Skin Corr. 1A	H330 H310 H300 H314	GHS06 GHS05 **Dgr**	H330 H310 H300 H314				CLP00/	Corr.1A Ac.tox	Skin Corr. 1A Ac. Tox. 1
1739	BENZYL CHLOROFORMATE	8		I		607-064-00-4	benzyl chloroformate	207-925-0	501-53-1	Skin Corr. 1B Aquatic Acute 1 Aquatic Chronic 1	H314 H400 H410	GHS05 GHS09 **Dgr**	H314 H410		STOT SE 3; H335		CLP00/	Corr.1A P	Skin Corr. 1B Aq.Ac.1 Aq.Chr.1
1744	BROMINE or BROMINE SOLUTION	8	6.1	I		035-001-00-5	bromine	231-778-1	7726-95-6	Acute Tox. 2 * Skin Corr. 1A Aquatic Acute 1	H330 H314 H400	GHS06 GHS05 GHS09 **Dgr**	H330 H314 H400				CLP00/	Corr.1A Ac.Tox.	Skin Corr. 1A Ac.Tox. 2 * Aq.Ac.1
1754	CHLOROSULPHONIC ACID (with or without sulphur trioxide)	8		I		016-017-00-1	chlorosulphonic acid	232-234-6	7790-94-5	Skin Corr. 1A STOT SE 3	H314 H335	GHS05 GHS07 **Dgr**	H314 H335	EUH014			CLP00/	Corr.1A	Skin Corr. 1A
1758	CHROMIUM OXYCHLORIDE	8		I		024-005-00-2	chromyl dichloride; chromic oxychloride	239-056-8	14977-61-8	Ox. Liq. 1 Carc. 1B Muta. 1B Skin Corr. 1A Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H271 H350i H340 H314 H317 H400 H410	GHS03 GHS08 GHS05 GHS07 GHS09 **Dgr**	H271 H350i H340 H314 H317 H410		Skin Corr. 1A; H314: C ≥ 10 % Skin Corr. 1B; H314: 5 % ≤ C < 10 % Skin Irrit. 2; H315: 0,5 % ≤ C < 5 % Eye Irrit. 2; H319: 0,5 % ≤ C < 5 % STOT SE 3; H335: 0,5 % ≤ C < 5 % Skin Sens. 1; H317: C ≥ 0,5 %	T 3	CLP00/	Corr.1A	Skin Corr. 1A Ox. Liq. 1 Aq.Ac.1 Aq.Chr.1
1777	FLUOROSULPHONIC ACID	8		I		016-018-00-7	fluorosulphonic acid	232-149-4	7789-21-1	Acute Tox. 4 * Skin Corr. 1A	H332 H314	GHS05 GHS07 **Dgr**	H332 H314				CLP00/	Corr.1A	Skin Corr. 1A Ac.Tox. 4 *
1790	HYDROFLUORIC ACID, with more than 60% hydrogen fluoride	8	6.1	I		009-003-00-1	hydrofluoric acid ... %	231-634-8	7664-39-3	Acute Tox. 2 * Acute Tox. 1 Acute Tox. 2 * Skin Corr. 1A	H330 H310 H300 H314	GHS06 GHS05 **Dgr**	H330 H310 H300 H314		Skin Corr. 1A; H314: C ≥ 7 % Skin Corr. 1B; H314: 1 % ≤ C < 7 % Eye Irrit. 2; H319: 0,1 % ≤ C < 1 %	B	CLP00/	Corr.1A Ac.Tox.	Skin Corr. 1A Ac.Tox. 1

UN Model Reg. Rev.16						CLP regulation												Classif. TDG - GHS	Classif. CLP -GHS
						Index No	Int. Chem. ID	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes	ATP inserted/ ATP Updated		
UN No	Proper shipping name/additional data FP = flash point BP = Boiling point	Class or Div.	Sub. risk	PG	SP					Haz Class +Cat	Haz Stat	Pict, SW	Haz stat	Suppl. Haz. St.				P= Marine pollutant PP= Severe marine pollutant	* highest minimum classif
1796	NITRATING ACID MIXTURE with more than 50% nitric acid	8	5.1	I															
1798	NITROHYDROCHLORIC ACID	8		I															
1826	NITRATING ACID MIXTURE, SPENT, with more than 50% nitric acid	8	5.1	I	113														
1828	SULPHUR CHLORIDES	8		I		016-012-00-4	disulphur dichloride; sulfur monochloride	233-036-2	10025-67-9	Acute Tox. 3 * Acute Tox. 4 * Skin Corr. 1A Aquatic Acute 1	H301 H332 H314 H400	GHS06 GHS05 GHS09 **Dgr**	H301 H332 H314 H400	EUH014 EUH029	STOT SE 3; H335		CLP00/	Corr.1A	Skin Corr. 1A Ac.Tox. 3 * Aq.Ac.1
		8		I		016-013-00-X	sulphur dichloride	234-129-0	10545-99-0	Skin Corr. 1B STOT SE 3 Aquatic Acute 1	H314 H335 H400	GHS05 GHS07 GHS09 **Dgr**	H314 H335 H400	EUH014	STOT SE 3; H335		CLP00/	*Corr. 1A*	*Skin Corr. 1B*
1829	SULPHUR TRIOXIDE, STABILIZED	8		I															
1831	SULPHURIC ACID, FUMING	8	6.1	I															
1836	THIONYL CHLORIDE	8		I		016-015-00-0	thionyl dichloride; thionyl chloride	231-748-8	7719-09-7	Acute Tox. 4 * Acute Tox. 4 * Skin Corr. 1A	H332 H302 H314	GHS05 GHS07 **Dgr**	H332 H302 H314	EUH014 EUH029	STOT SE 3; H335		CLP00/	Corr.1A	Skin Corr. 1A Ac.Tox. 4 *
1905	SELENIC ACID	8		I															
2029	HYDRAZINE, ANHYDROUS	8	3 6.1	I		007-008-00-3	hydrazine	206-114-9	302-01-2	Flam. Liq. 3 Carc. 1B Acute Tox. 3 * Acute Tox. 3 * Acute Tox. 3 * Skin Corr. 1B Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H226 H350 H331 H311 H301 H314 H317 H400 H410	GHS02 GHS06 GHS08 GHS05 GHS09 **Dgr**	H226 H350 H331 H311 H301 H314 H317 H410		Skin Corr. 1B; H314: C ≥ 10 % Skin Irrit. 2; H315: 3 % ≤ C < 10 % Eye Irrit. 2; H319: 3 % ≤ C < 10 %		CLP00/	Corr.1A Flam.Liq Ac.Tox.	Skin Corr. 1B Flam. Liq. 3 Ac.Tox. 3 * Aq.Ac.1 Aq.Chr.1
2030	HYDRAZINE AQUEOUS SOLUTION with more than 37% hydrazine, by mass	8	6.1	I															
2031	NITRIC ACID, other than red fuming, with more than 70% nitric acid	8	5.1	I		007-004-00-1	nitric acid ... %	231-714-2	7697-37-2	Ox. Liq. 3 Skin Corr. 1A	H272 H314	GHS03 GHS05 **Dgr**	H272 H314		Skin Corr. 1A; H314: C ≥ 20 % Skin Corr. 1B; H314: 5 % ≤ C < 20 % Ox. Liq. 3; H272: C ≥ 65 %	B	CLP00/	Corr.1A Ox. Liq.	Skin Corr. 1A Ox. Liq. 3

UN Model Reg. Rev.16						CLP regulation												Classif. TDG - GHS	Classif. CLP -GHS
						Index No	Int. Chem. ID	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes	ATP inserted/ ATP Updated		
UN No	Proper shipping name/additional data FP = flash point BP = Boiling point	Class or Div.	Sub. risk	PG	SP					Haz Class +Cat	Haz Stat	Pict, SW	Haz stat	Suppl. Haz. St.				P= Marine pollutant PP= Severe marine pollutant	* highest minimum classif
2032	NITRIC ACID, RED FUMING	8	5.1 6.1	I															
2054	MORPHOLINE	8	3	I		613-028-00-9	morpholine	203-815-1	110-91-8	Flam. Liq. 3 Acute Tox. 4 * Acute Tox. 4 * Acute Tox. 4 * Skin Corr. 1B	H226 H332 H312 H302 H314	GHS02 GHS05 GHS07 **Dgr**	H226 H332 H312 H302 H314				CLP00/	Corr.1A Flam. Liq.	Skin Corr. 1B Flam. Liq. 3 Ac.Tox. 4 *
2240	CHROMOSULPHURIC ACID	8		I															
2401	PIPERIDINE	8	3	I		613-027-00-3	piperidine	203-813-0	110-89-4	Flam. Liq. 2 Acute Tox. 3 * Acute Tox. 3 * Skin Corr. 1B	H225 H331 H311 H314	GHS02 GHS06 GHS05 **Dgr**	H225 H331 H311 H314		*		CLP00/	Corr.1A Flam. Liq.	Skin Corr. 1B Flam. Liq. 2 Ac.Tox. 3 *
2444	VANADIUM TETRACHLORIDE	8		I															
2604	BORON TRIFLUORIDE DIETHYL ETHERATE	8	3	I															
2692	BORON TRIBROMIDE	8		I		005-003-00-0	boron tribromide	233-657-9	10294-33-4	Acute Tox. 2 * Acute Tox. 2 * Skin Corr. 1A	H330 H300 H314	GHS06 GHS05 **Dgr**	H330 H300 H314	EUH014			CLP00/	Corr.1A	Skin Corr. 1A Ac.Tox. 2 *
2699	TRIFLUOROACETIC ACID	8		I		607-091-00-1	trifluoroacetic acid . . . %	200-929-3	76-05-1	Acute Tox. 4 * Skin Corr. 1A Aquatic Chronic 3	H332 H314 H412	GHS05 GHS07 **Dgr**	H332 H314 H412		*	B	CLP00/	Corr.1A	Skin Corr. 1A Ac.Tox. 4 * Aq.Chr.3
2879	SELENIUM OXYCHLORIDE	8	6.1	I															
3484	HYDRAZINE AQUEOUS SOLUTION, FLAMMABLE with more than 37% hydrazine, by mass	8	3 6.1	I		007-008-00-3	hydrazine	206-114-9	302-01-2	Flam. Liq. 3 Carc. 1B Acute Tox. 3 * Acute Tox. 3 * Acute Tox. 3 * Skin Corr. 1B Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H226 H350 H331 H311 H301 H314 H317 H400 H410	GHS02 GHS06 GHS08 GHS05 GHS09 **Dgr**	H226 H350 H331 H311 H301 H314 H317 H410		Skin Corr. 1B; H314: C ≥ 10 % Skin Irrit. 2; H315: 3 % ≤ C < 10 % Eye Irrit. 2; H319: 3 % ≤ C < 10 %		CLP00/	Corr.1A Ac.Tox. Flam.Liq.	Skin Corr. 1B Ac.Tox. 3 * Flam. Liq. 3 Aq.Ac.1 Aq.Chr.1

Table 2: List of substances in Dangerous Goods List (excluding generic/N.O.S. entries) together with corresponding GHS-CLP classification, for which a CLP-GHS classification as skin corrosive 1A is assigned

UN Model Reg. Rev.16						CLP regulation												Classif. TDG - GHS	Classif. CLP -GHS
										Classification		Labelling							
UN No	Proper shipping name/additional data FP = flash point BP = Boiling point	Class or Div.	Sub. risk	PG	SP	Index No	Int. Chem. ID	EC No	CAS No	Haz Class +Cat	Haz Stat	Pict, SW	Haz stat	Suppl. Haz. St.	Specific Conc. Limits, M-factors	Notes	ATP inserted/ ATP Updated	P= Marine pollutant PP= Severe marine pollutant	* highest minimum classif
1008	BORON TRIFLUORIDE LC50 (mg/m3) (4h) = 193.5	2.3	8			005-001-00-X	boron trifluoride	231-569-5	7637-07-2	Press. Gas Acute Tox. 2 * Skin Corr. 1A	H330 H314	GHS04 GHS06 GHS05 **Dgr**	H330 H314	EUH014		U	CLP00/	Press. Gas (liq,dis) Ac.Tox. 2 Corr.	Press. Gas Ac.Tox.2 * Skin Corr. 1A
1045	FLUORINE, COMPRESSED LC50 (mg/m3) (4h) = 92.5	2.3	5.1 8			009-001-00-0	fluorine	231-954-8	7782-41-4	Press. Gas Ox. Gas 1 Acute Tox. 2 * Skin Corr. 1A	H270 H330 H314	GHS04 GHS03 GHS06 GHS05 **Dgr**	H270 H330 H314				CLP00/ATP01	Press. Gas (comp.) Oxid.1 Ac.tox.1 Corr.	Press. Gas Ox. Gas 1 Ac.Tox. 2 * Skin Corr. 1A
1048	HYDROGEN BROMIDE, ANHYDROUS LC50 (mg/m3) (4h) = 1430	2.3	8			035-002-00-0	hydrogen bromide	233-113-0	10035-10-6	Press. Gas Skin Corr. 1A STOT SE 3	H314 H335	GHS04 GHS05 GHS07 **Dgr**	H314 H335			U	CLP00/	Press.Gas (liq,dis) Ac.tox.3 Corr.	Press. Gas Skin Corr. 1A
1050	HYDROGEN CHLORIDE, ANHYDROUS LC50 (mg/m3) (4h) = 1405	2.3	8			017-002-00-2	hydrogen chloride	231-595-7	7647-01-0	Press. Gas Acute Tox. 3 * Skin Corr. 1A	H331 H314	GHS04 GHS06 GHS05 **Dgr**	H331 H314			U 5	CLP00/	Press.Gas (liq,dis) Ac.tox.3 Corr.	Press. Gas Ac. Tox. 3 * Skin Corr. 1A
1052	HYDROGEN FLUORIDE, ANHYDROUS	8	6.1	I		009-002-00-6	hydrogen fluoride	231-634-8	7664-39-3	Acute Tox. 2 * Acute Tox. 1 Acute Tox. 2 * Skin Corr. 1A	H330 H310 H300 H314	GHS06 GHS05 **Dgr**	H330 H310 H300 H314				CLP00/	Corr.1A Ac.tox	Skin Corr. 1A Ac. Tox. 1
1125	n-BUTYLAMINE	3	8	II		612-005-00-0	butylamine	203-699-2	109-73-9	Flam. Liq. 2 Acute Tox. 4 * Acute Tox. 4 * Acute Tox. 4 * Skin Corr. 1A	H225 H332 H312 H302 H314	GHS02 GHS05 GHS07 **Dgr**	H225 H332 H312 H302 H314		STOT SE 3; H335		CLP00/	*Corr. 1B, 1C*	*Skin Corr. 1A*
1154	DIETHYLAMINE	3	8	II		612-003-00-X	diethylamine	203-716-3	109-89-7	Flam. Liq. 2 Acute Tox. 4 * Acute Tox. 4 * Acute Tox. 4 * Skin Corr. 1A	H225 H332 H312 H302 H314	GHS02 GHS05 GHS07 **Dgr**	H225 H332 H312 H302 H314		STOT SE 3; H335		CLP00/	Flam.2 Corr.1B, 1C	Flam. Liq. 2 Ac.Tox. 4 * Skin Corr. 1A
1295	TRICHLOROSILANE	4.3	3 8	I		014-001-00-9	trichlorosilane	233-042-5	10025-78-2	Flam. Liq. 1 Pyr. Liq. 1 Acute Tox. 4 * Acute Tox. 4 * Skin Corr. 1A	H224 H250 H332 H302 H314	GHS02 GHS05 GHS07 **Dgr**	H224 H250 H332 H302 H314	EUH014 EUH029	* STOT SE 3; H335: C ≥ 1 %	T	CLP00/	Water-react. 1 Flam. Corr.	 Flam. Liq. 1 Skin Corr. 1A Pyr. Liq. 1 Ac.Tox. 4 *

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UN No	Proper shipping name/additional data FP = flash point BP = Boiling point	Class or Div.	Sub. risk	PG	SP					Haz Class +Cat	Haz Stat	Pict, SW	Haz stat	Suppl. Haz. St.				P= Marine pollutant PP= Severe marine pollutant	* highest minimum classif
1296	TRIETHYLAMINE	3	8	II		612-004-00-5	triethylamine	204-469-4	121-44-8	Flam. Liq. 2 Acute Tox. 4 * Acute Tox. 4 * Acute Tox. 4 * Skin Corr. 1A	H225 H332 H312 H302 H314	GHS02 GHS05 GHS07 **Dgr**	H225 H332 H312 H302 H314		STOT SE 3; H335		CLP00/	Flam.2 Corr.1B, 1C	Flam. Liq. 2 Ac.Tox. 4 * Skin Corr. 1A
1381	PHOSPHORUS, WHITE or YELLOW, DRY or UNDER WATER or IN SOLUTION	4.2	6.1	I		015-001-00-1	white phosphorus	231-768-7	12185-10-3	Pyr. Sol. 1 Acute Tox. 2 * Acute Tox. 2 * Skin Corr. 1A Aquatic Acute 1	H250 H330 H300 H314 H400	GHS02 GHS06 GHS05 GHS09 **Dgr**	H250 H330 H300 H314 H400				CLP00/	Pyr. Sol. 1 Ac.Toxic P	Pyr. Sol. 1 Ac.Tox. 2 * Skin Corr. 1A Aq.Ac.1
1410	LITHIUM ALUMINIUM HYDRIDE	4.3		I		001-002-00-4	aluminium lithium hydride	240-877-9	16853-85-3	Water-react.1 Skin Corr. 1A	H260 H314	GHS02 GHS05 **Dgr**	H260 H314				CLP00/ATP01	Water-react. 1	Water-react. 1 Skin Corr. 1A
1463	CHROMIUM TRIOXIDE, ANHYDROUS	5.1	6.1 8	II		024-001-00-0	chromium (VI) trioxide	215-607-8	1333-82-0	Ox. Sol. 1 Carc. 1A Muta. 1B Repr. 2 Acute Tox. 2 * Acute Tox. 3 * Acute Tox. 3 * STOT RE 1 Skin Corr. 1A Resp. Sens. 1 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H271 H350 H340 H361f *** H330 H311 H301 H372 ** H314 H334 H317 H400 H410	GHS03 GHS06 GHS08 GHS05 GHS09 **Dgr**	H271 H350 H340 H361f *** H330 H311 H301 H372 ** H314 H334 H317 H410		STOT SE 3; H335		CLP00/	Ox. Sol. 2 Ac.Tox. Corr. *1B, 1C*	Ox. Sol. 1 Ac.Tox. 2 * Skin Corr. 1A Aq.Ac.1 Aq.Chr.1
1504	SODIUM PEROXIDE	5.1		I		011-003-00-1	sodium peroxide	215-209-4	1313-60-6	Ox. Sol. 1 Skin Corr. 1A	H271 H314	GHS03 GHS05 **Dgr**	H271 H314				CLP00/	Ox. Sol. 1	Ox. Sol. 1 Skin Corr. 1A
1744	BROMINE or BROMINE SOLUTION	8	6.1	I		035-001-00-5	bromine	231-778-1	7726-95-6	Acute Tox. 2 * Skin Corr. 1A Aquatic Acute 1	H330 H314 H400	GHS06 GHS05 GHS09 **Dgr**	H330 H314 H400				CLP00/	Corr.1A Ac.Tox.	Skin Corr. 1A Ac.Tox. 2 * Aq.Ac.1

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						Index No	Int. Chem. ID	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes	ATP inserted/ ATP Updated		
UN No	Proper shipping name/additional data FP = flash point BP = Boiling point	Class or Div.	Sub. risk	PG	SP					Haz Class +Cat	Haz Stat	Pict, SW	Haz stat	Suppl. Haz. St.				P= Marine pollutant PP= Severe marine pollutant	* highest minimum classif
1752	CHLOROACETYL CHLORIDE	6.1	8	I	354	607-080-00-1	chloroacetyl chloride	201-171-6	79-04-9	Acute Tox. 3 * Acute Tox. 3 * Acute Tox. 3 * STOT RE 1 Skin Corr. 1A Aquatic Acute 1	H331 H311 H301 H372 ** H314 H400	GHS06 GHS08 GHS05 GHS09 **Dgr**	H331 H311 H301 H372 ** H314 H400	EUH014 EUH029			CLP00/	Ac.tox.1 Corr.1A, *1B, 1C*	Ac.tox.3* Skin Corr.1A Aq.Ac.1
1754	CHLOROSULPHONIC ACID (with or without sulphur trioxide)	8		I		016-017-00-1	chlorosulphonic acid	232-234-6	7790-94-5	Skin Corr. 1A STOT SE 3	H314 H335	GHS05 GHS07 **Dgr**	H314 H335	EUH014			CLP00/	Corr.1A	Skin Corr. 1A
1758	CHROMIUM OXYCHLORIDE	8		I		024-005-00-2	chromyl dichloride; chromic oxychloride	239-056-8	14977-61-8	Ox. Liq. 1 Carc. 1B Muta. 1B Skin Corr. 1A Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H271 H350i H340 H314 H317 H400 H410	GHS03 GHS08 GHS05 GHS07 GHS09 **Dgr**	H271 H350i H340 H314 H317 H410		Skin Corr. 1A; H314: C ≥ 10 % Skin Corr. 1B; H314: 5 % ≤ C < 10 % Skin Irrit. 2; H315: 0,5 % ≤ C < 5 % Eye Irrit. 2; H319: 0,5 % ≤ C < 5 % STOT SE 3; H335: 0,5 % ≤ C < 5 % Skin Sens. 1; H317: C ≥ 0,5 %	T 3	CLP00/	Corr.1A	Skin Corr. 1A Ox. Liq. 1 Aq.Ac.1 Aq.Chr.1
1764	DICHLOROACETIC ACID	8		II		607-066-00-5	dichloroacetic acid	201-207-0	79-43-6	Skin Corr. 1A Aquatic Acute 1	H314 H400	GHS05 GHS09 **Dgr**	H314 H400				CLP00/	Corr.1B	Skin Corr. 1A Aq.Ac.1
1765	DICHLOROACETYL CHLORIDE	8		II		607-067-00-0	dichloroacetyl chloride	201-199-9	79-36-7	Skin Corr. 1A Aquatic Acute 1	H314 H400	GHS05 GHS09 **Dgr**	H314 H400				CLP00/	Corr.1B	Skin Corr. 1A Aq.Ac.1
1777	FLUOROSULPHONIC ACID	8		I		016-018-00-7	fluorosulphonic acid	232-149-4	7789-21-1	Acute Tox. 4 * Skin Corr. 1A	H332 H314	GHS05 GHS07 **Dgr**	H332 H314				CLP00/	Corr.1A	Skin Corr. 1A Ac.Tox. 4 *
1779	FORMIC ACID with more than 85% acid by mass	8	3	II		607-001-00-0	formic acid … %	200-579-1	64-18-6	Skin Corr. 1A	H314	GHS05 **Dgr**	H314		Skin Corr. 1A; H314: C ≥ 90 % Skin Corr. 1B; H314: 10 % ≤ C < 90 % Skin Irrit. 2; H315: 2 % ≤ C < 10 % Eye Irrit. 2; H319: 2 % ≤ C < 10 %	B	CLP00/	Corr.1B Flam.Liq.3	Skin Corr. 1A

UN Model Reg. Rev.16						CLP regulation												Classif. TDG - GHS	Classif. CLP -GHS
						Index No	Int. Chem. ID	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes	ATP inserted/ ATP Updated		
UN No	Proper shipping name/additional data FP = flash point BP = Boiling point	Class or Div.	Sub. risk	PG	SP					Haz Class +Cat	Haz Stat	Pict, SW	Haz stat	Suppl. Haz. St.				P= Marine pollutant PP= Severe marine pollutant	* highest minimum classif
1787	HYDRIODIC ACID	8		II		053-002-00-9	hydrogen iodide	233-109-9	10034-85-2	Press. Gas Skin Corr. 1A	H314	GHS04 GHS05 **Dgr**	H314		Skin Corr. 1A; H314: C ≥ 10 % Skin Corr. 1B; H314: 0,2 % ≤ C < 10 % Skin Irrit. 2; H315: 0,02 % ≤ C < 0,2 % Eye Irrit. 2; H319: 0,02 % ≤ C < 0,2 % STOT SE 3; H335: C ≥ 0,02 %	U 5	CLP00/	*Corr. 1B*	*Skin Corr. 1A*
1788	HYDROBROMIC ACID	8		II		035-002-00-0	hydrogen bromide	233-113-0	10035-10-6	Press. Gas Skin Corr. 1A STOT SE 3	H314 H335	GHS04 GHS05 GHS07 **Dgr**	H314 H335			U	CLP00/	Corr.1B	Press. Gas Skin Corr. 1A
1790	HYDROFLUORIC ACID, with more than 60% hydrogen fluoride	8	6.1	I		009-003-00-1	hydrofluoric acid ... %	231-634-8	7664-39-3	Acute Tox. 2 * Acute Tox. 1 Acute Tox. 2 * Skin Corr. 1A	H330 H310 H300 H314	GHS06 GHS05 **Dgr**	H330 H310 H300 H314		Skin Corr. 1A; H314: C ≥ 7 % Skin Corr. 1B; H314: 1 % ≤ C < 7 % Eye Irrit. 2; H319: 0,1 % ≤ C < 1 %	B	CLP00/	Corr.1A Ac.Tox.	Skin Corr. 1A Ac.Tox. 1
1802	PERCHLORIC ACID with not more than 50% acid, by mass	8	5.1	II		017-006-00-4	perchloric acid ... %	231-512-4	7601-90-3	Ox. Liq. 1 Skin Corr. 1A	H271 H314	GHS03 GHS05 **Dgr**	H271 H314		Skin Corr. 1A; H314: C ≥ 50 % Skin Corr. 1B; H314: 10 % ≤ C < 50 % Skin Irrit. 2; H315: 1 % ≤ C < 10 % Eye Irrit. 2; H319: 1 % ≤ C < 10 % Ox. Liq. 1; H271: C > 50 % Ox. Liq. 2; H272: C ≤ 50 %	B	CLP00/	Corr.1B Ox. Liq. 3	Skin Corr. 1A Ox. Liq. 1
1807	PHOSPHORUS PENTOXIDE	8		II		015-010-00-0	phosphorus pentoxide	215-236-1	1314-56-3	Skin Corr. 1A	H314	GHS05 **Dgr**	H314				CLP00/	Corr.1B	Skin Corr. 1A
1809	PHOSPHORUS TRICHLORIDE	6.1	8	I	354	015-007-00-4	phosphorus trichloride	231-749-3	7719-12-2	Acute Tox. 2 * Acute Tox. 2 * STOT RE 2 * Skin Corr. 1A	H330 H300 H373 ** H314	GHS06 GHS08 GHS05 **Dgr**	H330 H300 H373 ** H314	EUH014 EUH029			CLP00/	Ac.tox.1 Corr.1A, *1B, 1C*	Ac.tox.2* Skin Corr 1A

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						Index No	Int. Chem. ID	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes	ATP inserted/ ATP Updated		
UN No	Proper shipping name/additional data FP = flash point BP = Boiling point	Class or Div.	Sub. risk	PG	SP					Haz Class +Cat	Haz Stat	Pict, SW	Haz stat	Suppl. Haz. St.				P= Marine pollutant PP= Severe marine pollutant	* highest minimum classif
1810	PHOSPHORUS OXYCHLORIDE	6.1	8	I	354	015-009-00-5	phosphoryl trichloride	233-046-7	10025-87-3	Acute Tox. 2 * STOT RE 1 Acute Tox. 4 * Skin Corr. 1A	H330 H372 ** H302 H314	GHS06 GHS08 GHS05 **Dgr**	H330 H372 ** H302 H314	EUH014 EUH029			CLP00/	Ac.tox.1 Corr.*1A, 1B, 1C*	Ac.tox.2* Skin Corr 1A
1813	POTASSIUM HYDROXIDE, SOLID	8		II		019-002-00-8	potassium hydroxide; caustic potash	215-181-3	1310-58-3	Acute Tox. 4 * Skin Corr. 1A	H302 H314	GHS05 GHS07 **Dgr**	H302 H314		Skin Corr. 1A; H314: C ≥ 5 % Skin Corr. 1B; H314: 2 % ≤ C < 5 % Skin Irrit. 2; H315: 0,5 % ≤ C < 2 % Eye Irrit. 2; H319: 0,5 % ≤ C < 2 %		CLP00/	Corr.1B	Skin Corr. 1A Ac. Tox. 4 *
1814	POTASSIUM HYDROXIDE SOLUTION	8		II		019-002-00-8	potassium hydroxide; caustic potash	215-181-3	1310-58-3	Acute Tox. 4 * Skin Corr. 1A	H302 H314	GHS05 GHS07 **Dgr**	H302 H314		Skin Corr. 1A; H314: C ≥ 5 % Skin Corr. 1B; H314: 2 % ≤ C < 5 % Skin Irrit. 2; H315: 0,5 % ≤ C < 2 % Eye Irrit. 2; H319: 0,5 % ≤ C < 2 %		CLP00/	Corr.1B	Skin Corr. 1A Ac. Tox. 4 *
1823	SODIUM HYDROXIDE, SOLID	8		II		011-002-00-6	sodium hydroxide; caustic soda	215-185-5	1310-73-2	Skin Corr. 1A	H314	GHS05 **Dgr**	H314		Skin Corr. 1A; H314: C ≥ 5 % Skin Corr. 1B; H314: 2 % ≤ C < 5 % Skin Irrit. 2; H315: 0,5 % ≤ C < 2 % Eye Irrit. 2; H319: 0,5 % ≤ C < 2 %		CLP00/	Corr.1B	Skin Corr. 1A
1824	SODIUM HYDROXIDE SOLUTION	8		II		011-002-00-6	sodium hydroxide; caustic soda	215-185-5	1310-73-2	Skin Corr. 1A	H314	GHS05 **Dgr**	H314		Skin Corr. 1A; H314: C ≥ 5 % Skin Corr. 1B; H314: 2 % ≤ C < 5 % Skin Irrit. 2; H315: 0,5 % ≤ C < 2 % Eye Irrit. 2; H319: 0,5 % ≤ C < 2 %		CLP00/	Corr.1B	Skin Corr. 1A

UN Model Reg. Rev.16						CLP regulation												Classif. TDG - GHS	Classif. CLP -GHS
						Index No	Int. Chem. ID	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes	ATP inserted/ ATP Updated		
UN No	Proper shipping name/additional data FP = flash point BP = Boiling point	Class or Div.	Sub. risk	PG	SP					Haz Class +Cat	Haz Stat	Pict, SW	Haz stat	Suppl. Haz. St.				P= Marine pollutant PP= Severe marine pollutant	* highest minimum classif
1828	SULPHUR CHLORIDES	8		I		016-012-00-4	disulphur dichloride; sulfur monochloride	233-036-2	10025-67-9	Acute Tox. 3 * Acute Tox. 4 * Skin Corr. 1A Aquatic Acute 1	H301 H332 H314 H400	GHS06 GHS05 GHS09 **Dgr**	H301 H332 H314 H400	EUH014 EUH029	STOT SE 3; H335		CLP00/	Corr.1A	Skin Corr. 1A Ac.Tox. 3 * Aq.Ac.1
1830	SULPHURIC ACID with more than 51% acid	8		II		016-020-00-8	sulphuric acid ... %	231-639-5	7664-93-9	Skin Corr. 1A	H314	GHS05 **Dgr**	H314		Skin Corr. 1A; H314: C ≥ 15 % Skin Irrit. 2; H315: 5 % ≤ C < 15 % Eye Irrit. 2; H319: 5 % ≤ C < 15 %	B	CLP00/	Corr.1B	Skin Corr. 1A
1836	THIONYL CHLORIDE	8		I		016-015-00-0	thionyl dichloride; thionyl chloride	231-748-8	7719-09-7	Acute Tox. 4 * Acute Tox. 4 * Skin Corr. 1A	H332 H302 H314	GHS05 GHS07 **Dgr**	H332 H302 H314	EUH014 EUH029	STOT SE 3; H335		CLP00/	Corr.1A	Skin Corr. 1A Ac.Tox. 4 *
1839	TRICHLOROACETIC ACID	8		II		607-004-00-7	TCA (ISO); trichloroacetic acid	200-927-2	76-03-9	Skin Corr. 1A Aquatic Acute 1 Aquatic Chronic 1	H314 H400 H410	GHS05 GHS09 **Dgr**	H314 H410		STOT SE 3; H335		CLP00/	Corr.1B	Skin Corr. 1A Aq.Ac.1 Aq.Chr.1
1873	PERCHLORIC ACID with more than 50% but not more than 72% acid, by mass	5.1	8	I	60	017-006-00-4	perchloric acid ... %	231-512-4	7601-90-3	Ox. Liq. 1 Skin Corr. 1A	H271 H314	GHS03 GHS05 **Dgr**	H271 H314		Skin Corr. 1A; H314: C ≥ 50 % Skin Corr. 1B; H314: 10 % ≤ C < 50 % Skin Irrit. 2; H315: 1 % ≤ C < 10 % Eye Irrit. 2; H319: 1 % ≤ C < 10 % Ox. Liq. 1; H271: C > 50 % Ox. Liq. 2; H272: C ≤ 50 %	B	CLP00/	Ox. Liq. 1 Corr. *1A*, *1B*, *1C*	Ox. Liq. 1 Skin Corr. 1A
1938	BROMOACETIC ACID SOLUTION	8		II		607-065-00-X	bromoacetic acid	201-175-8	79-08-3	Acute Tox. 3 * Acute Tox. 3 * Acute Tox. 3 * Skin Corr. 1A Skin Sens. 1 Aquatic Acute 1	H331 H311 H301 H314 H317 H400	GHS06 GHS05 GHS09 **Dgr**	H331 H311 H301 H314 H317 H400				CLP00/ATP01	Corr.1B	Skin Corr. 1A Ac.Tox. 3 * Aq.Ac.1

UN Model Reg. Rev.16						CLP regulation												Classif. TDG - GHS	Classif. CLP -GHS
						Index No	Int. Chem. ID	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes	ATP inserted/ ATP Updated		
UN No	Proper shipping name/additional data FP = flash point BP = Boiling point	Class or Div.	Sub. risk	PG	SP					Haz Class +Cat	Haz Stat	Pict, SW	Haz stat	Suppl. Haz. St.				P= Marine pollutant PP= Severe marine pollutant	* highest minimum classif
2014	HYDROGEN PEROXIDE, AQUEOUS SOLUTION with not less than 20% but not more than 60% hydrogen peroxide (stabilized as necessary)	5.1	8	II		008-003-00-9	hydrogen peroxide solution ... %	231-765-0	7722-84-1	Ox. Liq. 1 Acute Tox. 4 * Acute Tox. 4 * Skin Corr. 1A	H271 H332 H302 H314	GHS03 GHS05 GHS07 **Dgr**	H271 H332 H302 H314		Ox. Liq. 1; H271: C ≥ 70 %**** Ox. Liq. 2; H272: 50 % ≤ C < 70 % **** * Skin Corr. 1A; H314: C ≥ 70 % Skin Corr. 1B; H314: 50 % ≤ C < 70 % Skin Irrit. 2; H315: 35 % ≤ C < 50 % Eye Dam. 1; H318: 8 % ≤ C < 50 % Eye Irrit. 2; H319: 5 % ≤ C < 8 % STOT SE 3; H335; C ≥ 35 %	B	CLP00/	Ox. Liq. 2 Corr. *1B, 1C*	Ox. Liq. 1 Ac.Tox. 4 * Skin Corr. 1A
2031	NITRIC ACID, other than red fuming, with more than 70% nitric acid	8	5.1	I		007-004-00-1	nitric acid ... %	231-714-2	7697-37-2	Ox. Liq. 3 Skin Corr. 1A	H272 H314	GHS03 GHS05 **Dgr**	H272 H314		Skin Corr. 1A; H314: C ≥ 20 % Skin Corr. 1B; H314: 5 % ≤ C < 20 % Ox. Liq. 3; H272: C ≥ 65 %	B	CLP00/	Corr.1A Ox. Liq.	Skin Corr. 1A Ox. Liq. 3
2186	HYDROGEN CHLORIDE, REFRIGERATED LIQUID	2.3	8			017-002-00-2	hydrogen chloride	231-595-7	7647-01-0	Press. Gas Acute Tox. 3 * Skin Corr. 1A	H331 H314	GHS04 GHS06 GHS05 **Dgr**	H331 H314			U 5	CLP00/	Press.Gas (Refrig., liq) Ac.tox. Corr. *1A, 1B, 1C*	Press. Gas Ac.Tox. 3 * Skin Corr. 1A
2197	HYDROGEN IODIDE, ANHYDROUS LC50 (mg/m3) (4h) = 1430	2.3	8			053-002-00-9	hydrogen iodide	233-109-9	10034-85-2	Press. Gas Skin Corr. 1A	H314	GHS04 GHS05 **Dgr**	H314		Skin Corr. 1A; H314: C ≥ 10 % Skin Corr. 1B; H314: 0,2 % ≤ C < 10 % Skin Irrit. 2; H315: 0,02 % ≤ C < 0,2 % Eye Irrit. 2; H319: 0,02 % ≤ C < 0,2 % STOT SE 3; H335: C ≥ 0,02 %	U 5	CLP00/	Press.Gas (liq,dis) Ac.tox.3 Corr. *1A, 1B, 1C*	Press. Gas Skin Corr. 1A

UN Model Reg. Rev.16						CLP regulation												Classif. TDG - GHS	Classif. CLP -GHS
						Index No	Int. Chem. ID	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes	ATP inserted/ ATP Updated		
UN No	Proper shipping name/additional data FP = flash point BP = Boiling point	Class or Div.	Sub. risk	PG	SP					Haz Class +Cat	Haz Stat	Pict, SW	Haz stat	Suppl. Haz. St.				P= Marine pollutant PP= Severe marine pollutant	* highest minimum classif
2218	ACRYLIC ACID, STABILIZED	8	3	II		607-061-00-8	acrylic acid; prop-2-enoic acid	201-177-9	79-10-7	Flam. Liq. 3 Acute Tox. 4 * Acute Tox. 4 * Acute Tox. 4 * Skin Corr. 1A Aquatic Acute 1	H226 H332 H312 H302 H314 H400	GHS02 GHS05 GHS07 GHS09 **Dgr**	H226 H332 H312 H302 H314 H400		STOT SE 3; H335	D	CLP00/	Corr.1B Flam. Liq.3	Skin Corr. 1A Flam. Liq. 3 Ac.Tox. 4 * Aq.Ac.1
2258	1,2-PROPYLENEDIAMINE	8	3	II		612-100-00-7	propylenediamine	201-155-9	78-90-0	Flam. Liq. 3 Acute Tox. 4 * Acute Tox. 4 * Skin Corr. 1A	H226 H312 H302 H314	GHS02 GHS05 GHS07 **Dgr**	H226 H312 H302 H314				CLP00/	Corr.1B Flam. Liq.3	Skin Corr. 1A Flam. Liq. 3 Ac.Tox. 4 *
2269	3,3'-IMINODIPROPYLAMINE	8		III		612-063-00-7	3,3'-iminodi(propylamine); dipropylenetriamine	200-261-2	56-18-8	Acute Tox. 2 * Acute Tox. 3 * Acute Tox. 4 * Skin Corr. 1A Skin Sens. 1	H330 H311 H302 H314 H317	GHS06 GHS05 **Dgr**	H330 H311 H302 H314 H317				CLP00/	Corr. 1C	Skin Corr. 1A Ac.Tox. 2 *
2383	DIPROPYLAMINE	3	8	II		612-048-00-5	dipropylamine	205-565-9	142-84-7	Flam. Liq. 2 Acute Tox. 4 * Acute Tox. 4 * Acute Tox. 4 * Skin Corr. 1A	H225 H332 H312 H302 H314	GHS02 GHS05 GHS07 **Dgr**	H225 H332 H312 H302 H314		STOT SE 3; H335		CLP00/	Flam.2 Corr.1B, 1C	Flam. Liq. 2 Ac.Tox. 4 * Skin Corr. 1A
2395	ISOBUTYRYL CHLORIDE	3	8	II		607-140-00-7	isobutyryl chloride	201-194-1	79-30-1	Flam. Liq. 2 Skin Corr. 1A	H225 H314	GHS02 GHS05 **Dgr**	H225 H314				CLP00/	Flam.2 Corr.1B, 1C	Flam. Liq. 2 Skin Corr. 1A
2447	PHOSPHORUS, WHITE, MOLTEN	4.2	6.1	I		015-001-00-1	white phosphorus	231-768-7	12185-10-3	Pyr. Sol. 1 Acute Tox. 2 * Acute Tox. 2 * Skin Corr. 1A Aquatic Acute 1	H250 H330 H300 H314 H400	GHS02 GHS06 GHS05 GHS09 **Dgr**	H250 H330 H300 H314 H400				CLP00/	Pyr. Sol. 1 Ac.Toxic P	Pyr. Sol. 1 Ac.Tox. 2 * Skin Corr. 1A Aq.Ac.1
2511	2-CHLOROPROPIONIC ACID	8		III	223	607-139-00-1	2-chloropropionic acid	209-952-3	598-78-7	Acute Tox. 4 * Skin Corr. 1A	H302 H314	GHS05 GHS07 **Dgr**	H302 H314				CLP00/	Corr. 1C	Skin Corr. 1A Ac.Tox. 4 *
2531	METHACRYLIC ACID, STABILIZED	8		II		607-088-00-5	methacrylic acid; 2-methylpropenoic acid	201-204-4	79-41-4	Acute Tox. 4 * Acute Tox. 4 * Skin Corr. 1A	H312 H302 H314	GHS05 GHS07 **Dgr**	H312 H302 H314		STOT SE 3; H335	D	CLP00/	Corr.1B	Skin Corr. 1A Ac.Tox. 4 *

UN Model Reg. Rev.16						CLP regulation												Classif. TDG - GHS	Classif. CLP -GHS
						Index No	Int. Chem. ID	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes	ATP inserted/ ATP Updated		
UN No	Proper shipping name/additional data FP = flash point BP = Boiling point	Class or Div.	Sub. risk	PG	SP					Haz Class +Cat	Haz Stat	Pict, SW	Haz stat	Suppl. Haz. St.				P= Marine pollutant PP= Severe marine pollutant	* highest minimum classif
2564	TRICHLOROACETIC ACID SOLUTION	8		II		607-004-00-7	TCA (ISO); trichloroacetic acid	200-927-2	76-03-9	Skin Corr. 1A Aquatic Acute 1 Aquatic Chronic 1	H314 H400 H410	GHS05 GHS09 **Dgr**	H314 H410		STOT SE 3; H335		CLP00/	Corr.1B	Skin Corr. 1A Aq.Ac.1 Aq.Chr.1
2692	BORON TRIBROMIDE	8		I		005-003-00-0	boron tribromide	233-657-9	10294-33-4	Acute Tox. 2 * Acute Tox. 2 * Skin Corr. 1A	H330 H300 H314	GHS06 GHS05 **Dgr**	H330 H300 H314	EUH014			CLP00/	Corr.1A	Skin Corr. 1A Ac.Tox. 2 *
2699	TRIFLUOROACETIC ACID	8		I		607-091-00-1	trifluoroacetic acid . . . %	200-929-3	76-05-1	Acute Tox. 4 * Skin Corr. 1A Aquatic Chronic 3	H332 H314 H412	GHS05 GHS07 **Dgr**	H332 H314 H412		*	B	CLP00/	Corr.1A	Skin Corr. 1A Ac.Tox. 4 * Aq.Chr.3
2789	ACETIC ACID, GLACIAL or ACETIC ACID SOLUTION, more than 80% acid, by mass	8	3	II		607-002-00-6	acetic acid … %	200-580-7	64-19-7	Flam. Liq. 3 Skin Corr. 1A	H226 H314	GHS02 GHS05 **Dgr**	H226 H314		Skin Corr. 1A; H314: C ≥ 90 % Skin Corr. 1B; H314: 25 % ≤ C < 90 % Skin Irrit. 2; H315: 10 % ≤ C < 25 % Eye Irrit. 2; H319: 10 % ≤ C < 25 %	B	CLP00/	Corr.1B Flam. Liq. 3	Skin Corr. 1A Flam. Liq. 3
2796	SULPHURIC ACID with not more than 51% acid or BATTERY FLUID, ACID	8		II		016-020-00-8	sulphuric acid ... %	231-639-5	7664-93-9	Skin Corr. 1A	H314	GHS05 **Dgr**	H314		Skin Corr. 1A; H314: C ≥ 15 % Skin Irrit. 2; H315: 5 % ≤ C < 15 % Eye Irrit. 2; H319: 5 % ≤ C < 15 %	B	CLP00/	Corr.1B	Skin Corr. 1A
2834	PHOSPHOROUS ACID	8		III		015-157-00-0	phosphonic acid; [1] phosphorous acid [2]	237-066-7 [1] 233-663-1 [2]	13598-36-2 [1] 10294-56-1 [2]	Acute Tox. 4 * Skin Corr. 1A	H302 H314	GHS05 GHS07 **Dgr**	H302 H314				CLP00/	Corr. 1C	Skin Corr. 1A Ac.Tox. 4 *
2851	BORON TRIFLUORIDE DIHYDRATE	8		II		005-001-00-X	boron trifluoride	231-569-5	7637-07-2	Press. Gas Acute Tox. 2 * Skin Corr. 1A	H330 H314	GHS04 GHS06 GHS05 **Dgr**	H330 H314	EUH014		U	CLP00/	Corr.1B	Press. Gas Skin Corr. 1A Ac.Tox. 2 *

UN Model Reg. Rev.16						CLP regulation												Classif. TDG - GHS	Classif. CLP -GHS
						Index No	Int. Chem. ID	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes	ATP inserted/ ATP Updated		
UN No	Proper shipping name/additional data FP = flash point BP = Boiling point	Class or Div.	Sub. risk	PG	SP					Haz Class +Cat	Haz Stat	Pict, SW	Haz stat	Suppl. Haz. St.				P= Marine pollutant PP= Severe marine pollutant	* highest minimum classif
2984	HYDROGEN PEROXIDE, AQUEOUS SOLUTION with not less than 8% but less than 20% hydrogen peroxide (stabilized as necessary)	5.1		III	65	008-003-00-9	hydrogen peroxide solution ... %	231-765-0	7722-84-1	Ox. Liq. 1 Acute Tox. 4 * Acute Tox. 4 * Skin Corr. 1A	H271 H332 H302 H314	GHS03 GHS05 GHS07 **Dgr**	H271 H332 H302 H314		Ox. Liq. 1; H271: C ≥ 70 %**** Ox. Liq. 2; H272: 50 % ≤ C < 70 % **** * Skin Corr. 1A; H314: C ≥ 70 % Skin Corr. 1B; H314: 50 % ≤ C < 70 % Skin Irrit. 2; H315: 35 % ≤ C < 50 % Eye Dam. 1; H318: 8 % ≤ C < 50 % Eye Irrit. 2; H319: 5 % ≤ C < 8 % STOT SE 3; H335; C ≥ 35 %	B	CLP00/	Ox. Sol. 3	Ox. Liq. 1 Ac.Tox. 4 * Skin Corr. 1A
3412	FORMIC ACID with not less than 10% but not more than 85% acid by mass	8		II		607-001-00-0	formic acid … %	200-579-1	64-18-6	Skin Corr. 1A	H314	GHS05 **Dgr**	H314		Skin Corr. 1A; H314: C ≥ 90 % Skin Corr. 1B; H314: 10 % ≤ C < 90 % Skin Irrit. 2; H315: 2 % ≤ C < 10 % Eye Irrit. 2; H319: 2 % ≤ C < 10 %	B	CLP00/	Corr.1B	Skin Corr. 1A
3425	BROMOACETIC ACID, SOLID	8		II		607-065-00-X	bromoacetic acid	201-175-8	79-08-3	Acute Tox. 3 * Acute Tox. 3 * Acute Tox. 3 * Skin Corr. 1A Skin Sens. 1 Aquatic Acute 1	H331 H311 H301 H314 H317 H400	GHS06 GHS05 GHS09 **Dgr**	H331 H311 H301 H314 H317 H400				CLP00/ATP01	Corr.1B	Skin Corr. 1A Ac.Tox. 3 * Aq.Ac.1
3437	CHLOROCRESOLS, SOLID	6.1		II		604-012-00-2	4-chloro-*o*-cresol; 4-chloro-2-methyl phenol	216-381-3	1570-64-5	Acute Tox. 3 * Skin Corr. 1A Aquatic Acute 1	H331 H314 H400	GHS06 GHS05 GHS09 **Dgr**	H331 H314 H400		STOT SE 3; H335		CLP00/	Ac.Tox. 2	Ac.Tox. 3 * Skin Corr. 1A Aq.Ac.1

Annex IV

Information submitted by the expert from the Netherlands including table comparing the skin corrosivity classifications for a few chemicals

Explanatory notes with the table comparing the skin corrosivity classifications for a few chemicals (RIVM, 11-03-2011)

The table compares the classification of several chemicals as listed in

- the DGL of the Model Regulations (16th edition);
- Annex VI of CLP (0 and 1st ATP);
- the CLP Inventory (March 2011); and
- the GESAMP Composite list EHS 47/9 (30 July 2010).

Explanations of the table columns

Chemical name: The common name of the chemical. This common chemical name does not take account of composition, impurity profile, concentration etc.

CAS No: The CAS number listed is taken from Annex VI of CLP or dossiers submitted to the OECD High Production Volume chemical assessment program.

TDG UN No: The UN number of the proper shipping names connected to the common chemical name, taken from the Model Regulations.

TDG Proper shipping name: The proper shipping names connected to the common chemical name, taken from the Model Regulations.

TDG Class: The DGL classification associated with the UN number and proper shipping name, taken from the Model Regulations.

TDG PG: The PG group assignment of the UN number and proper shipping name, taken from the Model Regulations.

Annex VI name: The name of the substance on Annex VI of the CLP Regulation.

Annex VI Classification: The skin corrosivity (or skin irritation) classification listed in Annex VI of the CLP Regulation for the (common) chemical name and CAS number listed. Annex VI lists harmonized classifications of chemicals. The classifications on this list are legally binding in Europe. If a chemical or an endpoint does not have an Annex VI entry, industry is required to self-classify for the chemical/endpoint.

Annex VI SCL: The SCL are the specific concentration limits for substances which are listed in Annex VI of the CLP Regulation. These SCLs are (usually) assigned to chemicals which are listed as ….% (i.e. solution) in Annex VI as the classification will depend on the concentration.

CLP Inventory: The classifications listed in the CLP Inventory for the CAS number specified. Where more than one classification for corrosivity was notified to the Inventory, all different classifications were written up in the table. The CLP inventory lists the classifications of all hazardous substances on the market in the EU. The producer or importer is responsible for notifying to the European Chemicals Agency (ECHA) the classification of substances that are brought on the market. Where a chemical/endpoint has

a harmonized classification, this harmonized classification must be used and notified to the inventory. For all other chemicals and endpoints, the producers/importers are responsible for deriving a classification (self-classification). The CLP regulation (Art. 41) requires the producers/importers of the same chemical to make every effort to come to an agreed entry to be included in the inventory.

GESAMP Comp List Name: The name used in the GESAMP composite list.

GESAMP Comp List Class.: The classification of the chemical with the common chemical name and CAS number as listed in the GESAMP composite list EHS 47/9 dated 30 July 2010. The GESAMP uses GHS criteria to classify; however, the categories have different names. The correlation between the GESAMP categories and the GHS categories are as follows: GESAMP 3A = GHS Cat 1C

GESAMP 3B = GHS Cat 1B

GESAMP 3C = GHS Cat 1A

Remarks: Any remarks on the entries.

Discussion

The table does contribute to item (c) in the terms of reference for the informal working group on corrosivity:

Identify differences in assignment to categories in lists provided by different regulations and guidance documents for a few representative common substances. Analyse the underlying data and origin of these differences and use these results for the work under paragraphs (a), (b), and (d).

The table shows that there are differences in classifications and/or concentration limits between the DGL, Annex VI, the CLP Inventory and GESAMP list.

The information in the table can not explain the reason for these differences as it only gives the result of the classification process and not the data or criteria used to classify.

The most likely reasons for these differences are

- substance identity (composition, impurity profile, concentration etc)
- data availability/data used to classify
- data interpretation
- derivation of the classification (criteria, experience etc)

The data used to derive the classification in the DGL, Annex VI, the Inventory and the GESAMP composite list are difficult to find since in many cases, the classification justifications are not well documented.

Data availability and the underlying data for each classification and the exact criteria used to derive the classification is not the most important issue for this purpose.

Data interpretation and criteria/methods used to derive the classification are most important. Furthermore, the substance identity needs to be carefully defined to prevent misinterpretation.

It is possible to examine in more detail a few chemicals using non-confidential (disseminated) data from REACH registrations and invite industry to supply any other additional data that may be available elsewhere in the world. The data can be compared with the criteria used in each framework to see if differences arise.

Other uses of the table (e.g. discussion on global lists)

The results illustrate the need for increased transparency and better documentation of the data and criteria used to classify, and the justification/reasoning for the classification.

The results also illustrate the need for further harmonization and updates of the lists as the discrepancies are difficult to justify.

		TDG					Annex VI				GESAMP Comp. List		
Chemical name	**CAS No.**	**UN No.**	**Proper shipping name**	**Class**	**PG**	**Name**	**Classif.**	**SCL**	**Conc. limits**	**CLP Inventory**	**Name**	**Class**	**Remarks**
Hydrogen chloride	7647-01-0	1050	HYDROGEN CHLORIDE, ANHYDROUS	2.3 (8)		Hydrogen chloride	Skin Corr. 1A			Not yet available			
Hydrochloric acid		1789	HYDROCHLORIC ACID	8	II or III	Hydrochloric acid ... %	Skin Corr. 1B*	C ≥ 25%, Skin Corr 1B		Not yet available	Hydrochloric acid	3C	UN1789 has SP223
								10% ≤ C < 25%, Skin Irr 2					
Sulphuric acid	7664-93-9	1830	SULPHURIC ACID with more than 51% acid	8	II	Sulphuric acid ... %	Skin Corr. 1A	C ≥ 15%, Skin Corr 1A	> 3%	Skin Corr 1A	Sulphuric acid	3C	
		1831	SULPHURIC ACID, FUMING	8 (6.1)	I			5% ≤ C < 15%, Skin Irr 2		Skin Corr 1C	Sulphuric acid, spent	3C	
		1832	SULPHURIC ACID, SPENT	8	II					No classification			
		2796	SULPHURIC ACID with not more than 51% acid or BATTERY FLUID, ACID	8	II								
Nitric acid	7697-37-2	1796	NITRATING ACID MIXTURES with more than 50% nitric acid	8 (5.1)	I	Nitric acid ... %	Skin Corr. 1A	C ≥ 20%, Skin Corr 1A		Skin Corr 1A	Nitric acid (70% and over)	3C	
		1796	NITRATING ACID MIXTURES with not more than 50% nitric acid	8	II			5% ≤ C < 20%, Skin Corr 1B		Skin Corr 1B	Nitric acid (less than 70%)	3C	
		1826	NITRATING ACID MIXTURES SPENT with more than 50% nitric acid	8 (5.1)	I					No classification			
		1826	NITRATING ACID MIXTURES SPENT with not more than 50% nitric acid	8	II								
		2031	NITRIC ACID, other than red fuming, with more than 70% nitric acid	8 (5.1)	I								
		2031	NITRIC ACID, other than red fuming, with at least 65% but not more than 70% nitric acid	8 (5.1)	II								
		2031	NITRIC ACID, other than red fuming, with less than 65% nitric acid	8	II								
		2032	NITRIC ACID, red fuming	8 (5.1, 6.1)	I								
Phosphoric acid	7664-38-2	1805	PHOSPHORIC ACID, SOLUTION	8	III	Phosphoric acid ... %, Orthophosphoric acid ... %	Skin Corr. 1B*	C ≥ 25%, Skin Corr 1B	≥ 25%	Skin Corr 1A	Phosphoric acid	3	UN1805 has SP223
		3453	PHOSPHORIC ACID, SOLID	8	III			10% ≤ C < 25%, Skin Irr 2		Skin Corr 1B			
										Skin Corr 1C			
										No classification			
Sodium hydroxide	1310-73-2	1823	SODIUM HYDROXIDE, SOLID	8	II	Caustic soda; sodium hydroxide	Skin Corr. 1A	C ≥ 5%, Skin Corr 1A		Skin Corr 1A			
		1824	SODIUM HYDROXIDE, SOLUTION	8	II			2% ≤ C < 5%, Skin Corr 1B		Skin Corr 1B	Sodium hydroxide solution	3C	
		1824	SODIUM HYDROXIDE, SOLUTION	8	III			0,5% ≤ C < 2%, Skin Irr 2		Skin Corr 1C			
										Skin Irr 2			
										No classification			
Potassium hydroxide	1310-58-3	1813	POTASSIUM HYDROXIDE, SOLID	8	II		Skin Corr. 1A	C ≥ 5%, Skin Corr 1A		Skin Corr 1A			
		1814	POTASSIUM HYDROXIDE, SOLUTION	8	II			2% ≤ C < 5%, Skin Corr 1B		Skin Corr 1B	Potassium hydroxide, solution	3C	
		1814	POTASSIUM HYDROXIDE, SOLUTION	8	III			0,5% ≤ C < 2%, Skin Irr 2		Skin Corr 1C			
										No classification			
Formic acid	64-18-6	1779	FORMIC ACID, with more than 85% acid by	8 (3)	II	Formic acid … %	Skin Corr. 1A	C ≥ 90%, Skin Corr 1A	≥ 5%	Skin Corr 1A	Formic acid	3C	
		3412	FORMIC ACID, with not less than 10% but not more than 85% acid by mass	8	II			10% ≤ C < 90%, Skin Corr 1B		Skin Corr 1B			
		3412	FORMIC ACID, with not less than 5% but less than 10% acid by mass	8	III			2% ≤ C < 10%, Skin Irr 2		Skin Corr 1C			
										No classification			

		TDG				Annex VI					GESAMP Comp. List		
Chemical name	**CAS No.**	**UN No.**	**Proper shipping name**	**Class**	**PG**	**Name**	**Classif.**	**SCL**	**Conc. limits**	**CLP Inventory**	**Name**	**Class**	**Remarks**
Acetic acid	64-19-7	2789	ACETIC ACID, GLACIAL or ACETIC ACID SOLUTION, more than 80% acid by mass	8 (3)	II	Acetic acid … %	Skin Corr. 1A	C ≥ 90%, Skin Corr 1A	> 10%	Skin Corr 1A	Acetic acid	3C	
		2790	ACETIC ACID SOLUTION, not less than 50% but not more than 80% acid, by mass	8	II			25% ≤ C < 90%, Skin Corr 1B		Skin Corr 1B			
		2790	ACETIC ACID SOLUTION, more than 10% but less than 50% acid, by mass	8	III			10% ≤ C < 25%, Skin Irr 2		Skin Corr 1C			
										No classification			
Propionic acid	79-09-4	1848	PROPIONIC ACID, with not less than 10% and less than 90% acid, by mass	8	III	Propionic acid … %	Skin Corr. 1B*	C ≥ 25%, Skin Corr 1B	≥ 10%	Skin Corr 1A	Propionic acid	3C	
		3463	PROPIONIC ACID, with not less than 90% acid, by mass	8 (3)	II			10% ≤ C < 25%, Skin Irr 2		Skin Corr 1B			
										Skin Corr 1C			
										Not corrosive			
Ammonia (anhydrous)	7664-41-7	1005	AMMONIA, ANHYDROUS	2.3 (8)		Ammonia, anhydrous	Skin Corr. 1B*	-		Skin Corr 1B			
Ammonia solutions	1336-21-6	2073	AMMONIA SOLUTION, relative density less than 0.880 at 15C in water, with more than 35% but not more than 50% ammonia	2.2		Ammonia … %	Skin Corr. 1B*	- (see remark)	> 10%	Skin Corr 1A	Ammonia aqueous (28% or less)	3	Table 3.2 has SCL C>10%, R34; 5%<C<10% R36/37/38
		2672	AMMONIA SOLUTION, relative density between 0.880 and 0.957 at 15C in water, with more than 10% but not more than 35% ammonia	8	III					Skin Corr 1B			Table 3.1 has no SCL for corrosivity
		3318	AMMONIA SOLUTION, relative density less than 0.880 at 15C in water, with more than 50% ammonia	2.3 (8)						Skin Corr 1C			
		1043	FERTILIZER AMMONIATING SOLUTION with free ammonia	2.2						No classification			
Formaldehyde	50-00-0	1198	FORMALDEHYDE SOLUTION, FLAMMABLE	3 (8)	III	Formaldehyde … %	Skin Corr. 1B*	C ≥25%, Skin Corr 1B		Skin Corr 1B	Formaldehyde (45% or less)	3	
		2209	FORMALDEHYDE SOLUTION, with not less than 25% formaldehyde	8	III			5% ≤ C < 25%, Skin Irr 2					
		2213	PARAFORMALDEHYDE	4.1	III								
Methylamine	74-89-5	1061	METHYLAMINE, ANHYDROUS	2.1		Mono-methylamine	Skin Irr 2	C ≥ 5%, Skin Irr 2		Skin Corr 1B			
										Skin Irr 2			
										No classification			
Methylamine	74-89-5	1235	METHYLAMINE, AQEOUS SOLUTION	3 (8)	II	Mono-methylamine … %	Skin Corr 1B*	- (see remarks)		Skin Corr 1B	Methylamine solution (42% or less)	3	Table 3.2 has SCL C>10%, R34; 5%<C<10% R36/37/38
										Skin Irr 2			Table 3.1 has no SCL for corrosivity
										No classification			
Ethylamine	74-04-7	1036	ETHYLAMINE	2.1		Ethylamine	- (see remarks)	-		Not yet available	Ethylamine	3	The entry for ethylamine contains no classification for corrosivity

		TDG					Annex VI				GESAMP Comp. List		
Chemical name	**CAS No.**	**UN No.**	**Proper shipping name**	**Class**	**PG**	**Name**	**Classif.**	**SCL**	**Conc. limits**	**CLP Inventory**	**Name**	**Class**	**Remarks**
Ethylamine		2270	ETHYLAMINE, AQEOUS SOLUTION with not less than 50% but not more than 70% Et-amine	3(8)	II			-		Not yet available	Ethylamine solution (72% or less)	3	
Isopropylamine	75-31-0	1221	ISOPROPYLAMINE	3 (8)	I	Isopropylamine; 2-aminopropane	Skin Irr 2	-		Skin Corr 1A	Isopropylamine	3	SIAM data and conclusion: corrosive
										Skin Irr 2	Isopropylamine (70% or less)	3	
Butylamine	109-73-9	1125	n-BUTYLAMINE	3 (8)	II	Butylamine	Skin Corr. 1A	- (see remark)		Skin Corr 1A	Butylamine	3C	Table 3.2 has SCL C>10%, R35; 5%<C<10% R34, 1%<C<5% R36/37/38
										No classification			Table 3.1 has no SCL for corrosivity
sec-Butylamine	13952-84-6	2733	AMINES, FLAMMABLE, CORROSIVE NOS	3 (8)	I, II or III	sec-Butylamine; 2-aminobutane	Skin Corr. 1A	-		Skin Corr 1A			UN2733 and UN2735 have SP223
	(racemic)	2734	AMINES, LIQUID, CORROSIVE, FLAMMABLE	8 (3)	I or II					No classification			
		2735	AMINES, LIQUID, CORROSIVE	8	I, II or III								
tert-Butylamine	75-64-9	2733	AMINES, FLAMMABLE, CORROSIVE NOS	3 (8)	I, II or III		no entry	no entry		Skin Corr 1A			UN2733 and UN2735 have SP223
		2734	AMINES, LIQUID, CORROSIVE, FLAMMABLE	8 (3)	I or II					Skin Corr 1B			
		2735	AMINES, LIQUID, CORROSIVE	8	I, II or III					Skin Corr 1C			
										No classification			
Octylamine	111-86-4	2733	AMINES, FLAMMABLE, CORROSIVE NOS	3 (8)	I, II or III		no entry	no entry		Skin Corr 1A			UN2733 and UN2735 have SP223
		2734	AMINES, LIQUID, CORROSIVE, FLAMMABLE	8 (3)	I or II					Skin Corr 1B			
		2735	AMINES, LIQUID, CORROSIVE	8	I, II or III					No classification			
2-ethylhexylamine	104-75-6	2276	2-ETHYLHEXYLAMINE	3 (8)	III		no entry	no entry		Skin Corr 1A		3	
										Skin Corr 1B			
										Skin Corr 1C			
										No classification			
4,4'-methylenebis cyclohexylamine	1761-71-3	2733	AMINES, FLAMMABLE, CORROSIVE NOS	3 (8)	I, II or III		no entry	no entry		Skin Corr 1A			UN2733 and UN2735 have SP223
		2734	AMINES, LIQUID, CORROSIVE, FLAMMABLE	8 (3)	I or II					Skin Corr 1B			
		2735	AMINES, LIQUID, CORROSIVE	8	I, II or III					Skin Irr 2			
										No classification			
3-methoxy-propylamine	5332-73-0	2733	AMINES, FLAMMABLE, CORROSIVE NOS	3 (8)	I, II or III		no entry	no entry		Skin Corr 1A			UN2733 and UN2735 have SP223
		2734	AMINES, LIQUID, CORROSIVE, FLAMMABLE	8 (3)	I or II					Skin Corr 1B			
		2735	AMINES, LIQUID, CORROSIVE	8	I, II or III					No classification			

		TDG					Annex VI				GESAMP Comp. List		
Chemical name	**CAS No.**	**UN No.**	**Proper shipping name**	**Class**	**PG**	**Name**	**Classif.**	**SCL**	**Conc. limits**	**CLP Inventory**	**Name**	**Class**	**Remarks**
1-amino-2-propylamine	78-96-6	2733	AMINES, FLAMMABLE, CORROSIVE NOS	3 (8)	I, II or III	Isopopanolamine; 1-aminopropan-2-ol	Skin Corr. 1B*	-		Skin Corr 1B			UN2733 and UN2735 have SP223
		2734	AMINES, LIQUID, CORROSIVE, FLAMMABLE	8 (3)	I or II					No classification			
		2735	AMINES, LIQUID, CORROSIVE	8	I, II or III								

* It is recommended to classify with 1V even if it could be possible that 1C could be applicable for certain cases. Going back to original data may not results in a possibility to distinguish between Cat 1B and 1C since the exposure period has normally been up to 4 hours according to Regulation (EC) no 440/2008.

Annex V

Comments and suggestions received from correspondence group members on how to proceed with the work of the group

Comments from the expert from the Netherlands

Regarding work element (c) we see the following possibility:

For some common substances we can compare the classifications regarding corrosivity as listed in: UN TDG, CLP Annex VI and the classifications as notified by industry (CLP obligation) to ECHA.

For those substances registered by REACH, we can check the underlying data.

An effort to elucidate the background of the present classifications as listed in UN TDG and CLP Annex VI can be made. However due to a lack of systematic archiving it may be very difficult to reveal the underlying data and argumentation.

To prevent double work, it is necessary to coordinate the substances on which we work within the working group.

Based on our experience with 5 substances, we will decide whether we expand our work to more substances.

Comments from the expert from Belgium

We need of course to consider the new proposed text for chapters 3.2 and 3.3. Even if the revision is normally only editorial, there is a big difference in the order how the data have to be considered in the tiered evaluation (pH considerations and QSAR coming at the end of the evaluation, after the in vitro tests)

Under point (b), as we need to analyse the assignment to the sub-categorisation not only for substances but also for mixtures, it would be useful to have access to data on mixtures to compare classifications based on the different methods: calculations, in vitro and in vivo tests,… but are there such data for corrosive mixtures ? Probably most mixtures are classified as irritant.

Under point (c), I share the views of NL, it was also not so easy to find back the data and argumentation of some classifications when writing the ECHA guidance document on health effect but finally with efforts we get them.

As the sub-categorisation is very important for the transport, I think we need to start with the comparison of at least 2 substances from each of the 3 sub-subcategories in transport, excluding substances classified in Packing Group III when the attribution to this Packing Group is due to the corrosive effect on metals. In the CLP, we have only substances classified either Skin Corr. 1A, either Skin Corr. 1B.

Comments from the expert from AISE

Definitions

The definitions of “dermal corrosion” in OECD 404 and “skin corrosion” GHS 3.2 are the same, apart from the titles: “the production of irreversible damage to the skin: namely visible necrosis through the epidermis and into the dermis, following the application of a test substance for up to 4 hours.”

Observation periods are quoted in 404 but there is no direct correlation to “sub-categories”.

Observation periods are quoted in 3.2 which along with exposure times are directly correlated with sub-categories.

Observation periods are *not* quoted in EC Regulation No. 440/2008 (formerly Annex V of DSD) *in the assignment of R35 and R34* - which is the test method for DSD and effectively a rewriting of 404, as is 3.2.

Definitions in RTDG 2.8 are different and are completely tied up with Packing Groups (degrees of hazard) e.g. “Packing group I is assigned to substances that cause full thickness destruction of intact skin tissue within an observation period of up to 60 minutes starting time after the exposure time of three minutes or less” An exposure time of three minutes or less is stated in 404: the “corrosive effect” is not defined beyond the general definition above. Table 3.2.1 in 3.2 seems to have been taken from 2.8 with PG replaced by 1A, 1B and 1C (which 3.2 says “only applies to some authorities”).

Questions:

(1) Is “irreversible damage” the same as “full thickness destruction”? [Need a toxicologist to answer that I expect]

(2) OECD 404 was first adopted in 1981, first revised in 1992, current edition 2002 (just prior to GHS First Ed. 2003). I suspect the RTDG text could be older than 1981 (can anyone confirm?) Which drove which?

(3) OECD 404’s 3 minute, 1 hour, 4 hour exposures seem to be driven by concerns for the humane treatment of animals. No argument with that aim. They do not seem to be used to grade hazard?

(4) The 3.2 text seems to be an unfortunate marriage of the other two – maybe a result of rushing the GHS text for the deadline?

Additional comments on Draft Document (12th April 2011) from AISE

We think the summary (Annex II) is a fair reflection of where we are and a good expression of how we should go forward. Specifically:

Work stream (a):

it does seem that investigation of whether the use of different terms does in fact lead to discrepancies between the criteria. This seems to have been identified as far back as the OECD paper (page 24) referenced in paragraph 7: “Current use of descriptors like full or whole thickness destruction may be somewhat of an overstatement, as tissue destruction need not include all of the dermis, only part of it. Likewise, visible necrosis or destruction is not very specific, as is irreversible or permanent injury. Attempts should be made to be more specific and use pathological terms where appropriate.”

We think the differences brought about, if any, by the presence or absence of observation periods needs to be resolved*. A toxicologist’s view should be sought?

Work stream b:

The proposed next step is difficult but necessary, though as put forward by the UK INF.12 in June 2009 it seems likely that extreme pH will not correlate with in vivo or in vitro test results. The positioning of extreme pH in Figure 3.2.1 in GHS 3.2 above such testing is somewhat perverse, especially as no assignment to PG in transport can be made from it.

Work stream c:

Following on from a*: the assignments made in the EU CLP Regulation Annex VI, based on Translation Table (Annex VII), ought to be reviewed? As previously noted subgroups make no difference to supply labelling, hazard statements, precautionary statements, pictograms, signal words in either GHS or CLP. Whether jurisdictions other than the EU have the same or similar assignments of subcategories, or none, we don't know.

The substances chosen for initial underlying data of classification investigation seem to be good choices.

Work stream d:

Agree this should be deferred – until the other work streams have been progressed.

Additional comments 2nd June 2011

Annex II paragraph 13: The limitation of TG435 in effect to acids and bases does not seem to us to be a severe drawback. Many of the important substances and mixtures are acids and bases and it is the use of "extreme pH" on these that produces a large number of controversial results;

We agree with the Belgian expert's view that extreme pH should be well down the order of consideration of data, and that the editorial revisions of 3.2 and 3.3 will be very important. We would advise that many mixtures are corrosive, not irritant.

Annex VI

Application of alternative methods in the regulatory assessment of chemical safety related to human skin corrosion & irritation: Current status and future prospects

(Document provided by the expert from Switzerland)

Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

Swiss Confederation

Federal Department of Home Affairs FDHA
Federal Office of Public Health FOPH

APPLICATION OF ALTERNATIVE METHODS IN THE REGULATORY ASSESSMENT OF CHEMICAL SAFETY RELATED TO HUMAN SKIN CORROSION & IRRITATION

CURRENT STATUS AND FUTURE PROSPECTS

Dr. Chantra Eskes

March 2010

The Orange House Partnership vzw
Kampendaal 83
1653 Dworp, Brussel

Sponsored by :
Swiss Federal Office of Public Health
Division of Chemical Products
Bern, Switzerland

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LIST OF ABBREVIATIONS

BfR	German Federal Institute for Risk Assessment
BfR-DSS	BfR Decision Support System
Cat.	Category
Cat. 1	Corrosive
Cat. 2	Irritant
Cat. 3	Mild irritant (optional)
CDS	Chemical Detection System
cm^2	Centimeter square
DMEM	Dulbecco's Modified Eagle'S Medium
DNELs	Derived No-Effect Levels
EC	European Communities
EC_{50}	Exposure time required to reduce cell viability by 50%
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ECHA	European Chemicals Agency
ECVAM	European Centre for the Validation of Alternative Methods
ESAC	ECVAM's Scientific Advisory Committee
EU	European Union
EU CLP	EU Regulation 1272/2008 on the Classification, Labelling and Packaging of Substances and Mixtures
EU DSD	EU Dangerous Substances Directive 67/548/EEC
EU DPD	EU Dangerous Preparation Directive 199/45/EC
g	Grams
GHS	Globally Harmonized System for Hazard Classification and Labeling
GLP	Good Laboratory Practices
h	hours
IC_{50}	Concentration at which a substance reduces the viability of the tissues by 50 % after a fixed exposure time
ICCVAM	US Interagency Coordinating Committee on Validation of Alternative Methods
ITS	Integrated Testing Strategies
M	Molar
min	Minutes
mg	Milligram
ml	Mililiter
MTT	3-(4,5-Dimethyl-2-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide, Thiazolyl blue; EINECS number 206-069-5, CAS number 298-93-1
N	Normal
NICEATM	US National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods
NSC	Non-Specific Colour
NSMTT	Non-Specific MTT reduction
NSS	Non-Specific Staining
OD	Optical Density
OECD	Organisation for Economic Co-operation and Development

(Q)SAR	(Quantitative) Structure-Activity Relationship
PBS	Phosphate Buffered Saline
PTFE	Polytetrafluoroethylene
R34	Causes burns
R35	Causes severe burns
R38	Irritating to skin
REACH	EU Regulation 1907/2006 on the Registration, Evaluation, Authorisation and restriction of Chemicals
RhE	Reconstructed human Epidermis
RT	Room Temperature
SD	Standard Deviation
SDS	Sodium Dodecyl Sulphate
SICRET	Skin Irritation Corrosion Rules Estimation Tool
SIFT	Skin Integrity Function Test
SIT	Skin Irritation Test
SIVS	ECVAM Skin Irritation Validation Study
SOP	Standard Operating Procedures
TER	Transcutaneous Electrical Resistance Test
TG	Test Guideline
UN	United Nations
US	United States
US DOT	US Department of Transportation
v/v	Volume/volume
w/v	Weight/volume
µl	Microliter
µg	Microgram

1 Regulatory Context in the European Union

1.1. Definitions

Dermal corrosion is generally defined within the regulatory context as “the production of irreversible damage of the skin; namely, visible necrosis through the epidermis and into the dermis, following the application of a test substance for up to 4 hours. Corrosive reactions are typified by ulcers, bleeding, bloody scabs, and, by the end of observation at 14 days, by discolouration due to blanching of the skin, complete areas of alopecia, and scars. Histopathology should be considered to evaluate questionable lesions.” (OECD, 2002; UN, 2003, 2009; EC, 2008a,b).

Dermal irritation is generally defined within the regulatory context as “the production of reversible damage of the skin following the application of a test substance for up to 4 hours” (OECD, 2002; UN, 2003, 2009; EC, 2008a,b).

1.2. Regulatory requirements for skin corrosion and irritation testing

The European Union (EU) chemicals policy 1907/2006 adopted in 2006 for the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) establishes standard information requirements that need be submitted for the registration and evaluation of chemicals. Such information requirements are specified in details in the REACH Annexes VI to XI (EC, 2006). According to Annex VI, the registrant should gather and evaluate all available information before considering further testing. These include physico-chemical properties, (Quantitative) Structure-Activity Relationship ((Q)SAR), grouping, *in vitro* data, animal studies, and human data (for details see chapter 1.6). Information on exposure, use and risk management measures should also be collected and evaluated. If these data are inadequate for hazard and risk assessment, further testing should be carried out in accordance with the requirements of REACH Annexes VII and VIII, which are based on the tonnage levels of the manufactured or imported chemicals.

The standard toxicological information requirements for substances manufactured or imported in quantities between one tonne and 10 tonnes are laid down in Annex VII. If new testing data are necessary, these must be derived from *in vitro* methods only. Annex VII does not foresee *in vivo* testing for skin corrosion and irritation. The standard information required at this tonnage level for skin corrosion and irritation can be satisfied by following four consecutive steps:

1) assessment of the available human and animal data,
2) assessment of the acid or alkaline reserve,
3) *in vitro* study for skin corrosion,
4) *in vitro* study for skin irritation.

Specific rules for adaptation are given that specify when steps 3 and 4 do not need to be conducted, which are:

- the available information indicates that the criteria are met for classification as corrosive to the skin or irritating to eyes, or
- the substance is flammable in air at room temperature, or
- the substance is classified as very toxic in contact with skin, or
- an acute toxicity study by the dermal route does not indicate skin irritation up to the limit dose level (2000 mg/kg body weight).

For substances manufactured or imported in quantities of ≥ 10 tonnes, the toxicological information requirements are laid down in Annex VIII. Such information is additional to that required in annex VII, and requires:

1) *in vivo* testing for skin irritation.

No *in vivo* testing is required for skin corrosion. Moreover, the following specific rules are given that specify when the *in vivo* study for skin irritation does not need to be conducted:

- the substance is classified as corrosive to the skin or as a skin irritant, or
- the substance is a strong acid (pH<2) or base (pH>11.5), or
- the substance is flammable in air at room temperature, or
- the substance is classified as very toxic in contact with skin, or
- an acute toxicity study by the dermal route does not indicate skin irritation up to the limit dose level (2000 mg/kg body weight).

Importantly, Annex VI also states that new tests on vertebrates shall only be conducted or proposed as a last resort when all other data sources have been exhausted. In particular it states that the *in vivo* testing requirement of Annex VIII can be adapted by the rules laid down in Annex XI allowing to avoid unnecessary animal testing. Annex XI establishes amongst others, the conditions in which the standard testing may not be scientifically necessary. *In vitro* test methods fall within this category. Annex XI states that "if the results obtained from the use of an *in vitro* methods do not indicate a certain dangerous properties," a confirmatory test according to annex VII To X "may be waived if the following conditions are met:

1. results are derived from an *in vitro* method whose scientific validity has been established by a validation study, according to internationally agreed principles
2. results are adequate for the purpose of classification and labelling and/or risk assessment, and,
3. adequate and reliable documentation of the applied method is provided."

In addition, it states that "results obtained from a suitable *in vitro* method" may be used to "indicate the presence of a certain dangerous property, or may be important in relation to a mechanistic understanding which may be important for the assessment". "Suitable" *in vitro* methods "means sufficiently well developed according to internationally agreed test development criteria (e.g., criteria from the European Centre for the Validation of Alternative Methods (ECVAM) for the entry of a test into the pre-validation process)". However, depending on the potential risk, immediate or proposed confirmation (based on the tonnage levels) may be necessary requiring tests beyond the information foreseen in Annexes VII to X.

In order to apply the information testing requirements as laid down in REACH, the European Chemicals Agency has issued an Endpoint Specific Guidance on the REACH Information Requirements and Chemical Safety Assessment (ECHA, 2008a). For skin corrosion and irritation, a testing strategy is proposed to be followed, including a step-wise approach that takes into consideration information from the physico-chemical properties of the test substance, existing human data, animal data, QSAR and *in vitro* data on the test material, a weight-of-evidence evaluation, the generation of new *in vitro* data and only as a last resort, the generation of new *in vivo* testing (for details see chapter 1.6).

1.3. The need for alternative test methods

At the EU level, not only REACH but also the cosmetics legislation has accelerated the need for alternative methods to toxicological testing. As mentioned above, within REACH, *in vitro* testing is required as standard information for substances marketed in volumes between 1 and 10 tonnes per year (EC, 2006). Such requirement could lead to testing of up to 20,000 existing chemicals using *in vitro* methods. Moreover REACH regulation whereas 1 and article 1 promote alternative methods for safety testing. Article 25 states that animal testing must be used as a last resort, which encourages the exploitation of useful alternative methods. Article 13 states, that information on hazards (regarding positive results) and risks may be generated by suitable alternative methods that have not yet been taken up as official regulatory test methods, upon the condition that such methods fulfil the requirements of Annex XI (e.g., ECVAM criteria for the entry of a test into the prevalidation process). If such methods are moreover validated, they may be used for identifying positives as well as negatives (EC, 2006).

The 7th amendment to the EU Cosmetics Directive (Directive 2003/15/EC) went further and prohibited animal testing of finished products from 2004 and of ingredients from 2009. The animal testing ban is reinforced by marketing bans of cosmetics tested on animals from 2004 (finished products), 2009 (acute effects) or 2013 (repeated-dose toxicity, toxicokinetics, reproductive toxicity; EC, 2003).
In addition, the European Union Directive 86/689 on animal protection also promotes the use of alternative methods. It states that "an experiment shall not be performed if another scientifically satisfactory method of obtaining the result sought, not entailing the use of an animal, is reasonably and practicably available" (EC, 1986).

As a consequence, there is a strong need for *in vitro* alternative methods to fulfil these current regulatory requirements within the European Union. In particular, the European Chemicals Agency has issued a guidance for the evaluation of available information for REACH, in which it states that there are two ways for using data from *in vitro* studies:

1) information from validated *in vitro* tests: may be used to determine whether a substance has or not dangerous properties, allowing to fully or partly replace an animal test. In that case one of the criteria for acceptance is the adequacy of the information generated using such test(s) for the purpose of classification and labelling and/or risk assessment,
2) information derived from suitable *in vitro* methods: can be used for determining the presence of a certain dangerous property, adapting the standard testing regime as set out in annex XI.

Finally, information from *in vitro* tests may be also used to provide with mechanistic insights (ECHA, 2008b). As such, the scientific validation of such *in vitro* methods certify their level of relevance and reliability to be used in the regulatory framework for detecting both positive and negative results, as full replacement, partial replacement, reduction or refinement of the animal testing.

The area of skin corrosion and irritation represents one of the most advanced areas for the validation of alternative test methods. Replacement alternatives have been validated and adopted in the regulation as early as 1998 and 2000. The present document describes in details the *in vitro* methods endorsed as scientific validated for regulatory use as replacement of the skin corrosion and skin irritation animal test.

1.4. Regulatory test methods and sequential testing strategies

The traditional methods recommended in Europe for assessing *in vivo* acute dermal corrosion and irritation used to be the EU test method B.4 which is actually equivalent to the Test Guideline (TG) 404 from the Organisation for Economic Co-operation and Development (OECD TG 404, 2002; EC, 2008a).

However, for skin corrosion, validated alternative methods that allow replacement of the traditional *in vivo* test for skin corrosion have been adopted by the EU as the test method B.40 and B.40bis since 2000, and by the OECD as the TG 430, 431 and 435 since 2004 (EC, 2000; OECD, 2004, 2006).

In addition, more recently alternative test methods that allow full replacement of the classical Draize test for predicting skin irritation have also been officially validated and adopted in the EU as the test method B.46, and were proposed as a OECD draft test guideline (ESAC, 2007; EC, 2009).

As a consequence, *in vivo* test methods for the assessment of acute dermal corrosion and irritation shall no longer be used in the European Union. A summary of the validated and adopted alternatives for skin corrosion and skin irritation are given in table 1.

Table 1. Validated and adopted *in vitro* methods for skin corrosion and skin irritation, their purposes, status, application and limitations

Purpose	Test Method	Status	Application and Limitations
Identification of skin corrosives *Positive results lead to skin corrosion classification.* *Negative results lead to no classification as corrosive*	Reconstructed human Epidermis models - EPISKIN™ - EpiDerm™ - SkinEthic™ - EST-1000	Validated and adopted (EU B.40bis, OECD TG 431)	Applicable to chemicals and mixtures. Allows identification of corrosives (GHS cat. 1), and EPISKIN™ able to distinguish the two categories R35 and R34. An EPISKIN™ prediction model also exists for the three UN packaging groups (similar to the GHS subcategories 1A, 1B, 1C) but its validity has not been evaluated in specific. Other models not able to distinguish the three GHS subcategories. Method not compatible with highly volatile substances, however possible to test volatile chemicals on separate plates. Not designed to provide information on skin irritation. Method may be incompatible to test materials presenting non-specific interaction with MTT greater than 30% of negative control
	Transcutaneous Electrical resistance (TER) test	Validated and adopted (EU B.40, OECD TG 430)	Applicable to chemicals and mixtures. Allows identification of corrosives (GHS cat. 1). Not able to distinguish the three GHS subcategories (1A, 1B and 1C). Not designed to provide information on skin irritation.
	Membrane barrier test - Corrositex®	Validated and adopted by OECD (OECD TG 435)	Applicable to chemicals and mixtures. Allows identification of corrosives (GHS cat. 1) and sub-categorisation into the three GHS subcategories (1A, 1B and 1C). In EU, method not adopted in legislation but considered valid for acids, bases and their derivatives. Test materials not causing detectable changes in the chemical detection system cannot be tested. Not designed to provide information on skin irritation.
Identification of skin irritants *Positive results lead to skin irritation classification.* *In EU, negative results lead to no classification.*	Reconstructed human Epidermis models - EPISKIN™ Skin Irritation Test (SIT) - EpiDerm™ EPI-300-SIT - SkinEthic™ SIT^{42bis}	Validated and adopted in EU (EU B.46, draft OECD TG)	Classify skin irritants according to GHS Cat. 2. In the EU, where non-category 2 are considered non-classified, the method is considered as a skin irritation replacement test. It allows hazard identification of irritant substances and non-classified substances. Applicable to mono-constituent substances (in draft OECD TG proposed also for mixtures). The method is not designed to distinguish: the optional GHS cat. 3 for mild irritants, corrosive substances. Not applicable to gases and aerosols. In addition, the method may be incompatible to test materials presenting non-specific interaction with MTT greater than 30% of negative control

At the OECD level, the *in vivo* acute dermal irritation / corrosion test guideline 404 could still be used, although a revision of such guideline is currently planned. However, before the *in vivo* test is undertaken, a sequential testing strategy should be followed as recommended in the OECD TG 404 (2002). This strategy is based in a stepwise order on: a weight of evidence analysis, pH considerations, the use of validated and accepted *in vitro* tests, and finally, the refinement of the animal testing (see figure 1). The following are some examples of decision-making according to the testing strategy:

1) A substance with a pH below 2.0 or above 11.5 should not be tested, due to its suspected corrosivity.
2) A substance found to be corrosive in one of the validated and accepted alternative corrosivity tests (i.e., OECD TG 430, 431 and 435) should not be tested in the Draize test.
3) A substance found to be irritant in one of the validated and accepted alternative irritation tests (i.e., EU B.46 and OECD, under discussions) should not be tested in the Draize test.

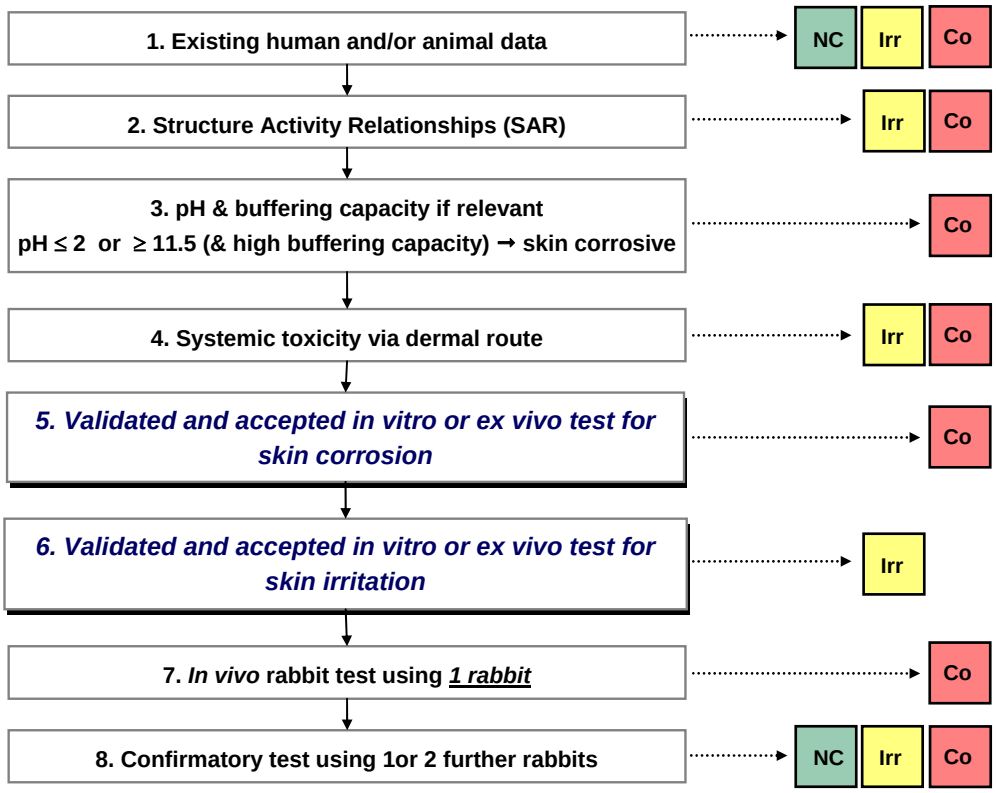

Figure 1. Summary steps of the testing strategy as recommended in OECD TG 404 (2002 adapted). NC: Non Classified; Irr: Irritating to skin; Co: Corrosive to skin

The United Nations Globally Harmonized System for classification and labeling proposed a strategy which is similar to the one proposed by the OECD TG 404 (UN, 2003, 2009; OECD, 2002), with the only difference that a human test is suggested as the last step after the *in vivo* test, if the test material has been shown to be non irritant and non corrosive.

Since then, additional sequential testing strategies have been proposed for the testing of skin irritation and/or corrosion for specific applications. For example, in the Endpoint Specific Guidance to the REACH Regulation, a sequential strategy has been proposed for skin irritation and/or corrosion as summarized in figure 3 (ECHA, 2008a). If the building blocks are similar to the ones recommended in the OECD TG 404, this test strategy introduces some new elements:

- the use of physico-chemical properties,
- the use of existing *in vitro* data,
- the use of weight-of-evidence analysis of all existing and relevant data,
- and the use of validated and accepted *in vitro* methods for the identification of non irritants in addition to the identification of irritants and corrosives, so that the *in vivo* test might be avoided.

That strategy also foresees the use of non-validated *in vitro* methods for the identification of irritants with eventual confirmation depending on potential risk as defined in annex XI of REACH (EC, 2006). However since then, validated *in vitro* methods have been adopted as full replacements in the EU. As the validated and adopted assays should be used formerly to the non-validated assays, the use of non-validated methods might be unnecessary.

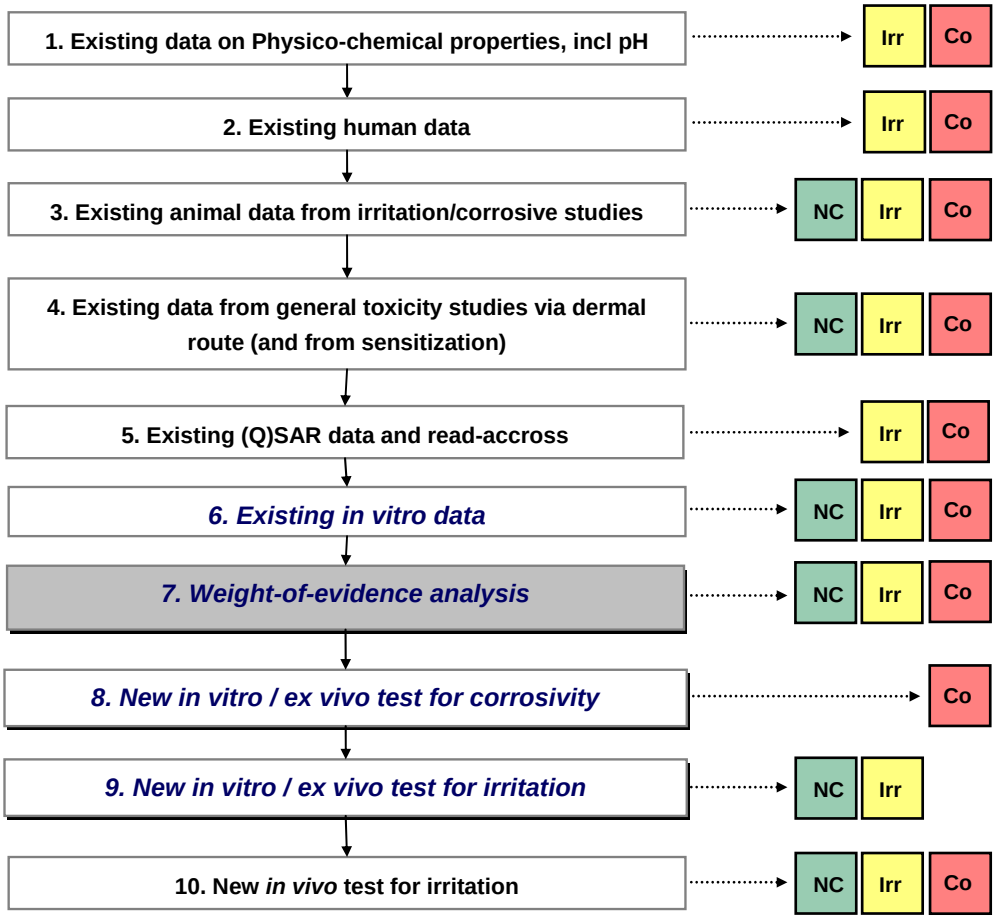

Figure 3. Summary steps of the testing strategy as recommended in Endpoint Specific Guidance to the REACH Regulation (ECHA, 2008a adapted).
NC: Non Classified; Irr: Irritating to skin; Co: Corrosive to skin

Louekari and co-workers (2006) have evaluated four test substances as case studies to assess the applicability of a testing strategy similar to the proposed strategy as depicted in figure 3. The results support the relevance of incorporating weigh-of-evidence approaches as part of the sequential testing strategies. A further integrated testing strategy has been proposed for skin corrosion and irritation in the framework of REACH by Grandon and co-authors (2008). In this test strategy the authors exclude the *in vivo* testing as the last step, and use *in vitro* test methods instead as the last step in the integrated testing strategy.

Specific test strategies have also been proposed for the hazard and risk assessment of cosmetic ingredients as described by Macfarlane and co-authors (2009). Here again the use of weight-of-evidence analysis is proposed to evaluate all available data such as physicochemical properties, literature, animal, *in vitro*, human, read-across, SAR. Such evaluation is then followed by an *in vitro* test for skin corrosion and an *in vitro* test for skin irritation. No *in vivo* and human testing are used for hazard assessment. However, human testing is proposed for risk assessment. Furthermore, Robinson and co-workers (2002) have showed in 2002 the general testing strategies implemented within industry to assess skin corrosion and irritation of ingredients and finished products without the need to test in animals.

A feasibility study was carried out by Hoffmann *et al.* (2008) for developing integrated testing strategies (ITS) for the assessment of skin irritation hazard based on a combination of *in silico*, *in vitro* and *in vivo* information. The authors showed that the best performing animal-free test strategy was found to be a combination of TOPKAT, BfR-Decision Support System and the EPISKIN™ *in vitro* model. However such combination resulted in predictive capacity values almost identical as the

EPISKIN™ *in vitro* model as a stand-alone test. The difference in costs was also considered marginal by the authors since the number of chemicals to be tested in EPISKIN™ was reduced only by eight when taking into account the expert system information. The authors also discussed the complexity of systematic construction of ITS, and recommended that further investigation is carried out to explore optimal combinations of methods within ITS, and that further guidance is developed on construction and multi-parameter evaluation in order to facilitate and promote ITS (Hoffmann *et al.*, 2008).

1.5. Classification systems: towards the Globally Harmonized System (GHS)

At the level of the United Nations (UN), a Globally Harmonised System (GHS) for classification and labelling has been proposed since 2003 to be applied by its member countries (UN, 2003, 2009).

At the EU level, the classification and labelling system used has been defined in the past by 1) for substances, the Dangerous Substances Directive 67/548/EEC (EU DSD; EC, 2001), and 2) for mixtures, by the Dangerous Preparation Directive 199/45/EC (EU DPD; EC, 1999). Since 2008 however, the UN GHS classification and labelling system has been introduced so that the EU DSD and EU DPD classification system is being progressively replaced by the novel classification system according to new Regulation 1272/2008 on the Classification, Labelling and Packaging of Substances and Mixtures (EU CLP; EC, 2008b). The progressive implementation of the EU CLP will be carried out as described below and summarized in figure 2.

Until 1 December 2010

Substances and mixtures shall be classified, labelled and packaged in accordance with EU DSD and EU DPD, respectively. They may also be classified, labelled and packaged in accordance with EU CLP. In that case they shall not be labelled and packaged according to EU DSD or EU DPD. When a substance or mixture is classified, labelled and packaged according to EU CLP the classification information according to both systems shall be provided in the Safety Data Sheet.

From 1 December 2010 to 1 June 2015

Substances shall be classified, labelled and packaged in accordance with EU CLP, but also classified in accordance with EU DSD in order to allow these classifications to be used in the classifications of mixtures. Classifications in accordance with both systems shall be included in Safety Data Sheet, but classifications in accordance with EU DSD shall not appear on the label. Mixtures shall be classified, labelled and packaged in accordance with EU DPD. They may also be classified, labelled and packaged in accordance with EU CLP. In that case they shall not be labelled and packaged according to EU DPD. When a mixture is classified, labelled and packaged according to EU CLP the classification information according to both systems shall be provided in Safety Data Sheet.

From 1 June 2015

Both substances and mixtures shall be classified, labelled and packaged in accordance with EU CLP. EU DSD and EU DPD are repealed from 1 June 2015 and classification according to these directives is not allowed. However, substances classified, labelled and packaged in accordance with EU DSD and already placed on the market (“on the shelves”) before 1 December 2010, and mixtures classified, labelled and packaged in accordance with EU DPD and already placed on the market (“on the shelves”) before 1 June 2015, do not have to be relabelled and repackaged in accordance with EU CLP until 1 December 2012 and 1 June 2017, respectively.

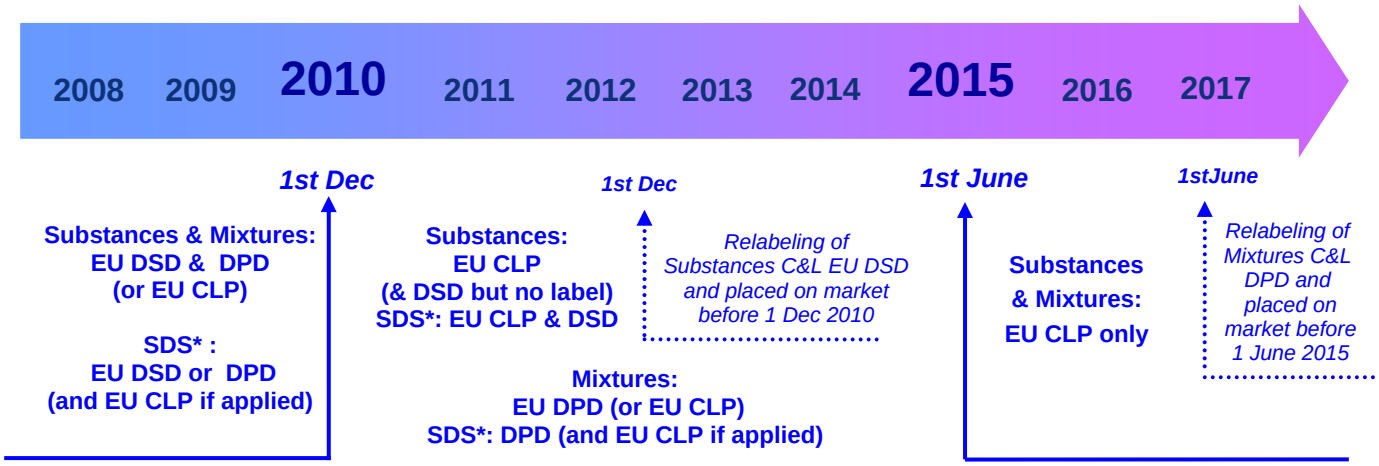

Figure 2. Schematic view of the progressive implementation in the EU of the new EU CLP classification system, and phasing out of the EU DSD and DPD. *SDS: Safety Data Sheet

The *in vivo* observations used for classifying substances for skin corrosion / irritation according to the UN GHS, EU DSD and EU CLP are given in chapter 3.

2 *In vivo* Acute Dermal Corrosion and Irritation test

Dermal irritation and corrosion were originally evaluated by the method described by John Draize and his colleagues in 1944 (Draize *et al*., 1944). In the United States, the Federal Hazardous Substance Act which deals with agricultural and industrial chemicals, adopted a modification of the Draize using 6 animals. In this method the material was tested for 24 hours on two sites on the same animal, one with intact skin and another in which the skin was abraded by using the tip of a needle drawn across the skin repeatedly, so that the *stratum corneum* was opened but no bleeding was produced; and the removal of test material was not specified. The reproducibility of this procedure and the relevance to human experience have been questioned so that numerous modifications have been proposed including changing the species tested, reducing the exposure period, using fewer animals, and testing on intact skin only (for review see Patrick and Maibach, 1994).

Althought vesiculation, ulceration and severe eschar formations were not included in the original Draize scoring scales, the Draize test has been used to evaluate corrosion as well as irritation. When severe reactions that may not be reversible were noted, test sites were observed for a longer period, usually made on days 7 and 14. Further modifications were made to the basic exposure procedures for skin irritation and corrosion of OECD guidelines in order to test for corrosion during shorter periods as in the first OECD test guideline 404 adopted in 1981 (Patrick and Maibach, 1994). Under a directive of the European Community, a 3-minutes exposure time was added, and the United Nations recommendations for transport of dangerous goods were based on exposure times of 4 hours, 1 hour, and 3 minutes (UN, 2001). Finally, more recently, the OECD TG 404 has been modified to include for refinement and/or reduction tiered testing strategies including the use of validated and adopted *in vitro* test methods (OECD, 2002). Today, the OECD TG 404 method for skin corrosion and irritation makes use of 3 animals, with albino rabbit as the preferred species. The test material is applied for 3 minutes, 1 hour and 4hours in a sequential way and the animals are observed for 14 days or until reversibility is seen as described in chapter 2.2.

2.1. Mechanisms of skin irritation and corrosion

The human skin is divided in three distinct regions: the epidermis as the outer region, the dermis and the deeper localized subcutis. The epidermis represents 5% of the full thickness of the skin, and is subdivided into 5 to 6 layers based on cellular characteristics (see figure 4). The outer layer represents the *stratum corneum*, whereas the inner layer represents the *stratum basale*, subdivided into the basal layer (outer part) and the basal lamina (inner part).

The epidermis layers are formed by keratinocytes having ordered differentiation of cells from the basal layer keratinocytes which are metabolically active and have the capacity to divide. Some daughter cells of the basal layer move upward and differentiate. The outermost layer, the *stratum corneum* consists of cornified keratinocytes that have elongated and flattened with respect to the basal layer keratinocytes, and have lost their nucleus and all capacity for metabolic activity. The dominant constituent of these cells is keratin. In addition to keratinocytes, the epidermis contains two dendritic cell types, melanocytes and Langerhans cells. Melanocytes produce melanin, the principal pigment of human skin, whereas Langherans cells express Ia (immune recognition) antigen and receptors of IgG and C3 on their surface (for review see Patrick and Maibach, 1994).

The *stratum corneum* represents an effective barrier against a vast number of substances. Apart this, keratinocytes play crucial roles in the immune surveillance of the epidermis, as after stimulation they can trigger inflammatory responses (for review see Welss *et al.*, 2004).

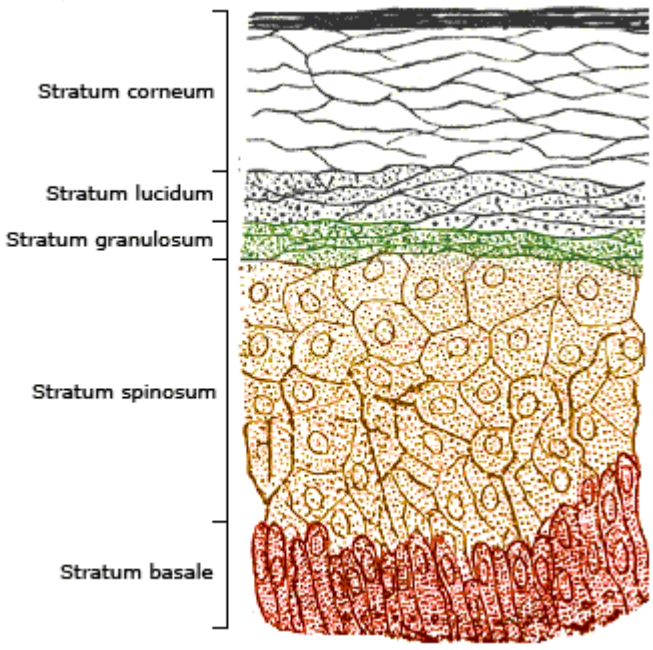

Figure 4. The epidermis cell layers

If skin corrosion assesses the potential of a substance to cause visible necrosis through the epidermis and into the dermis that may lead to irreversible damage to skin, acute skin irritation is characterised by the local and reversible non-immunological inflammatory response of the living skin.

Chemical-induced skin irritation manifested by erythema and oedema, is the result of a cascade of events beginning with penetration of the *stratum corneum* and damage to the underlying layers of keratinocytes. The dying keratinocytes release mediators that begin the inflammatory cascade which acts on the cells in the dermis, particularly the stromal and endothelial cells, It is the dilation and increased permeability of the endothelial cells that produce the observed erythema and oedema. However, the underlying mechanisms of skin irritation, linked to the molecular and cellular responses, are still poorly understood. Probably different pathways may be involved, such as damage to the barrier function of the *stratum corneum*, and the direct effects of irritants on cells of the skin (for review see Welss *et al.*, 2004).

2.2. Method description according to OECD TG 404

The procedure is described in detail in the OECD TG 404 (2002). The principal steps of the *in vivo* testing are described here below.

a) Animals used
Albino rabbit is the preferable laboratory animal.

b) Dose and application of the test substance
A dose of 0.5 ml of liquid or 0.5 g of solid or paste is applied.

The test substance is applied to a small area (approximately 6 cm^2) of skin and covered with a gauze patch, which is held in place with non-irritating tape.

Liquids are generally tested undiluted. Solids should be moistened with water to ensure good skin contact. When vehicles other than water are used, the potential influence of the vehicle on irritation of the skin by the test substance should be minimal, if any.

c) Sequential testing
An initial test using one animal is recommended. If no corrosive effects are observed but an irritant effect is observed, a confirmatory test using additional one or two animals may be conducted in a sequential manner. In any case an irritant or negative response in the initial test should be confirmed using up to two animals.

d) Exposure time
If a substance is not expected to produce corrosion but may be irritating, a single patch should be applied to one animal for four hours, and then proceed the confirmatory testing.

In the past, if a substance was suspected of being corrosive but testing was warranted because of insufficient evidence, up to three patches were applied sequentially to the animal. The first patch was removed after three minutes, if no serious reactions were observed, a second patch was applied at a different site and removed after one hour. If observations indicated that exposure could humanely be allowed, a third patch was applied and removed after four hours.

e) Observation period
If no corrosive effects are observed after patch removal, the animal is observed for 14 days or until reversibility is seen. If corrosive effect were observed, the test should be immediately terminated.

f) Grading of skin reactions
Animals are examined for signs of erythema and oedema and the responses are scored at 60 min, and then at 24h, 48h and 72h after patch removal as described in Table 2 (OECD, 2002). The dermal irritation scores are to be evaluated in conjunction with the nature and severity of lesions and their reversibility or lack of reversibility. Examples of other observations which can be made from the study include:
- All local toxic effects such as defatting of the skin
- Any systematic adverse effects such as effects on clinical signs of toxicity and body weight
- Persistence of responses such as alopecia (limited area), hyperkeratosis, hyperplasia and scaling (in this case the substance should be considered an irritant.
Finally, histopathological examination may be carried out to clarify equivocal responses.

Table 2: Grading of skin reactions according to OECD TG 404 (2002)

Erythema and Eschar Formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness) to eschar formation preventing grading of erythema	4
Maximum possible: 4	

Oedema Formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well defined by definite raising)	2
Moderate oedema (raised approximately 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4
Maximum possible: 4	

2.3. Limitations of the OECD TG 404 (Draize rabbit test)

The Draize rabbit test for skin corrosion and irritation was originally developed and included in the guidelines with the purpose to identify chemicals that posed a severe hazard to the public. The test

has been used for about half a century since its introduction in 1944 (Draize *et al*., 1944), and provided value in warning consumers, workers, and manufacturers of potential dangers associated with specific chemicals so that appropriate precautions could be taken. The Draize rabbit test was however not originally developed to compare products. In addition, at that time the current scientific standards required for the validation and evaluation of test methods such as those outlined in the OECD Guidance Document 34 since did not exist (OECD, 2005). Perhaps because of this, the Draize rabbit skin corrosion and irritation test has often been a target for criticism due to the several drawbacks it may present as described below.

It is generally recognised that the Draize method has erred on the safe of safety in that it overpredicts the severity of skin damage produced by chemicals. York and co-workers (1994) have shown that over 50% (8 out of 15) materials classified as irritants or corrosives based on the Draize rabbit test, did not show effects on humans using the human patch test, suggesting that the Draize test overpredicts the effects on human. Similarly, Robinson and co-workers (2000) have showed the case study of a substance classified as corrosive with the *in vivo* skin corrosion and irritation test, whereas the *in vitro* and human studies showed no effects or irritation. Furthermore, Hoffmann and co-workers (2005) have shown that the practical use of the European classification system seems to introduce a bias by itself towards overclassification of those chemicals having Draize scores close to but below the threshold for assigning skin irritation classification.

Scientific concerns about the variability of the Draize rabbit acute skin irritation and corrosion have also been raised (Worth and Cronin, 2001a; Weil and Scala, 1971). In particular, Weil and Scala (1971) have shown that considerable variation existed between laboratories. The irritation scores given by the participating laboratories were shown to vary from the lowest non-irritation extreme to the most severe irritation/corrosion extreme in 3 out of 10 tested materials (Figure 5). Moreover the authors found that some laboratories consistently rated materials more irritating while other laboratories just as consistently rated the same materials less irritating than the majority of the 30 participating laboratories. However, an old protocol of the Draize skin irritation test was used with 24 hours exposure to the test material, and it is also not clear whether all laboratories have applied the same test protocol or variants were used (Weil and Scala, 1971). On the other hand, Hoffmann and co-workers (2005) have evaluated the *in vivo* skin irritation data for around 3000 chemicals registered in the 'New Chemicals Database' of the EC European Chemicals Bureau as notified from the 80's which made use of a more recent version of the OECD TG 404. The authors have shown that the within-test variability of Draize skin irritation test rarely resulted in misclassification. However, some principle aspects of within- and between-laboratory variability could not be assessed (Hoffmann *et al.*, 2005). Finally, more recently, the Explanatory Background Document to the OECD Draft test guideline on *in vitro* skin irritation testing show that high variability was also evident in the database from the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) on chemicals containing high quality data for skin irritation produced with the standardised OECD TG 404 and following Good Laboratory Practices (GLP; see figure 6). The authors suggest that variations may be due to subjectivity in scoring or to the intrinsic variability of responses in animals, or to both factors (Griesinger *et al*., 2009).

Another criticism to the *in vivo* skin irritation and corrosion testing is that the adverse skin responses associated with repetitive, low-dose exposure to industrial chemicals and consumer products are not predicted accurately by the current regulatory assays (Patrick and Maibach, 1994). Indeed, chemically induced skin irritation can be divided into acute, cumulative and delayed acute skin irritation, where the cumulative type is the most common skin irritation and arises after repetitive exposure to mild irritants. It often occurs in humans who do repetitive wet work and subsequently is often a cause of occupational skin disease (Welss *et al.*, 2004).

Finally, from an ethical point of view, testing for skin corrosion and irritation in laboratory animals has the potential to cause them considerable discomfort or pain. For these reasons, several alternative methods for trying to identify skin corrosives and irritants have been developed, validated and accepted for regulatory use.

MINIMUM-MAXIMUM SCORE FOR REFERENCE PROCEDURE SKIN TEST—INDIVIDUAL RABBITS

Laboratory No.*	Material										Any material
	E	F	G	I	J	K	L	M	N	O	
12	0–4	21–46	2–8	1–10	5–13	7–14	41–46	12–46	4–12	6–12	0–46
14	3–10	5–22	2–7	1–5	1–22	11–16	17–28	3–46	4–10	2–8	1–46
11	5–7	6–44	0–8	0–5	6–25	6–11	12–46	8–29	0–6	3–9	0–46
13	2–19	20–46	2–16	2–30	3–28	11–23	22–36	0–4	All 0	0–9	0–46
10	0–4	23–31	1–5	2–6	4–12	6–13	20–42	2–34	0–24	0–5	0–42
25	2–9	4–44	0–3	0–3	5–23	4–9	30–42	2–35	0–6	1–5	0–44
31	0–8	31–33	0–20	0–6	1–22	6–32	All 29	0–32	All 0	0–4	0–33
23	0–4	7–30	0–3	2 15	6 25	24 27	14 31	0 24	0 1	0 3	0 30
26	1–8	6–13	1–8	2–6	1–3	7–11	6–11	3–10	2–9	2–7	1–13
5	5–7	6–11	0–4	0–1	5–9	5–9	9–12	3–5	1–4	1–4	0–12
9	1–4	6–16	2–8	0–2	7–13	9–12	15–16	0–12	All 0	0–5	0–16
2	4–9	21–38	0–1	All 0	3–7	4–8	17–21	0–25	0–3	0–2	0–38
19	3–8	6–10	1–5	1–4	2–6	4–8	10–26	1–4	0–3	0–2	0–26
24	1–4	5–9	0–2	0–3	4–8	4–7	8–11	0–4	0–3	1–4	0–11
22	0–2	8–32	0–2	1–3	4–15	9–25	25–32	0–1	0–1	0–1	0–32
7	2–5	2–6	All 0	2–19	0–21	1–20	1–21	0–2	0–2	0–2	0–21
8	2–7	2–13	0–3	0–4	4–7	4–6	3–11	0–4	All 0	0–2	0–13
4	0–3	3–6	0–1	1–3	2–10	4–9	10–13	1–4	0–1	All 0	0–13
18	0 1	0–6	All 0	All 0	0–5	2–9	9–19	0–18	0–3	0–7	0–19
30	0–2	1–5	0–1	0–9	3–7	4–8	6–13	0–2	0–1	1–3	0–13
27	0–1	5–12	0–1	0–2	0–9	0–8	8–12	0–4	All 0	0–3	0–12
21	All 0	All 0	All 0	All 0	All 0	All 0	All 0	0–6	All 0	All 0	0–6
Any laboratory	0–19	0–46	0–20	0–30	0–28	0–32	0–46	0–46	0–12	0–12	—

* Laboratories ordered by sum of ranks for primary irritation as in Table 47.

Figure 5. Extract from Weil and Scala (1971). Variability between-laboratories on the observed skin corrosion and irritation scores for individual rabbits (minimum score=0, maximum score = 46).

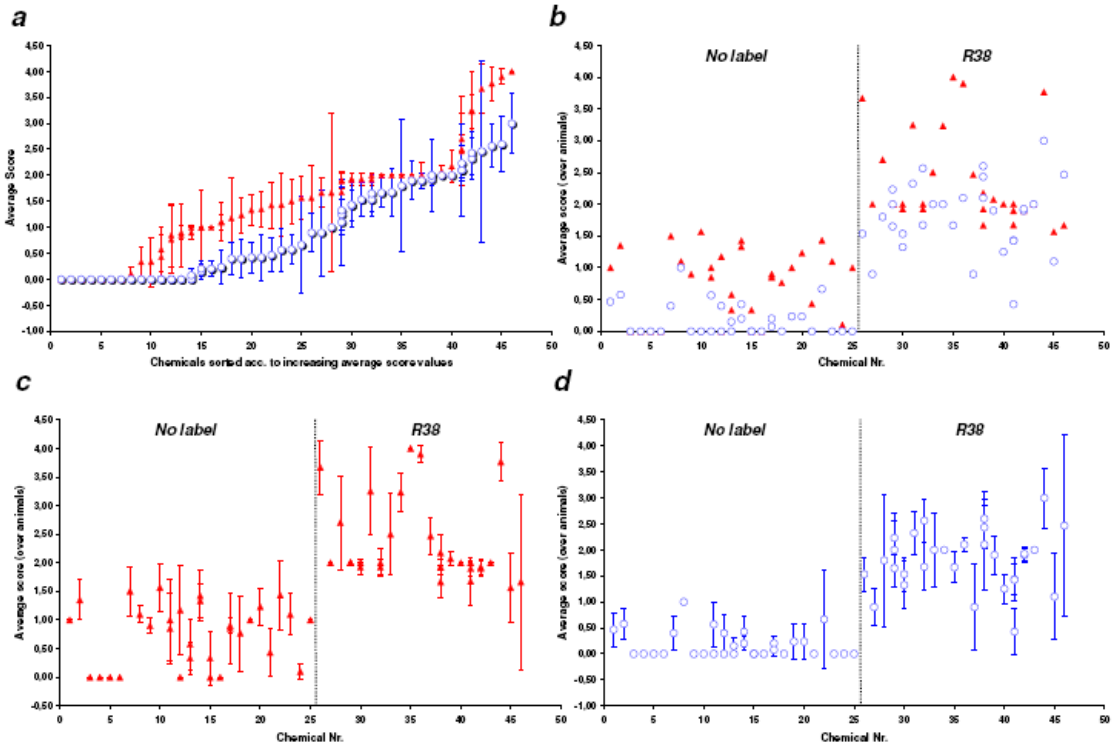

Figure 6. Extract from the Explanatory Background Document to the OECD Draft test guideline on *in vitro* skin irritation testing (Griesinger *et al*., 2009). Erythema (red triangles) and Edema (blue circles) irritancy scores of 45 chemicals from the ECETOC skin irritancy dataset produced in agreement with OECD TG 404 and under GLP. (a) Averages of animal scores ± Standard Devidation (SD) of test results. (b-c) Scatter plots of the averages of animal scores with chemicals plotted according to the number attributed in the ECETOC report. Considerable variability is observed in particular of edema test results (circles) for irritant (R38). The stippled line separates label from R38 substances. Average erythema and edema scores without SD (b), average erythema (c) and edema (d) scores across animals (± SD).

3 Classification for Skin Corrosion & Irritation based on the Draize data

The *in vivo* observations used for classifying substances for skin corrosion / irritation according to the UN GHS, EU DSD and EU CLP are described below. The Swiss legislation for the protection against dangerous substances and preparations n. 813.11 (CFS, 2009) when introducing the GHS classification system according to UN (2003, 2009) recalls the EU CLP Regulation (EC, 2008b), both explained here below.

These classification systems apply to the *in vivo* Draize rabbit test which is to be considered within the framework of sequential testing strategies as recommended by the UN, OECD and EU guidelines (see chapter 1.6). Moreover, in the EU the *in vivo* test may no longer be needed due to the available of full replacement alternatives (see chapter 1.2). Details for the EU DPD are not shown here since it is not based on classifiable *in vivo* observed effects. For details on the EU DPD, please refer to EC (1999).

3.1. The UN Globally Harmonised System (GHS) for classification & labelling (UN, 2003, 2009)

Skin Corrosion

A single harmonized corrosion category is provided by the UN GHS system based on the results of animal testing (Category 1). However, for those authorities wanting more than one designation for corrosivity, up to three subcategories are provided within the corrosive category (Category 1, see Table 3): subcategory 1A, where responses are noted following up to 3 minutes exposure and up to 1 hour observation; subcategory 1B, where responses are described following exposure between 3 minutes and 1 hour and observations up to 14 days; and subcategory 1C - where responses occur after exposures between 1 hour and 4 hours and observations up to 14 days.

Table 3. UN GHS Skin Corrosive category and subcategories

Category 1: Corrosive	Corrosive subcategories	Corrosive in > 1 of 3 animals	
(applies to authorities not using subcategories)	(only applies to some authorities)	Exposure	Observation
Corrosive	1A	≤ 3 minutes	≤ 1 hour
	1B	> 3 minutes -- ≤ 1 hour	≤ 14 days
	1C	> 1 hour -- ≤ 4 hours	≤ 14 days

Skin Irritation

A single irritant category is provided by the UN GHS system (Category 2, see table 4). However, an additional mild irritant category is available for those authorities wanting to have more than one skin irritant category. The major criterion for the irritant category is that at least 2 of 3 tested animals have a mean score for either erythema/eschar or oedema of > 2.3 (Table 4). For the optional mild irritant category, the mean score cut-off values are > 1.5 and < 2.3 for at least 2 tested animals.

In addition to severity of effects, reversibility of skin lesions is another consideration in evaluating irritant responses. When inflammation persists to the end of the observation period in 2 or more test animals, taking into consideration alopecia (limited area), hyperkeratosis, hyperplasia and scaling, then a material shall be considered to be an irritant.

Animal irritant responses within a test can be quite variable, as they are with corrosion. A separate irritant criterion accommodates cases when there is a significant irritant response but less than the mean score criterion for a positive test. For example, a test material might be designated as an irritant

if at least 1 of 3 tested animals shows a very elevated mean score throughout the study, including lesions persisting at the end of an observation period of normally 14 days. Other responses could also fulfil this criterion. However, it should be ascertained that the responses are the result of chemical exposure.

Table 4. UN GHS Skin irritation categories

Categories	**Criteria**
Irritant (Category 2) (applies to all authorities)	(1) Mean value of ≥ 2,3 ≤ 4,0 for erythema/eschar or for oedema in at least 2 of 3 tested animals from gradings at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions; or (2) Inflammation that persists to the end of the observation period normally 14 days in at least 2 animals, particularly taking into account alopecia (limited area), hyperkeratosis, hyperplasia, and scaling; or (3) In some cases where there is pronounced variability of response among animals, with very definite positive effects related to chemical exposure in a single animal but less than the criteria above.
Mild irritant (Category 3) (applies to only some authorities)	Mean value of > 1.5 < 2.3 for erythema/eschar or for oedema from gradings in at least 2 of 3 tested animals from grades at 24, 48 and 72 hours or, if reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions (when not included in the irritant category above).

3.2. The new EU CLP classification system (EC, 2008b)

Skin corrosion

The EU CLP is equivalent to the UN GHS as shown in Table 3, and makes use of the three subcategories within the corrosive category (1A, 1B and 1C).

Skin irritation

The EU CLP is equivalent to the UN GHS as shown in Table 4, but makes use of a single category (Category 2). The optional mild irritant category 3 is not required. Substances falling in the UN GHS category 3, would require No Classification under the EU CLP.

3.3. The EU DSD classification system (EC, 2001)

Skin Corrosion

According to the EU DSD, a substance or preparation shall be classified as corrosive in accordance with the following criteria (EC, 2001):

- a substance or a preparation is considered to be corrosive if, when it is applied to healthy intact animal skin, it produces full thickness destruction of skin tissue on at least one animal during the test for skin irritation cited in Annex V or during an equivalent method (equivalent to OECD TG 404)
- classification can be based on the results of a validated *in vitro* test, such as that cited in Annex V (B.40. Skin corrosion: rat skin transcutaneous electrical resistance assay and human skin model assay),
- a substance or a preparation should also be considered corrosive if the result can be predicted, for example from strongly acid or alkaline reactions indicated by a pH of 2 or less or 11,5 or greater. However, where extreme pH is the basis for classification, acid/alkali reserve may also be taken into consideration. If consideration of alkali/acid reserve suggests the substance or preparation may not

be corrosive then further testing should be carried out to confirm this, preferably by use of an appropriate validated *in vitro* test. Consideration of acid/alkali reserve should not be used alone to exonerate substances or preparations from classification as corrosive.

Risk phrases are assigned in accordance with the following criteria:

R35 Causes severe burns
- if, when applied to healthy intact animal skin, full thickness destruction of skin tissue occurs as a result of *up to three minutes exposure*, or if this result can be predicted.

R34 Causes burns
- if, when applied to healthy intact animal skin, full thickness destruction of skin tissue occurs as a result of *up to four hours exposure*, or if this result can be predicted,
- organic hydroperoxides, except where evidence to the contrary is available.

Notes: Where classification is based on results of a validated *in vitro* test R35 or R34 should be applied according to the capacity of the test method to discriminate between these. Where classification is based upon consideration of extreme pH alone, R35 should be applied.

Skin irritation
Substances and preparations shall be classified as irritant and assigned the following risk phrase in accordance with the criteria given below.

R38 Irritating to skin
- Substances and preparations which cause significant inflammation of the skin which persists for at least 24 hours after an exposure period of up to four hours determined on the rabbit according to the cutaneous irritation test method B.4.

  Inflammation of the skin is significant if:
  (a) the mean value of the scores for either erythema and eschar formation or oedema formation, calculated over all the animals tested, is 2 or more; or
  (b) in the case where the *in vivo* test has been completed using three animals, either erythema and eschar formation or oedema formation equivalent to a mean value of 2 or more calculated for each animal separately has been observed in two or more animals.

  In both cases all scores at each of the reading times (24, 48 and 72 hours) for an effect should be used in calculating respective mean values.
  Inflammation of the skin is also significant if it persists in at least two animals at the end of the observation time. Particular effects e.g. hyperplasia, scaling, discoloration, fissures, scabs and alopecia should be taken into account.
  Relevant data may also be available from non-acute animal. These are considered significant if the effects seen are comparable to those described above.

- Substances and preparations which cause significant inflammation of the skin, based on practical observations in humans on immediate, prolonged or repeated contact.

- Organic peroxides, except where evidence to the contrary is available.

3.4. Comparison of classification systems

Skin Corrosion
In principle, the *in vivo* testing for skin corrosion shall no longer be used due to the availability of replacement *in vitro* tests. However, table 5 is shown for comparison between the UN GHS, EU CLP and EU DSD classification systems based on the traditional *in vivo* effects (UN, 2003, 2009; EC, 2001, 2008b).

Table 5: Corrosion classification based in the traditional *in vivo* test according to the EU DSD, EU CLP and UN GHS classification systems.

EU DSD*	**UN GHS*** & EU CLP***	Corrosive in > 1 of 3 animals
R35*	**Cat. 1A**	≤ 3 minutes
R34**	**Cat. 1B**	> 3 minutes -- ≤ 1 hour
	Cat. 1C	> 1 hour -- ≤ 4 hours

*Classification might be assigned based on the results from adopted and validated *in vitro* test methods (e.g., EU B.40, OECD TG 430, 431, 435), or based on pH measurements
**Classification might be assigned based on the results from adopted and validated *in vitro* test methods (e.g., EU B.40, OECD TG 430, 431, 435), or if substance is an organic hydroperoxide (except if evidence to the contrary is available)
*** Before the *in vivo* test is carried out a sequential testing strategy shall be carried out including pH, (Q)SAR considerations as well as the use of validated and accepted *in vitro* test methods (e.g., EU B.40, OECD TG 430, 431, 435).

Skin Irritation

Figure 7 shows a summary of the cut-offs applied in the UN GHS, EU CLP and EU DSD classification systems for skin irritation (UN, 2003, 2009; EC, 2001, 2008b).

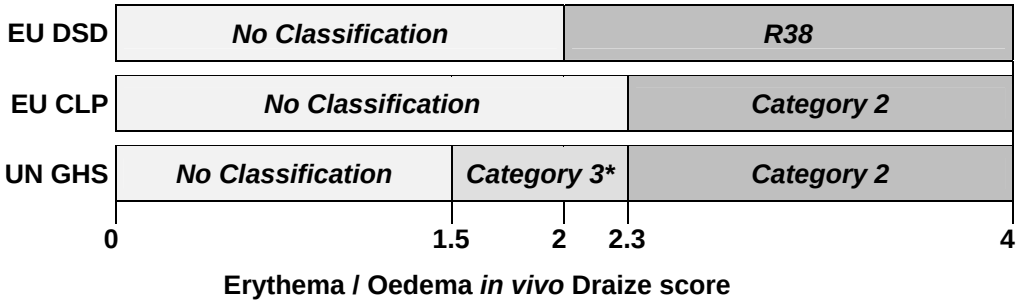

Figure 7: Erythema/oedema Draize score ranges defining EU DSD, EU CLP and UN GHS classification of skin irritation. Scores refer to the mean value from gradings at 24, 48 and 72hours; observed in at least two out of three animals or, for the EU DSD only, the mean value over all tested animals in case of more than 3 animals used. * Category 3 is an optional category available for those authorities that want to have more than one skin irritant category.

It is important to note that the three classification systems also consider a substance irritant if effects persist at the end of the observation period (d14) in 2 or more test animals, and other effects such as hyperplasia, scaling, discoloration, fissures, scabs and alopecia are also taken into account.

Furthermore, the EU DSD and the Guidance on the application of the EU CLP criteria consider organic peroxides as skin irritants and hydroperoxides as skin corrosives, except where evidence to the contrary is available (EC, 2001; ECHA, 2009). Finally, the EU CLP & UN GHS may use in some cases, where there is pronounced variability, of a separate irritant criterion when there is a significant irritant response but less than the mean score criterion for a positive test.

4 *In vitro* Alternative Methods for Skin Corrosion

4.1. Validation status

Several *in vitro* assays for skin corrosion have undergone prevalidation (Botham *et al*, 1995) and validation studies in the '90s (Fentem *et al.*, 1998; Liebsch *et al.*, 2000). Such efforts led to the formal endorsement of the scientific validity of three *in vitro* alternatives (ESAC, 1998a, 1998b, 2000, 2001; NIH, 1999) which were adopted and included in the EU test guidelines in 2000 and in the OECD testing guidelines in 2004 and 2006 (EC, 2000; OECD, 2004a, 2004b, 2006). These assays are:

- The human skin model tests EPISKIN™ and EpiDerm™ based on Reconstructed human Epidermal (RhE) equivalents which use the cell viability (MTT-test) as an endpoint (EU B.40bis and OECD TG 431).
- The *in vitro* skin corrosion rat skin transcutaneous electrical resistance (TER) test, which uses excised rat skin as a test system and its electrical resistance as an endpoint (EU B.40 and OECD TG 430).
- The Corrositex® test, which uses penetration of test substances through a hydrogenated collagen matrix (biobarrier) and supporting filter membrane, which was considered to be useful for acids, bases and their derivates (OECD TG 435).

In addition, two other skin models, the SkinEthic™ RhE and the Epidermal Skin Test EST-1000 methods have been more recently endorsed as a scientific valid to be used for regulatory purposes within the OECD TG 431 and the EU R.40bis (Kandárová *et al.*, 2006; ESAC, 2006, 2009a). They are to be considered as similar RhE tests for skin corrosion assessment.

It is generally recommended that these assays are used in a sequential (stepwise) testing strategy as recommended in the OECD TG 404 (see figure 1), where the hazard assessment of skin corrosive substances includes the use of a pH test and measurement of acid-alcaline reserve, where appropriate (Worth and Cronin., 2001b), and the use of validated *in vitro* tests (OECD, 2002).

4.2. Reconstructed human Epidermis (RhE) test methods

The assay is described in details in the OECD TG 431 (2004) and in the EU B.40bis test method, which is actually equivalent to the OECD TG 431 (EC, 2008a). The principle of the test method, the validated human skin models and a summary of the test method procedure and their known applicability and limitations are described below.

4.2.1. Principles of the test

The principles of the RhE test method is based on the premise that corrosive chemicals are able to penetrate the *stratum corneum* by diffusion or erosion, and are cytotoxic to the cells in the underlying layers. Corrosive materials are identified by their ability to produce a decrease in cell viability below defined threshold levels (i.e., $\leq$ 50%). The test material is applied topically to Reconstructed human Epidermis. Cell viability is measured by dehydrogenase conversion of the vital dye MTT, into a blue formazan salt that is quantitatively measured after extraction from tissues.

4.2.2. Human skin models currently validated

Four commercially available models based on reconstructed human epidermis have been endorsed as scientific valid to be used for regulatory purposes. These are:

- EPISKIN™, validated in 1998 following a formal prospective validation studies (ESAC, 1998a),

- EpiDerm™, from Mattek validated in 2000 following a formal prevalidation and catch-up validation studies (ESAC, 2000),
- SkinEthic™ Reconstituted Human Epidermis (RHE) from SkinEthic, validated in 2006 for having met the Performance Standards as required in the OECD TG 431 (ESAC, 2006),
- Epidermal Skin Test EST-1000 from CellSystems, validated in 2009 for having met the Performance Standards as required in the OECD TG 431 (ESAC, 2009a).

These three-dimensional RhE models are comprised of normal, human-derived epidermal keratinocytes, which have been cultured to form a multilayered, highly differentiated model of the human epidermis. It consists of organized basal, spinous and granular layers, and a multilayered *stratum corneum* containing intercellular lamellar lipid layers arranged in patterns representing main lipid classes analogous to those found *in vivo*.

4.2.3. Method description according to OECD TG 431 and prediction models used for classification

a) Non-specific interaction with MTT
A preliminary assessment of potential interaction of the test material with MTT is required, named non-specific MTT reduction. If the test material directly acts on MTT, appropriate additional controls should be carried out to detect and correct for test substance interference with the viability measurement.

b) Number of replicates
Two replicates for each exposure time.

c) Dose and application of the test substance
Liquids: should uniformly cover the skin surface. Minimum: 25 μl/ cm^2.
Solids: should be moistened with deionised or distilled water and applied to evenly cover the skin. If needed may be grounded to powder.

d) Positive and negative controls
Concurrent positive and negative controls should be used for each study to ensure adequate performance of the experimental model.
Example of negative controls: 0.9% NaCl or water
Example of positive control: 8N KOH

e) Exposure time
The exposure time can vary depending on each RhE model protocols (e.g., 3 min, 1 h and/or 4h).

f) Washing
At end of exposure time the test material must be carefully washed with appropriate buffer or 0.9% NaCl.

g) Cell viability measurement
Apply MTT at appropriate concentration (e.g., 0.3 to 1 mg/ml) for 3 hours at appropriate temperature. Extract the formazan product using a solvent (e.g., isopropanol). Measure the Optical Density (OD) between 540 and 595 nm.

h) Interpretation of results
OD values obtained for each test sample are used to calculate the percentage of viability relative to the negative control, which is set at 100%.
The Prediction Model used to classify the test material as corrosive or non-corrosive should be clearly stated. It refers to the cut-off values used to classify a test material. The cut-off values defined in the validated assays are shown in Figure 8.

EPISKIN™

Treatment time (min)	Viability (%)	Prediction	EU risk phrase	UN packing Group*
3	<35	corrosive	R35	I
3 / 60	≥35 / <35	corrosive	R34	II
60 / 240	≥35 / <35	corrosive	R34	III
240	≥35	non-corrosive	No label	-

EpiDerm™, SkinEthic™ RHE and EST-1000

mean tissue viability (expressed as % of negative control)	Prediction
3 min < 50	corrosive
3 min ≥ 50 and 1 hour < 15	corrosive
3 min ≥ 50 and 1 hour ≥ 15	non-corrosive

SkinEthic™ RHE Prediction for inorganic acids

Mean tissue viability (% negative ctrl.)	Prediction
3 min < 50	corrosive
3 min ≥ 50 **and** 1 hour < 15	corrosive
3 min ≥ 50 **and** 4 hours < 15	corrosive
3 min ≥ 50 **and** 1 hour ≥ 15	non-corrosive

Figure 8: Prediction Models applied by the validated Reconstructed human Epidermis test methods. (*)The test method was validated for corrosive / non-corrosive predictions and R34/no label EU risk phrases. Prediction of UN packing groups I, II and III (which correspond respectively to the UN GHS categories 1A, 1B and 1C; UN, 2001, 2003, 2009) were not evaluated in specific (Fentem et al., 1998).

4.2.4. Comparison of validated RhE protocols for skin corrosion

The details of the principal protocol components of the four validated RhE models (EPISKIN™, EpiDerm™, SkinEthic™ RHE and EST-1000) to be used within OECD TG 431 and EU B.40bis are shown in Table 6. Moreover the practical steps to detect and correct for non-specific interaction of the test material with the MTT reduction are described in Table 7 for the four validated RhE models.

Table 6a: Principal protocol components of the RhE models EPISKIN™ and EpiDerm™ for skin corrosion testing based on updated Invittox protocols 118 and 119 (see appendixes 1 & 2 for the non-updated Invittox protocols 118 and 119).

	EPISKIN™	**EpiDerm™**
MTT interference pre-check	See table 7.	See table 7.
Tissue conditioning	Follow Standard Operating Procedures (SOP)	Follow SOP instructions
N. of replicates	3 replicates for each exposure time.	2 replicates for each exposure time.
Treatment doses and application	Liquids: 50 µl with positive displacement pipette. Solids: 20 ± 2 mg crushed to powder + 100 µl NaCl 0.9% to improve contact with epidermis. Viscous/sticky: 20 ± 2 mg applied with curved spatula.	Liquids: 50 µl, spread with bulb headed Pasteur pipette. If needed use nylon mesh to improve spreading of test article. Semisolids: 50 µl using a positive displacement pipette. Solids: 25 mg crushed to powder + 25 µl H_2O to improve contact with epidermis (or more if needed). Waxes: form a flat cookie like piece of about 8 mm diameter and place atop the tissue, wetted with 15 µl H_2O.
Controls	Negative control: 50 µl NaCl 0.9% applied 4h, in 3 replicates. Positive control: 50 µl glacial acetic acid in 3 replicates.	Negative control: 50µl H_2O applied 3 min & 1 hour, in duplicate. Positive control: 8N KOH applied 3 min & 1 hour, in duplicate.
Exposure time	3 min, 1 hour and 4 hours in ventilated cabinet, Room Temperature (RT, 18-28°C).	3 min at RT, and 1hour at 37°C, 5% CO_2, 95%rh.
Washing	Rinse thoroughly with 25 ml Phosphate Buffered Solution (PBS) 1x solution to remove test material Place units on absorbent paper and remove remaining PBS by gently taping. Sweep surface with cotton-bud.	Rinse tissue with PBS (fill and empty insert 20 times with a constant soft stream of PBS). Remove excess PBS by gently shaking the insert and blot bottom on blotting paper. Dry the surface with cotton swab.
Cell viability	Place insert with treated tissues together with MTT solution (0.3 mg/ml in assay medium). Incubate 3 hours (± 5 min) at RT (20-28°C) protected from light. Place tissue units on absorbent paper, take a tissue biopsy and gently separate epidermis from collagen matrix with the aid of forceps and place both parts (turn epidermis topical side against the collagen) into microtubes. Add e.g., 500 µl acidified isopropanol (0.04 N HCl in isopropanol) and mix thoroughly with vortex. Extract either 72h at 4°C or 4h at RT (19-25°C), with gently mixing protected from light. Mix with vortex and if suspended cell fragments centrifuge at 500 rpm. Take the necessary sample from each tube (e.g., 2x 200 µl per tissue in 96-well plate) and read OD between 545 and 595 nm (preferentially at 570 nm). Use acidified isopropanol as blank	Place insert with treated & control tissues together with 300 µl of MTT solution (1 mg/ml in DMEM based medium). Incubate 3 hours at 37°C, 5% CO_2, 95%rh. Rinse twice with PBS, and ensure tissues are dry. Immerse inserts by adding 2 ml Isopropanol in each insert, and seal the plate (e.g., with a zip bag). Extract either overnight at RT without shaking, or 2h at RT with shaking (~ 120rpm). Pierce the inserts with an injection needle and allow the extract to run into well (discard inserts). Shake 15 min for colour to be homogeneously distributed. Take the necessary sample from each well (i.e., 3x 200 µl in 96-well plate) and read OD at 570 nm without reference filter (the classical filter of 630 nm could interfere with the OD measurements of formazan). Alternatively ODs can be read at 540 to 595 nm.
Data interpretation	% viability = (OD treated tissue –OD blank) x100 / (mean OD negative controls – mean OD blanks)	% viability = (mean OD treated tissues) x100 / (mean OD negative controls)
Acceptance Criteria	1. Negative control (NaCl) exposed for 4h: OD > 0.4. 2. Positive control (glacial acetic acid) exposed for 3min: mean tissue viability < 20%.	1. Negative control (H_2O): OD_{570} > 0.8. 2. Positive control (8N KOH) exposed for 1 hour: tissue viability < 15%. 3. Mean difference between two tissues treated identically ≤ 30 % in the range 20 - 100% cell viability.
Prediction Model	See figure 8.	See figure 8.

Table 6b: Principal protocol components of the RhE models SkinEthic™ and EST-1000 for skin corrosion testing based on the SOPs from the validation study (see appendixes 3 & 4).

	SkinEthic™ RHE	**EST-1000**
MTT interference pre-check	See table 7.	See table 7.
Tissue conditioning	Follow SOP instructions	Follow SOP instructions
N. of replicates	Three replicates used per treatment.	Three tissues used per treatment.
Treatment doses and application	Liquids: 50 µl spread atop the tissue and then place nylon mesh on surface. Semisolids: 50 µl using a positive displacement pipette Solids: 25 mg crushed and grind material + 25 µl H_2O to improve contact with epidermis (or more if needed). Waxy consistence: form a flat cookie-like piece of about 8 mm diameter and place atop the tissue, wetted with 15 µl H_2O *To test if a test chemical interacts with the nylon mesh, place the mesh on a slide and apply 50 µL test substance. After 60 minutes exposure, check using a microscope: if an interaction between test substance and the mesh is noticed, the test substance has to be applied without using the nylon mesh as spreading aid.*	Liquids: 50 µl spread atop the tissue and then place nylon mesh on surface. Semisolids: 50 µl using a positive displacement pipette Solids: 25 mg crushed and grind material + 25 µl H_2O to improve contact with epidermis (or more if needed). Waxy consistence: form a flat cookie-like piece of about 8 mm diameter and place atop the tissue, wetted with 15 µl H_2O *To test if a test chemical interacts with the nylon mesh, place the mesh on a slide and apply 50 µL test substance. After 60 minutes exposure, check using a microscope: if an interaction between test substance and the mesh is noticed, the test substance has to be applied without using the nylon mesh as spreading aid*
Controls	- Negative control: 50µl H_2O applied 3 min and 1 hour, 3 replicate tissues - Positive control: 8N KOH applied 3 min and 1 hour, 3 replicate tissues	- Negative control: 50µl H_2O applied 3 min and 1 hour, 3 replicate tissues - Positive control: 8N KOH applied 3 min and 1 hour, 3 replicate tissues
Exposure time	3 min at RT, and 1 hour at 37°C, 5% CO_2. For aqueous solutions of inorganic acids an additional 4h exposure.	3 min at RT and 1 hour at 37°C, 5% CO_2.
Washing	Remove insert and gently rinse tissue with PBS using a wash bottle (fill & empty insert 20 times) Remove excess PBS by gently shaking the insert and blot bottom with blotting paper	Remove insert and gently rinse tissue with PBS using a wash bottle (fill & empty insert 20 times) Remove excess PBS by gently shaking the insert and blot bottom with blotting paper
Cell viability	Place insert with treated & control tissues together with 300 µl of MTT solution (1 mg/ml in maintenance medium). Incubate 3 hours at 37°C, 5% CO_2. Aspirate MTT, refill wells with PBS and aspirate, repeat rinsing twice, and ensure tissues are dry. Immerse inserts by gently adding 2 ml Isopropanol in each insert and seal the plate. Extract either overnight without shaking at RT, or 2h with shaking (~ 120rpm) at RT. Pierce the inserts with an injection needle and allow the extract to run into well (discard inserts). Shake plate for 15 min so that solution is homogeneous in colour. Take the necessary samples (i.e., 2x 200 µl for each tissue into 96 -well plate) and read OD at 570 nm without reference filter (the classical filter of 630 nm could interfere with the OD measurements of formazan). Alternatively ODs can be read at 540 nm.	Place insert with treated & control tissues together with 300 µl of MTT solution (1 mg/ml in MTT assay medium). Incubate 3 hours at 37°C, 5% CO_2. Aspirate MTT, refill wells with PBS and aspirate, repeat rinsing twice, and ensure tissues are dry. Immerse inserts by gently adding 2 ml Isopropanol in each insert and seal the plate. Extract either overnight without shaking at RT, or 2h with shaking (~ 120rpm) at RT. Pierce the inserts with an injection needle and allow the extract to run into well (discard inserts). Shake plates for 15 min so that solution is homogeneous in colour. Take the necessary samples (i.e., 2x 200 µl for each tissue into 96-well plate) and read OD at 540 to 570 nm without reference filter (the classical filter of 630 nm could interfere with the OD measurements of formazan).
Data interpretation	Relative mean tissue viabilities obtained after 3 min or 1 hour treatment compared to the negative control tissues concurrently treated with H_20.	Relative mean tissue viabilities obtained after 3 min or 1 hour treatment compared to the negative control tissues concurrently treated with H_20.
Acceptance Criteria	1. Negative control (H_2O): mean OD $\geq$ 0.8 2. Positive control (8N KOH) exposed for 1 hour: tissue viability < 20% 3. Coefficient of Variation $\leq$ 30 % in the range between 20 - 100% viability.	1. Negative control (H_2O): mean OD $\geq$ 0.8 2. Positive control (8N KOH) exposed for 1 hour: tissue viability < 20% 3. Coefficient of Variation $\leq$ 30 % in the range between 20 - 100% viability.
Prediction Model	See figure 8	See figure 8

Table 7a: Practical steps to be taken to detect and correct for non-specific interaction of the test substance with the MTT reduction according to the EPISKIN™ and EpiDerm™ test protocols.

		EPISKIN™	**EpiDerm™**
Direct interaction of test material with MTT		2ml MTT (0.3 mg/ml) + 50µl (liquid) or 20mg (solid) test material Incubate 3 hours at 37°C in dark Read OD and compare with MTT solution	1ml MTT (1 mg/ml) + 50µl (liquid) or 25 mg (solid) test material Incubate 1 hour at RT in dark Read OD and compare with MTT solution
Test material interference with the viability	Preparation of killed tissues	Replace culture medium with 2 ml distilled water Incubate 48 ± 1h at 37°C, 5% CO_2, 95% humidity Discard water and freeze dried epidermis at -18°C to 20°C (can be stored up to 6 months)	Place untreated tissues in a freezer (-18°C) overnight.
	Treatment of killed tissues	De-freeze tissues at RT: 1h with 2ml culture medium. Use thawed tissues similar to living tissues. Three killed issues from 1 batch are used for each exposure time.	Apply MTT reducing chemical in two freeze-killed tissues. Use two untreated freeze-killed tissues as controls
	Data analysis	In case of unspecific colouration, there is a need to correct the results using killed tissues as follows: True specific viability (%) = [mean OD of treated viable cells – (mean OD of treated killed tissues – mean OD killed control tissues)] x100 / (mean OD negative controls – mean OD blanks) *Note: Controls and treated killed tissues must be from the same batch, but not necessarily from the same batch as living controls and treated tissues.*	To obtain the true amount of MTT reduction that reflects metabolic conversion only, the net OD obtained from killed tissues (treated with the test substance) is subtracted from the mean OD obtained with treated viable tissues. Data are corrected as follows: True viability (%) = [(OD of treated viable tissue – OD of treated freeze killed tissues) x 100%] / (OD of Negative Control)
Limitations		If the non specific colour (NSC) is > 30%, either additional steps must be taken if possible, or the chemical must be considered as non compatible with the assay NSC (%) = (mean OD of treated killed tissues – mean OD killed control tissues)] x100 / (mean OD negative controls – mean OD blanks)	If the direct reduction by the test substance is greater than 30% of the negative control value, additional steps must be taken into account or the test substance may be considered incompatible with this test system.

Table 7b: Practical steps to be taken to detect and correct for non-specific interaction of the test substance with the MTT reduction according to the SkinEthic™ and EST-1000 test protocols. N.d.: no details given in the SOP.

		SkinEthic™ RHE	**EST-1000**
Direct interaction of test material with MTT		1ml MTT (1 mg/ml) + 50µl (liquid) or 25 mg (solid) test material Incubate 1 hour at RT in dark Use untreated MTT solution as control. If treated MTT solution turns blue/purple, the test material is presumed to have reduced the MTT and a functional check should be performed.	1ml MTT (1 mg/ml) + 50µl (liquid) test material Incubate 1 hour in incubator *Note in case of observed MTT reduction.*
Test material interference with the viability	Preparation of killed tissues	Place untreated tissues in a freezer (-18°C) overnight. Tissues can be stored indefinitely in the freezer.	n.d.
	Treatment of killed tissues	Apply MTT reducing chemical in two freeze-killed tissues. Use two untreated freeze-killed tissues as controls.	n.d.
	Data analysis	True viability = OD of treated viable tissue – (mean OD of treated killed tissues – mean OD untreated killed tissues)	n.d.
Limitations		If the direct reduction by the test substance is greater than 30% of the negative control value, additional steps must be taken into account or the test substance may be considered incompatible with this test system.	n.d.

4.2.5. Proficiency testing and performance standards

In its current version, the OECD requires general and functional model conditions to be met, as described here after, for the assay to be used for the purposes of the guideline.

- *General model conditions*

  Human keratinocytes should be used to construct the epithelium. Multiple layers of viable epithelial cells should be present under a functional *stratum corneum*. The skin model may also have a stromal component layer. *Stratum corneum* should be multi-layered with the necessary lipid profile to produce a functional barrier with robustness to resist rapid penetration of cytotoxic markers. The containment properties of the model should prevent the passage of material around the *stratum corneum* to the viable tissue. Passage of test chemicals around the *stratum corneum* will lead to poor modelling of the exposure to skin. The skin model should be free of contamination with bacteria (including mycoplasma) or fungi.

- *Functional model conditions*

  The magnitude of viability is usually quantified by using MTT or other metabolically converted vital dyes. In these cases the optical density of the extracted (solubilised) dye from the negative control tissue should be at least 20 fold greater than the OD of the extraction solvent alone. The negative control tissue should be stable in culture (provide similar viability measurements) for the duration of the test exposure period. The *stratum corneum* should be sufficiently robust to resist the rapid penetration of certain cytotoxic marker chemicals (e.g., 1% Triton X-100). This property can be estimated by the exposure time required to reduce cell viability by 50% (ET50) (e.g. for the EpiDerm™ and EPISKIN™ models this is > 2 hours). The tissue should demonstrate reproducibility over time and preferably between laboratories. Moreover it should be capable of predicting the corrosive potential of the recommended reference chemicals (table 8).

Table 8: Reference chemicals recommended in the OECD TG 431 and TG 430

1,2-Diaminopropane	CAS-No. 78-90-0	Severely Corrosive
Acrylic Acid	CAS-No. 79-10-7	Severely Corrosive
2-tert. Butylphenol	CAS-No. 88-18-6	Corrosive
Potassium hydroxide (10%)	CAS-No. 1310-58-3	Corrosive
Sulfuric acid (10%)	CAS-No. 7664-93-9	Corrosive
Octanoic acid (caprylic acid)	CAS-No. 124-07-02	Corrosive
4-Amino-1,2,4-triazole	CAS-No. 584-13-4	Not corrosive
Eugenol	CAS-No. 97-53-0	Not corrosive
Phenethyl bromide	CAS-No. 103-63-9	Not corrosive
Tetrachloroethylene	CAS-No. 127-18-4	Not corrosive
Isostearic acid	CAS-No. 30399-84-9	Not corrosive
4-(Methylthio)-benzaldehyde	CAS-No. 3446-89-7	Not corrosive

However, a revised version of the OECD TG 431 has been proposed, where, in addition to the above mentioned required general and functional model conditions, two new requirements are proposed (OECD, 2009a):
- Demonstration of proficiency, by testing a set of proficiency chemicals before routine use of the assay in the laboratory (Table 9),
- Performance Standards requirements that a new or modified similar *in vitro* test methods needs to meet for being considered valid for regulatory skin corrosion testing. The performance standards generally include structurally and mechanistically requirements, as well as performance requirements based on the testing of a set of recommended reference chemicals. In that case, generally a dossier with the description of the new or modified test method and all relevant information and results are submitted to an international validation body, such as ECVAM in Europe or in the United States (US), the Interagency Coordinating Committee on Validation of Alternative Methods (ICCVAM), which in their turn make an official statement on the validity of the test method for regulatory uses. The SkinEthic™ RHE and EST-1000 skin models are two examples of similar tests that were validated for having met the performance standards as required in the test guideline.

Table 9. Proficiency Chemicals recommended in the proposed draft OECD TG 431 and TG 430. (*) UN packing groups I, II and III correspond respectively to the UN GHS categories 1A, 1B and 1C (UN, 2001)

Chemical	CASRN	UN *In vivo* Packing Group*	pH
1,2-Diaminopropane	78-90-0	II	8.3
Dimethyldipropylenetriamine	10563-29-8	I	8.3
2-tert-Butylphenol	88-18-6	II/III	3.9
Potassium hydroxide (10% aq.)	1310-58-3	II	13.1
Sulfuric acid (10%)	7664-93-9	II/III	1.2
Octanoic acid (caprylic acid)	124-07-2	II/III	3.6
4-Amino-1,2,4-triazole	584-13-4	NC	5.5
Eugenol	97-53-0	NC	3.7
Phenethyl bromide	103-63-9	NC	3.6
Tetrachloroethylene	127-18-4	NC	4.5
Isostearic acid	30399-84-9	NC	3.6
4-(Methylthio)benzaldehyde	3446-89-7	NC	6.8

4.2.6. Known applicability and limitations

The test method described in the OECD TG431 and EU B.40bis guidelines allows the identification of corrosive chemical substances and mixtures. Moreover the EPISKIN™ RhE model was considered scientific valid to distinguish the two EU DSD corrosive categories R34 and R35. The test method also proposes a prediction model to distinguish the three subcategories for corrosive substances as permitted in the Globally Harmonised Classification System (GHS cat. 1A, 1B and 1C), however the model has not been validated for such GHS sub-categorization in specific. The other RhE models also do not allow such sub-categorisation, however the most severe category could be applied as precautionary principle (e.g., Cat 1A).

The RhE models that fall within the OECD TG431 and EU B.40bis guidelines also enable the identification of non-corrosive substances and mixtures when supported by a weight of evidence determination using other existing information (e.g., pH, structure-activity relationships, human and/or animal data). However, they do not normally provide adequate information on skin irritation.

The methods were not designed to be compatible with highly volatile test substances. However, possible toxic interference across plate wells can be avoided by sealing the wells with an adhesive cover sheet, or testing volatile chemicals on separate plates.

Furthermore, if the direct MTT reduction by the test substance is greater than 30% of the negative control value, additional steps must be taken into account or the test substance may be considered incompatible with this test system.

4.3. Transcutaneous Electrical Resistance test (TER)

The *in vitro* skin corrosion transcutaneous electrical resistance test is described in details in the OECD TG 430 (2004) and in the EU B.40 test method which is equivalent to the OECD TG 430 (EC, 2008a). The principles of the test method, a summary of its procedure and its known applicability and limitations are described hereafter.

4.3.1. Principles of the test

The Transcutaneous Electrical Resistance is a measure of the electrical impedance of the skin, as a resistance value in kilo Ohms. The test material is applied for up to 24 hours to the epidermal surfaces of skin discs in a two-compartment test system in which the skin discs function as the separation between the compartments. The skin discs are taken from humanely killed rats aged 28-30 days.

Corrosive materials are identified by their ability to produce a loss of normal *stratum corneum* integrity and barrier function, which is measured as a reduction in the TER below a threshold level. For rat TER, a cut-off value of 5k has been selected based on extensive data for a wide range of chemicals where the vast majority of values were either clearly well above (often > 10 k), or well below (often < 3 k) this value. Generally, materials that are non-corrosive in animals but are irritating or non-irritating do not reduce the TER below this cut-off value.

Furthermore, a dye-binding step is incorporated into the test procedure for confirmation testing of positive results in the TER including values around 5 k. The dye-binding step determines if the increase in ionic permeability is due to physical destruction of the *stratum corneum*. Indeed, exposure of certain non-corrosive materials can result in a reduction of resistance below the cut-off of 5 k allowing the passage of ions through the *stratum corneum*, thereby reducing the electrical resistance. For example, neutral organics and chemicals that have surface-active properties (including detergents, emulsifiers and other surfactants) can remove skin lipids making the barrier more permeable to ions. In case of skin corrosive effects where the *stratum corneum* is disrupted, the dye sulforhodamine B, when applied to the skin surface rapidly penetrates and stains the underlying tissue. This particular dye is stable to a wide range of chemicals and is not affected by the extraction

procedure. As a consequence, obtaining high dye contents may indicate a corrosive effect (see details below).

4.3.2. Method description according to OECD TG 430 and prediction model used for classification

a) Preparation of skin discs
Skin discs are prepared from young rats as described in the test guideline. Around 10-15 skin discs, with a diameter of approximately 20-mm each, can be obtained from a single rat skin. The skin may be stored before disks are used where it is shown that positive and negative control data are equivalent to that obtained with fresh skin. The skin discs are placed in a polytetrafluoroethylene (PTFE) tube which is supported by a spring clip inside a receptor chamber containing $MgSO_4$ solution (154 mM). The skin disc should be fully submerged in the $MgSO_4$ solution.

b) Quality control of skin discs
Before testing begins, the electrical resistance of two skin discs are measured as a quality control procedure for each animal skin. Both discs should give resistance values greater than (or equal to) 10 kΩ for the remainder of the discs to be used for the test. If the resistance value is less than 10 kΩ, the remaining discs from that skin should be discarded.

c) Number of replicates
Three skin discs for each test and control substance.

d) Dose and application of the test substance
Liquids: 150 µl applied uniformly to the epidermal surface of the skin discs inside the tube.
Solids: a sufficient amount is applied to ensure that the whole surface of the epidermis is covered. 150 µl of deionised water is added on top of the solid. To achieve maximum contact with the skin, solids may need to be warmed to 30°C to melt or soften, or ground.

e) Positive and negative controls
Positive and negative controls should be used for each study, where skin discs from a single animal should be used.
Suggested negative controls: distilled water
Suggested positive control: 10M hydrochloric acid

f) Exposure time
Test substances are applied for 24 hours at 20-23°C.

g) Washing
At end of exposure time, the test substance is removed by washing with a jet of tap water at up to 30°C.

h) TER measurements
The skin impedance is measure as TER by using a low-voltage, alternating current Wheatstone bridge. The assay measurements are recorded in resistance, at a frequency of 100 Hz and using series values. The distance between the spring clip and the bottom of the PTFE tube is maintained as a constant because this distance affects the resistance value obtained (for more details, see OECD TG 430, 2004)

The properties and dimensions of the test apparatus and the experimental procedure used may influence the TER values obtained. Different threshold and control values may apply if the test conditions are altered or a different apparatus is used. Therefore, it is necessary to calibrate the methodology and resistance threshold values by testing a series of reference standards chosen from the chemicals used in the validation study, or from similar chemical classes to the chemicals being investigated. A set of suitable reference chemicals is provided in the OECD TG 430 (see table 8).

i) Dye binding method
If the TER values of test substances are less than or equal to 5 kΩ in the absence of visual damage, an assessment of dye penetration should be carried out on the control and treated tissues to determine if the TER values obtained were the result of increased skin permeability, or due to skin corrosion. In case of the latter where the *stratum corneum* is disrupted, the dye sulforhodamine B, when applied to the skin surface rapidly penetrates and stains the underlying tissue.

For evaluating the dye penetration, following TER assessment the magnesium sulphate is discarded from the tube and the skin is carefully examined for obvious damage. If there is no obvious major damage, 150μL of a 10% (w/v) dilution of sulforhodamine B dye in distilled water, is applied to the epidermal surface of each skin disc for 2 hours. These skin discs are then washed with tap water at up to room temperature for approximately 10 seconds to remove any excess/unbound dye. Each skin disc is carefully removed from the PTFE tube and placed in a vial containing deionised water. The vials are agitated gently for 5 minutes to remove any additional unbound dye. This rinsing procedure is then repeated, after which the skin discs are removed and placed into vials containing 5ml of 30% (w/v) sodium dodecyl sulphate (SDS) in distilled water and are incubated overnight at 60° C. After incubation, each skin disc is removed and discarded and the remaining solution is centrifuged. A 1ml sample of the supernatant is diluted 1 in 5 (v/v) with 30% (w/v) SDS in distilled water. The OD of the solution is measured at 565nm.
The sulforhodamine B dye content per disc is calculated from the OD values and by using the appropriate calibration curve. The mean dye content is then calculated for the replicates.

j) Acceptable ranges

Controls	**Resistance (TER) range (kΩ)**	**Dye content range (μg/disc)**
Positive: 10 M Hydrochloric acid	0.5 - 1.0	40 - 100
Negative: Distilled water	10 - 25	15 – 35

k) Interpretation of results

Observed results	**Prediction**
TER > 5 kΩ	Non corrosive
TER ≤ 5 kΩ, AND no obvious damage to skin disc, AND the mean disc dye content well below the values obtained with positive control	
TER ≤ 5 kΩ AND obvious damage to skin disc	Corrosive
TER ≤ 5 kΩ AND no obvious damage to skin disc, AND the mean disc dye content is greater than or equal to the values obtained with positive control	

4.3.3. Proficiency testing and performance standards

In its original version, the OECD TG 430 proposes a list of 12 reference chemicals (see Table 8) that are to be used to calibrate the methodology and resistance threshold values in case if the test conditions are altered or a different apparatus is used. Furthermore, it states that the use of other skin preparations or other equipment may alter the cut-off value, necessitating further validation.

However, a revised version of the OECD TG 430 has been proposed where the following two requirements are suggested as follows (OECD, 2009b):
- Demonstration of proficiency where a subset of 12 proficiency chemicals is recommended to be used by laboratories prior to routine use of any TER test method that adheres to the TG 430 to demonstrate technical proficiency (see Table 9).
- Performance Standards requirements for determining the validation status of new and revised skin corrosion test methods that are structurally and mechanistically similar to the TER, in accordance with the principles of Guidance Document No. 34. The proposed performance standards include 1) the essential test method components that should be included in the protocol for the test method to be considered structurally and mechanistically similar, 2) a list of 24 reference chemicals by which to evaluate assay performance, and 3) the minimum accuracy and reliability necessary for the test method to be considered comparable to the TER. In that case, generally a dossier with all relevant information and results are submitted to an international validation body, such as ECVAM or ICCVAM, which in their turn make an official statement on the validity of the test method for regulatory uses.

4.3.4. Known applicability and limitations

The *in vitro* skin corrosion TER test allows the identification of corrosive chemical substances and mixtures. It further enables the identification of non-corrosive substances and mixtures when supported by a weight of evidence determination using other existing information (e.g. pH, structure-activity relationships, human and/or animal data).

However, it does not provide information on skin irritation, nor does it allow the sub-categorisation of corrosive substances as permitted in the Globally Harmonised System for Hazard Classification and Labelling (GHS). In the later case, the most severe category could be applied (e.g., Cat 1A).

4.4. *In vitro* membrane barrier test method for skin corrosion (Corrositex®)

The *in vitro* membrane barrier test method for skin corrosion is described in details in the OECD TG 435 (2006). It is also legal test adopted by the US Department of Transport (US DOT). The only commercial available *in vitro* membrane barrier method currently endorsed as valid is Corrositex®. In Europe, although it was endorsed by the ECVAM's Scientific Advisory Committee (ESAC), it has not been taken up in the EU legislation perhaps due to the fact that Corrositex® was validated as useful only for acids, bases and their derivates (ESAC, 2001; NIH, 1999). The principle of the test method, a summary of its procedure and its known applicability and limitations are described hereafter.

4.4.1. Principles of the test

The basis of the *in vitro* membrane barrier test method for skin corrosion is that it detects membrane barrier damage caused by corrosive test substances after the application of the test substance to the surface of the artificial membrane barrier, presumably by the same mechanism(s) of corrosion that operate on living skin.

The classification assigned is based on the time it takes a substance to penetrate through the hydrogenated collagen matrix (biobarrier) and supporting filter membrane. Penetration of the membrane barrier (or breakthrough) is measured by a number of procedures, including a change in the colour of a pH indicator dye or in some other property of the indicator solution below the barrier. In the case of Corrositex®, the endpoint assessed is a colour change in the Chemical Detection System (CDS).

The time required for this change to occur (the breakthrough time) is reported to be inversely proportional to the degree of corrosivity of the test material, i.e., the longer it takes to detect a change, the less corrosive is the substance.

4.4.2. Method description according to OECD TG 435 and prediction model used for classification

The test system is composed of two components:
- a synthetic macromolecular bio-barrier consisting of 1) a proteinaceous macromolecular aqueous gel serving as the target for the test substance, and 2) a permeable supporting membrane.
- a Chemical Detection System (CDS) which is an indicator solution that responds to the presence of a test substance with the help of a pH indicator dye or a combination of dyes, e.g., cresol red and methyl orange, or other types of chemical or electrochemical reactions.

a) Test substance compatibility test
Prior to performing the membrane barrier test, a compatibility test is carried out to determine if the test substance is detectable by the CDS. If the CDS does not detect the test substance, the membrane barrier test method is not suitable for evaluating the potential corrosivity of that particular test substance and a different test method should be used. The CDS and the exposure conditions used for the compatibility test should reflect the exposure in the subsequent membrane barrier test.

In the case of Corrositex®, 150 µl or 100 mg of test material are added to the 'Qualify' test tube. If the test material fails to produce a colour or physical change in the Qualify test, it cannot be analysed with Corrositex®.

b) Test substance timescale category test
A timescale category test is carried out to distinguish between weak and strong acids or bases. In the case of Corrositex® the timescale categorization test is made based on whether significant acid or alkalai reserve is detected. A total of 150 µl or 100 mg of test material is added in the "tube A" and "tube B" provided in the kit. Tubes are mixed and the resulting colours are compared to a colour chart provided to determine the category. If no colour change is observed in either tube, two drops of the 'confirm' reagent are added to tube B, which is mixed and the resulting colour used to determine the category.
- Category 1 materials: materials having high acid/alkaline reserves
- Category 2 materials: materials having low acid/alkaline reserves

Such categorisation is then used to indicate which of two prediction models should be used for determining corrosivity subcategories (see paragraph i).

c) Number of replicates
The number of replicates should be appropriate, e.g., four for each test substance in the case of Corrositex®, two repeats in two different batches. The qualification screen and the categorisation screen should also be undertaken in two separate occasions.

d) Dose and application of the test substance
A suitable amount of the test substance, e.g., for Corrositex® 500 µl of a liquid or 500 mg of finely powdered solid are added directly to the membrane disc placed on the top of a vial containing CDS at RT (17 – 25°C).
The biobarriers should not be in the vial for longer than 2 min before adding the test sample, and the tray containing the biobarrier discs before exposure should be kept on crushed ice if not in the refrigerator.

e) Positive, negative, vehicle and blank controls
Positive control should have an intermediate corrosivity, e.g., Sodium hydroxide (GHS Cat 1B). A second positive control of the same chemical class as the test substance may be useful. Furthermore, a weak corrosive (GHS cat 1C) might also be employed as a positive control to measure the ability of a test method to distinguish between weakly corrosives and non-corrosive substances. An acceptable positive control response range should be developed based on historical range, such as the mean ± 2 to 3 SDs.

Negative control should be non-corrosive, e.g., 10% citric acid, 6% propionic acid, and demonstrate functional integrity of the membrane barrier.

Vehicles or solvents: should be tested concurrently with the test substance to demonstrate the compatibility with the membrane barrier system, i.e., it should not alter the integrity of the membrane barrier system, and should not alter the corrosivity of the test substance.

Blank: CDS colour control.

f) Exposure time and barrier penetration
The elapsed time between test material application and the first change in the indicator solution, i.e., barrier penetration, is recorded (minutes). In the case of Corrositex®, the changes observed can be either in colour or in physical appearance such as flaking or precipitation in the CDS compared to the blank control.

g) Acceptability criteria
For a study to be considered acceptable, the concurrent positive control should give the expected penetration response time, the concurrent negative control should not be corrosive, and, when included, the concurrent solvent control should neither be corrosive nor should it alter the corrosivity potential of the test substance.

h) Interpretation of results
The average time (in minutes) of the four replicates elapsed between application of the test substance to the membrane and its barrier penetration is used to classify the test substance in terms of corrosivity and, if applicable, UN Packing Group.

In the case of Corrositex® the following two prediction models are used depending on the category of the test material based on whether significant acid or alkalai reserve is detected for the material in the timescale category test.

Category 1 Test Materials

Corrosivity	UN Packing Group*	Risk Phrase	Mean Time
Corrosive	I	R35	0 - 3 min
Corrosive	II	R34	> 3 min – 1 h
Corrosive	III	R34	> 1 – 4 h
Non Corrosive	Not applicable	No label	> 4 h

Category 2 Test Materials

Corrosivity	Packing Group	Risk Phrase	Mean Time
Corrosive	I	R35	0 - 3 min
Corrosive	II	R34	> 3 – 30 min
Corrosive	III	R34	> 30 – 60 min
Non Corrosive	Not applicable	No label	> 60 min

* UN packing groups I, II and III correspond respectively to the UN GHS categories 1A, 1B and 1C (UN, 2001)

4.4.3. Proficiency testing and performance standards

The OECD TG 435 requests that the membrane barrier used should be demonstrated to be valid, i.e., relevant and reliable, for its intended use. This includes ensuring that different preparations are consistent in regard to barrier properties, e.g., capable of maintaining a barrier to non-corrosive substances, able to categorize the corrosive properties of chemicals across the various subcategories of corrosivity.

For this purpose, prior to routine use of a test method that adheres to this test guideline, laboratories may wish to demonstrate technical proficiency, using twelve recommended chemicals as shown in Table 10.

Table 10: Proficiency Chemicals recommended in the OECD TG 435

Chemical	CASRN	Chemical Class	UN GHS Subcategory*
Nitric acid	7697-37-2	Inorganic acids	1A
Phosphorus pentachloride	10026-13-8	Precursors of inorganic acids	1A
Selenic acid	7783-08-6	Inorganic acids	1A
Valeryl chloride	638-29-9	Acid chlorides	1B
Sodium Hydroxide	1310-73-2	Inorganic bases	1B
1-(2-Aminoethyl) piperazine	140-31-8	Aliphatic amines	1B
Benzenesulfonyl chloride	98-09-9	Acid chlorides	1C
Hydroxylamine sulphate	10039-54-0	Organic ammonium salts	1C
Tetraethylenepentamine	112-57-2	Aliphatic amines	1C
Eugenol	97-53-0	Phenols	NC
Nonyl acrylate	2664-55-3	Acrylates/methacrylates	NC
Sodium bicarbonate	144-55-8	Inorganic salts	NC

* The corresponding UN Packing groups are I, II and III, respectively, for the UN GHS 1A, 1B and 1C.
NC; Non-corrosive.

In addition, for new “me-too” test methods developed under this OECD TG 435 that are structurally and functionally similar to the validated reference test method the performance standards described in the TG should be used to demonstrate the reliability and accuracy of the new test method prior to its use for regulatory testing. This includes a list of 40 reference chemicals to be tested and used to evaluate the relevance and reliability of the “me-too” proposed membrane barrier test method for skin corrosion (for details see OECD TG 435, 2006). In that case, generally a dossier with the description of the new or modified test method and all relevant information and results are submitted to an international validation body, such as the ECVAM or ICCVAM, which in their turn make an official statement on the validity of the test method for regulatory uses.

4.4.4. Known applicability and limitations

The *in vitro* membrane barrier test method for skin corrosion was considered valid for the subcategorisation of corrosive substances into the UN Transport Packing Groups for corrosivity hazard, which are similar to the three GHS subcategories of corrosivity..

The *in vitro* membrane barrier test method may be used to test solids (soluble or insoluble in water), liquids (aqueous or non-aqueous), and emulsions. Moreover, test samples may be pure chemicals, dilutions, formulations, or waste. No prior treatment of the sample is required.

However, test chemicals and chemical mixtures not causing a detectable change in the compatibility test (i.e., colour change in the CDS of the validated reference test method) cannot be tested with the membrane barrier test method and should be tested using other test methods. Indeed, a limitation of the validated reference test method that is the basis for this Test Guideline is that, based on the results of the initial compatibility test, many non-corrosive chemicals and chemical mixtures and some corrosive chemicals and chemical mixtures may not qualify for testing. Aqueous substances with a pH in the range of 4.5 to 8.5 often do not qualify for testing; however, 85% of chemicals tested in this pH range were non-corrosive in animal tests (NIH, 1999).

Furthermore, in Europe, the test was considered scientifically validated only for acids, bases and their derivates which meet the technical requirements of the assay (ESAC, 2001).

4.5. Comparison to the *in vivo* test method

A summary of the major components of the regulatory *in vivo* and *in vitro* tests for skin corrosion is shown in Table 11.

Morphologically, the adopted *in vitro* reconstructed human epidermis methods are closer to the human epidermis as compared to the rabbit skin. Although these models do not present all functional complexity that exist *in vivo*, i.e., the dermis and its features such as hair follicules, subaceous glands, nerve and immune cells, such features seem to play a less important role in the mechanisms of skin corrosion than in the inflammatory reactions that could lead to skin irritation. On the other hand, the adopted *in vitro* TER method makes use of excised rat skin which does include the dermis, but no blood circulation. Finally, the adopted *in vitro* membrane barrier assay does only mimic the morphological features of the *in vivo* skin.

The various adopted *in vitro* models for regulatory purposes also mimic the mechanisms of skin corrosion occurring in the *in vivo* test. These encompass:

- Cell viability (reconstructed human epidermis models) based on the principle that corrosive chemicals are able to penetrate the *stratum corneum* and are cytotoxic to the underlying layers.
- Loss of barrier function and integrity (TER assay), based on the principle that corrosive materials can produce loss of *stratum corneum* integrity and barrier function.
- Membrane barrier damage (membrane barrier test) presumably by the same mechanism(s) of corrosion that operate on living skin.

With the exception of TER, the exposure times used with the adopted *in vitro* assays are comparable to those used *in vivo* (3 min, 1 h and 4 hours), and the doses applied *in vitro* are similar or greater than those applied *in vivo* (for details see table 11).

Unlike the *in vivo* test, the *in vitro* assays make systematically use of positive and negative controls to check for the functionality of the test method. In addition, recent proposals for updating the OECD TG 430 and 431 recommend also ensuring the technical proficiency of the assays, by the laboratory, prior to the routine use of the *in vitro* assays by testing a list of recommended proficiency chemicals.

Overall, the adopted *in vitro* assays for skin corrosion are considered as full replacements to the traditional *in vivo* assay. All assays are able to distinguish between corrosives and non corrosives test materials according to the GHS classification system. However, only the membrane barrier test was considered valid to distinguish the three UN packaging groups, which are similar to the three GHS subcategories, for acids, bases and their derivates which meet the technical requirements of the assay. The EPISKIN™ RhE proposes a prediction model for such subcategorisation but was not specifically evaluated for that purpose (Fentem et al., 1998). However, if there is a need to use the three GHS subcategories, the most severe category may be used (i.e., category 1A).

Furthermore, it is to be noted that the *in vitro* assays for skin corrosion were validated for the purposes of testing chemicals, following internationally agreed principles of validation, which recommends that the tested chemicals are of the highest available purity (OECD, 2005). As a consequence their use was demonstrated to be relevant and reliable for testing chemicals within the framework of the EU Dangerous Substance Directive. Up to day, their applicability to test formulation and/or dilutions was not evaluated by international centers of validation such as ECVAM and ICCVAM, due to the scientific difficulties in including the large existing variety of possible formulations and/or dilutions in a comprehensive formal validation study. As a consequence, the applicability of the *in vitro* assays for testing formulations and/or dilutions of the test substances is to be evaluated on a case-by-case basis, taking into consideration the demonstrated performances of the assay for the types of formulations and/or dilutions tested.

Table 11. Comparison of the principal method components of the regulatory accepted *in vivo and in vitro* tests for skin corrosion.

	***In vivo* test for skin corrosion (OECD TG 404)**	***In vitro* human skin model (OECD TG 431)**	***In vitro* TER (OECD TG 430)**	***In vitro* membrane barrier test (OECD TG 435)**
Model used	Albino rabbit	Three-dimensional reconstructed human epidermis, consisting of organized basal, spinous and granular layers, and a multilayered *stratum corneum* (0.38 to 0.63 cm^2 surface depending on the model).	Skin disks prepared from young rats, where 10-15 skin discs can be obtained per rat skin (0.79 cm^2 surface)	A macromolecular biobarrier and a chemical detection system (CDS) which indicates the presence of a test substance.
Number of replicates	1 to 3 animals based on severity of effects	2 replicates for each exposure time	3 skin disks	2 repeats in 2 batches
Dose and application of test substance	0.5 ml (liquids) or 0.5 g (solids) applied to ~ 6 cm^2 of skin and covered with a gauze patch (~ 83.3 µl or mg / cm^2). Solids might be moisten to ensure good skin contact.	Liquids: 50 µl (79.4 to 131.6 µl/cm^2 depending on model). Solids: 20-25 mg (39.7 to 52.6 mg / cm^2 depending on model). Solids should be moisten to ensure good contact with the RhE.	Liquids: 150 µl (~ 189.9 µl/cm^2). Solids: sufficient amount to cover surface, and 150 µl of deionised water added on top of the solid.	0.5 ml (liquids) or 0.5 g (solids) applied on membrane.
Controls	Potential influence of the vehicle on irritation of the skin by the test substance should be minimal, if any.	Negative control: 0.9% NaCl or water Positive control: 8N KOH	Negative control: distilled water Positive control: 10M hydrochloric acid	- Negative control: e.g., 10% citric acid or 6% propionic acid - Positive control: e.g., sodium hydroxide - Vehicles or solvents should not alter integrity of the membrane barrier system, and should not alter the corrosivity of the test substance.
Exposure time	3 min, 1 hour, 4 hours applied in a sequential way, so that if corrosive effects are observed the test is terminated. If no corrosive effects seen after 4 h exposure, the animal is observed up to 14 days.	3 min at RT 1 hour at RT or at 37°C depending on the model. In one model, also 4 hours at RT	24 hours at RT	The time needed for a material to penetrate the membrane barrier is used to predict corrosivity. It is reported to be inversely proportional to the degree of corrosivity, i.e., the longer it takes to penetrate, the less corrosive is the substance.
Washing	At the end of exposure time to remove test substance	At the end of exposure time to remove test substance	At the end of exposure time to remove test substance	Not necessary
Endpoint assessed	- Grading of skin reactions. - Other reactions such as: defatting of skin, clinical signs of toxicity and body weight, persistence of alopecia, hyperkeratosis, hyperplasia and scaling. - Histopathology may be carried out in case of equivocal responses	Cell viability: based on the principle that corrosive chemicals are able to penetrate the *stratum corneum* and are cytotoxic to the underlying layers.	- Transcutaneous Electrical Resistance: based on the principle that corrosive materials can produce loss of *stratum corneum* integrity and barrier function, measured by the TER. - Dye binding: to determine if TER values below the cut-off but in absence of visual damage, are due to increase in permeability or to skin corrosion.	The time it takes a substance to penetrate through the membrane barrier. Penetration is measured by colour or physical change of the Chemical Detection System.
Interpretation of results	Classification schemes. For corrosion, three subcategories depending on the time to produce corrosive effects (3 min, 1 hour, and 4 hours)	Able to distinguish between corrosive and non corrosive. EPISKIN™ also able to distinguish the two EU DSD risk phrases R34 and R35, but not specifically evaluated to distinguish the three corrosive subcategories (1A, 1B and 1C).	Able to distinguish between corrosive and non corrosive.	Able to distinguish the three GHS subcategories
Limitations	- May overpredict human responses. - May be variable between laboratories. - Does not assess repetitive low-dose exposure. - Has the potential to cause considerable discomfort or pain to laboratory animals	- Not applicable to test materials presenting non-specific interaction with MTT greater than 30% of negative control. - Not compatible with highly volatile substances, however possible to test volatile chemicals on separate plates.	Not able to distinguish the 3 GHS subcategories.	Test materials not causing detectable change in the CDS cannot be tested. In Europe, assay considered valid only for acids, bases and their derivatives.

5 *In vitro* Alternative Methods for Skin Irritation

In August 2009, the *in vitro* skin irritation test based on reconstructed human epidermis models has been adopted as the EU test method B.46 (EU, 2009). It is to be considered a stand-alone replacement test within a testing strategy, in a weight of evidence approach in agreement to the REACH guidance on information requirements and chemical safety assessment (ECHA, 2008b). The assay allows the hazard identification of irritant substances in accordance with GHS Category 2, and or non classified substances for skin irritation (no GHS category). A draft OECD Test guideline is also currently under discussion (OECD, 2009c). This chapter will describe the validation process the led to the adoption of this assay, as well as provide with a description of the assay, its known applicability and limitations and its comparison to the *in vivo* test method.

5.1. Validation process

Because systemic reactions play a minor role in modulating local skin toxicity potential of chemicals, *in vitro* systems which are sufficiently complex to mimic human skin barrier and cell reactivity, were considered as potential models to predict skin irritation potential of substances and have been evaluated over the last decade (Zuang *et al.*, 2005). Following an extensive review of existing *in vitro* systems and toxicological endpoints (Botham *et al.*, 1998; Van de Sandt *et al.*, 1999), an ECVAM prevalidation study was conducted during 1999 - 2000 where five promising *in vitro* methods were evaluated, i.e., EpiDerm™, EPISKIN™, Prediskin™, the non-perfused pig ear model, and the *in vitro* mouse skin integrity function test (SIFT). The study concluded that although the reproducibility of the two human skin model tests (EpiDerm™ and EPISKIN™) and of the SIFT test was acceptable, their predictive capacity needed further improvement (Fentem *et al.*, 2001). ECVAM and its task force on skin irritation recommended therefore the optimization of the protocols and prediction models of the three assays (Zuang *et al.*, 2002). Subsequent refinements were made to the three assays so that the optimized test protocols and/or prediction models met the criteria for inclusion in a formal validation study (Heylings *et al.*, 2003; Cotovio *et al.*, 2005; Portes *et al.*, 2002; Kandárová *et al.*, 2004; Kandárová *et al.*, 2005).

The ECVAM skin irritation validation study (SIVS) then took place from 2003 to 2006. The aim of the study was to evaluate whether the EpiDerm™, EPISKIN™ and the SIFT assays were able to reliably identify skin irritant and non-irritant chemicals and as such to replace the rabbit Draize test for skin irritation. Further to the outcome of the validation study (Spielmann *et al.*, *2007*) and to an independent peer review, the scientific validity of the two test methods was endorsed as follows (ESAC, 2007):

(1) the EPISKIN™ assay was considered to be a reliable and relevant stand-alone test for predicting rabbit skin irritation, when the endpoint is evaluated by MTT reduction, and to be used as a replacement for the Draize Skin Irritation Test (OECD TG 404) for the purposes of distinguishing between R38 skin irritating and no-label (non-skin irritating) test substances.

The IL-1α endpoint was regarded as a useful adjunct to the MTT assay, as it had the potential to increase the sensitivity of the test, without reducing its specificity. This endpoint could be used to confirm negatives obtained with the MTT endpoint.

(2) The EpiDerm™ assay was considered to reliably identify skin irritants due to its high specificity, but negative results might require further testing (e.g. according to the tiered strategy, as described in the OECD TG 404). Further improvements to increase the level of sensitivity of the EpiDerm™ protocol were recommended.

With regard to the SIFT assay, it fail to fulfil the predefined criteria to enter in phase II of the validation study, and further investigations on the assay were recommended.

Following the ESAC statement, modifications of the EpiDerm™ assay were made leading to the EpiDerm™ Skin Irritation Test (SIT) modified protocol. Moreover, a similar assay based on RhE, the SkinEthic™ RHE test method, was proposed for skin irritation testing. Both assays are based on reconstructed human epidermis and measure or predict the same biological effect as the EPISKIN™

validated and accepted method, and could therefore be considered to be 'similar' tests to the validated assay. To evaluate the scientific validity of these assays, external studies were carried out to determine whether the two assays met the requirements of the performance standards as defined by ECVAM for *in vitro* skin irritation testing. Following review by ESAC, both assays were endorsed as scientific valid for having met the criteria outlined in the performance standards, and to have sufficient accuracy and reliability for prediction of R38 skin irritating and no label (non-skin irritating) test substances compared to the validated EPISKIN™ assay, including the limitations associated with it (ESAC, 2008).

In December 2008, the EU adopted the UN GHS by means of the CLP Regulation (see chapter 1.5). The performance of all three test methods (EPISKIN™, modified EpiDerm™ EPI-200 and SkinEthic™ RHE) has been re-evaluated taking into account the shift of the cut-off value for the classification of skin irritants (a cut-off of 2 for R38 classification versus a cut-off of 2.3 for the GHS Cat.2, see also figure 7), and has been shown to be satisfactory. The ESAC statements relating to the scientific validity of the three test methods therefore continued to be accurate and were extended to the EU CLP (GHS) classification system (ESAC, 2009b).

An EU Test Guideline on "*In vitro* Skin Irritation: Reconstructed Human Epidermis Model" has been adopted on these validated assays and included in EU Test Method Regulation (EU test method B.46; EC, 2009). Furthermore an equivalent draft guideline proposed at the OECD level is currently under discussions (OECD, 2009c).

5.2. Reconstructed human Epidermis (RhE) test method

5.2.1. Principles of the test

The test substance is applied topically to a three-dimensional reconstructed human epidermis model, comprised of normal, human-derived epidermal keratinocytes, which have been cultured to form a multilayered, highly differentiated model of the human epidermis. It consists of organised basal, spinous and granular layers, and a multilayered *stratum corneum* containing intercellular lamellar lipid layers arranged in patterns analogous to those found *in vivo*.

Chemical-induced skin irritation, manifested by erythema and oedema, is the result of a cascade of events beginning with penetration of the *stratum corneum* and damage to the underlying layers of keratinocytes. The dying keratinocytes release mediators that begin the inflammatory cascade which acts on the cells in the dermis, particularly the stromal and endothelial cells. It is the dilation and increased permeability of the endothelial cells that produce the observed erythema and oedema (Wells *et al.*, 2004).

The RhE-based test methods measure the initiating events in the cascade, and are based on the premise that irritant substances are able to penetrate the *stratum corneum* by diffusion and are cytotoxic to the cells in the underlying layers. Cell viability is measured by dehydrogenase conversion of the vital dye MTT, into a blue formazan salt that is quantitatively measured after extraction from tissues.

Irritant substances are identified by their ability to decrease cell viability below defined threshold levels (i.e. ≤ 50 %, for UN GHS category 2 irritants). Depending on the regulatory requirements, substances that produce cell viabilities above the defined threshold level, may not be classified (i.e. > 50 %, no category).

5.2.2. Reconstructed human epidermis models validated

Three commercially available models based on reconstructed human epidermis have been endorsed as scientific validated for regulatory testing:

- EPISKIN™ Skin Irritation Test (SIT), validated following an ECVAM prospective validation study (ESAC, 2007),

- modified EpiDerm™ EPI-200-SIT protocol, validated for having met the performance standards as required by ECVAM (ESAC, 2008),
- SkinEthic™ RHE SIT-42bis, validated for having met the performance standards as required by ECVAM (ESAC, 2008).

These three RhE models are based on three-dimensional reconstituted human epidermis and are generated by growing keratinocyte cultures at the air-liquid interface on various substrates, enabling the topical application of either neat or diluted test materials (Botham *et al*., 1998; van de Sandt *et al*., 1999). The three models all fulfil the structural characteristics as described in chapter 5.2.1. However, they differ regarding e.g., i) the origin of the cells and their characteristics such as hydrophobicity, of the reconstituted tissues, ii) the surface of the models (0.38 cm^2 for Episkin™-SIT, 0.63 cm^2 for EpiDerm™ 200-SIT and 0.5 cm^2 for SkinEthic™ SIT), and iii) the support used to culture the reconstituted tissues. With regard to the last point, the Episkin™-SIT RhE tissues are seeded on a dermal substitute consisting of a collagen type I matrix coated with type IV collagen; whereas the EpiDerm™ 200-SIT are cultured on specially prepared cell culture inserts; and the SkinEthic™ SIT on an inert 0.5 cm2 polycarbonate filter. The detailed protocols for the three RhE models are described hereafter.

5.2.3. Method description according to EU Guideline B.46 & proposed OECD TG

a) Functional model conditions

- The OD of the extracted solvent alone should be <0.05, and the OD of the negative control (NC) should fall within the acceptable ranges established by the RhE model developer / supplier.
- The *stratum corneum* should be sufficient to resist the rapid penetration of cytotoxic marker substances, e.g., SDS or Triton X-100, as estimated by IC_{50} OR ET_{50}.
- Histological examination should demonstrate human epidermis-like structure (including multilayered *stratum corneum*).
- Reproducibility over time with an appropriate batch control should be demonstrated
- Quality control: cell viability and/or barrier function measured for each batch of the epidermal model used should fall within pre-established acceptability ranges of the validated reference methods. Only results produced with qualified tissues can be accepted for reliable prediction of irritation effects.

b) Number of replicates

At least three replicates should be used for each test substance and for the controls in each run. One run should be sufficient when the classification is unequivocal. However, in cases of borderline results, a second run should be considered, as well as a third one in case of discordant results between the first two runs.

c) Dose and application of the test substance

Liquids: should uniformly cover the skin surface. Minimum: 25 µl/ cm^2.

Solids: should uniformly cover the skin surface. Minimum: 25 mg/ cm^2. The epidermis should be moistened with deionised or distilled water and then the solid substance applied to evenly cover the skin. Whenever possible, solids should be tested as a fine powder.

d) Positive and negative controls

Concurrent negative and positive controls should be used for each study

Suggested positive control: 5% aqueous SDS

Suggested negative controls: water or phosphate buffered saline (PBS)

e) Exposure time

The exposure time should be optimised for each RhE model, e.g., 15 to 60 min, at 20 to 37°C (for details see table 12).

f) Washing

At end of exposure time, the test substance should be carefully washed from the epidermis surface with aqueous buffer or 0.9% NaCl.

g) Post-treatment incubation
Viability measurements are not performed immediately after exposure to the test substance. A sufficiently long post-treatment time should allow for recovery from weakly irritant effects and for appearance of clear cytotoxic effects, e.g., 42 hours is used with EPISKIN™ SIT.

h) Cell viability measurement
Skin samples are placed in MTT solution of appropriate concentration (e.g., 0.3 to 1 mg/ml) for 3 hours. The precipitated blue formazan product is then extracted using a solvent (e.g., isopropanol, acidic isopropanol), and the concentration of formazan determined by measuring the OD at 570 nm.

i) Non-specific interaction with MTT
If a test substance 1) acts directly on the MTT, or 2) has a natural colour or becomes coloured during tissue treatment in a way that interferes with the MTT measurements, additional controls should be used to detect and correct for test substance interference with the viability measurements. Detailed description of how to test and correct direct MTT reduction is shown in table 13. Non-specific interferences due to these interferences should not exceed 30 % of the negative control (for corrections). If non specific interferences are > 30 %, the test substance might be considered as incompatible with the test.

j) Acceptability criteria
- tissues treated with negative controls should exhibit OD not below historical established lower boundaries
- tissues treated with positive controls (e.g., 5% aqueous SDS) should fall within pre-defined ranges that reflect the ability of the tissues to respond to an irritant substance under the conditions of the test method (e.g., viability $\leq$ 40%).
- variability between tissue replicates should be appropriate and fall within pre-defined ranges (e.g., SD $\leq$ 18).

k) Interpretation of results
The OD values obtained for each test sample are used to calculate the percentage of viability relative to the negative control, which is set at 100%. The cut-off value of percentage cell viability distinguishing irritant from non-classified test substances, and the statistical procedure(s) used to evaluate the results, should be defined, documented and proven to be appropriate. For example, the cut-off values for the prediction of irritation associated with the three RhE validated models are the following.

EPISKIN™ SIT, EpiDerm™ EPI-200-SIT and SkinEthic™ SIT-42bis

***In vitro* result**	***In vivo* prediction**
Mean tissue viability* $\leq$ 50%	Irritant (GHS & EU CLP Cat. 2, EU DSD R38)
Mean tissue viability* $>$ 50%	Non-irritant (no category, no classification)

*after exposure and post-treatment incubation

5.2.4. Comparison of validated RhE protocols
The details of the principal protocol components for the validated RhE models EPISKIN™ SIT, EpiDerm™ EPI-200-SIT, and SkinEthic™ SIT-42bis to be used within EU guideline B.46 and the draft proposed OECD Test Guideline for *in vitro* skin irritation testing are shown in Table 12. The practical steps to be taken to detect and correct for non-specific MTT reduction and/or colour interference are described in Table 13.

Table 12: Principal protocol components of the RhE models for skin irritation testing based on the SOPs of the validation and catch-up studies (see appendixes 7 - 9)

	EPISKIN™ SIT	**EpiDerm™ EPI-200-SIT***	**SkinEthic™ SIT[-42bis]**
Non specific MTT interference prior check	See table 13 for procedures.	See table 13 for procedures. *Note: If the test substance interfering with the MTT measurements (due to interfering colouration or MTT reduction) is classified as irritant by the SIT (tissue viability < 50%), the correction procedures may not be necessary.*	See table 13 for procedures. *Note: A table grid is used to decide which controls to be carried out depending on whether medium colouration, tissue staining or MTT interaction are observed (see SOP in annex 9).*
Tissue conditioning	Follow SOP instructions.	Follow SOP instructions.	Follow SOP instructions.
N. of replicates	3 tissues (replicates) for each test material.	3 tissues (replicates) for each test material.	3 tissues(replicates) per test substance, negative control and positive controls.
Treatment doses and application	Liquids: 10 µl (26.3 µl/cm²) with positive displacement pipette applied on the top of epidermis, and gently spread to ensure covering of all surface. Solids: 10 ± 2 mg (26.3 µl/cm²) crushed to fine powder applied to the epidermis surface to which 5 µl of distilled water was added to improve contact between powder and epidermis surface. Gently spread if necessary. Viscous/sticky materials: 10 ± 2 mg (26.3 µl/cm²) applied with curved edge spatula (weigh 12± 2 mg to compensate the product remained in spatula). Gently spread onto the surface to cover all surface.	Liquids: 30 µl applied atop the tissue and spread. If necessary, place nylon mesh on tissue surface to improve spreading. Semisolids: 30 µl using positive displacement pipette. Spread, if necessary. Solids: 25 mg crushed and grinded to powder into tissues where 25 µl DPBS was applied shortly before application to improve contact of test material with epidermis. Gently shake the inserts to improve spreading of solids. Spread if necessary. Waxes: form a flat 'cookie like' piece of about 8 mm diameter and place atop the tissue, wetted with sterile DPBS. *Note: Compatibility of the test material with the nylon mesh needs to be checked. For that purpose, place mesh on slide and apply 30 µl test sample. After 60 min exposure check using microscope whether mesh was damaged. If so, test material shall be applied without the mesh.*	Liquids: 16 ± 0.5 µl (32µg/cm^2) on the top of epidermis using a positive displacement pipette. Gently spread the substance on the epidermis surface. Carefully apply a nylon mesh. Solids: 16 ± 2 mg (32mg/cm^2) crushed and grind material when necessary, applied into tissues where 10 µl distilled water was applied before application to improve contact between the powder and the epidermis. Sticky substances: 16 ± 2 mg (32mg/cm^2) spread on tarred nylon mesh. Apply the test material coated side of the nylon mesh on the epidermal surface and spread gently.
Controls	- Negative control: PBS, 3 tissues. - Positive control: 5% aq. SDS applied 15 min, re-spread after 7 min. One positive control with 3 tissue replicates, should be included in each run (1 per day).	- Negative control: DPBS, 3 tissues. - Positive control: 5% aq. SDS to be tested concurrently with the test samples in each assay. No more than one positive control with three replicates is required per day.	- Negative control: PBS, 3 tissues. - Positive control: 5% aq. SDS, 3 tissues.
Exposure time	15 ± 0.5 min in ventilated cabinet at RT (19-23°C).	60 ± 1 min. The first 35 min after treatment at 37°C, 5% CO_2, 95% relative humidity. The remaining time at RT in sterile hood.	42 ± 1 min at RT.
Washing	Rinse thoroughly with 25 ml sterile PBS filling and emptying the tissue inserts to remove all test material. Place units on absorbent paper and remove remaining PBS by gently taping, and sweep surface with cotton-bud.	Rinse tissues with sterile DPBS filling and emptying inserts 15 times to remove test material. Submerge the inserts 3 times in 150 ml DPBS and shake to remove all test material. Then, rinse tissue from inside and from outside with DPBS. Remove excess DPBS by gently shaking the insert and blot insert on sterile blotting paper. Dry the surface with cotton swab.	Rinse thoroughly 25 times with 1 ml PBS to remove all test material from the epidermal surface. Empty the insert and dry the insert bottom on sterile absorbent paper or gauze. Sweep the surface of epidermis with both ends of a cotton tip.

Table 12 continued: Principal protocol components of the RhE models for skin irritation testing based on the SOPs of the validation and catch-up studies (see appendixes 7 - 9)

	EPISKIN™ SIT	**EpiDerm™ EPI-200-SIT***	**SkinEthic™ SIT-42bis**
Post-treatment incubation time	42 ± 1 hours at 37°C, 5% CO_2, 95% humidified atmosphere (in culture medium).	42 ± 2 hours at 37°C, 5% CO_2, 95% relative humidity (in culture medium).	42 ± 1 hours at 37°C, 5% CO_2, 95% humidified atmosphere (in culture medium).
Released mediators (optional)	After the post-treatment incubation time, shake 15 ± 2 min at 300 rpm to homogenise the released mediators in the medium before sampling. Freeze and stock 1.6 ml of incubation medium.	Collect medium at 24± 2 hours after treatment: shake plates 10 min at 500 rpm/min (or pipette medium up & down 3 times), collect the medium (samples can be stored at -20°C), and transfer inserts in new plates with fresh medium and place back in incubator for 18± 2 hours.	After the post-treatment incubation time, homogenize the culture medium by gentle agitation (300 rpm), transfer 3x 500µl for each tissue in tubes and freeze at -20°C until analyses. Freeze also the culture medium used as diluent.
Cell viability	Place insert with treated tissues together with 2 ml MTT solution (0.3 mg/ml in assay medium). Incubate 3 hours (± 5 min) at 37°C, 5% CO_2, 95% humidified atm. Place tissue units on absorbent paper, make a biopsy of the epidermis by using biopsy punch, separate epidermis from collagen matrix with the aid of forceps and place both parts (turn epidermis topical side against the collagen matrix) into microtubes. Add 500 µl acidic isopropanol (0.04 N HCl in isopropanol). Plug tube, mix thoroughly using a vortex mixer and ensure all biological material is immersed in the solvent. Extract formazan either 4h at RT (18-23°C) with vortex mixing at the middle of the incubation period, or 72h at 4°C, protected from light. Mix with vortex until solution colour becomes homogeneous. If suspended cell fragments are present centrifuge at 500 rpm. Transfer 2 x 200 µl per tissue in 96-well plate (2 wells per tissue) and read OD at 570±30 nm (without the classical 630 nm reference filter since it is still within the absorption curve of formazan).	Place insert in wells containing 300 µl of MTT solution (1 mg/ml in assay medium). Incubate 3 hours (± 5 min) at 37°C, 5% CO_2, 95%rh. Rinse three times with DPBS, and ensure tissues are dry. Immerse inserts by adding 2 ml isopropanol in each insert. Extract formazan for at least 2 hours at RT with shaking (~ 120rpm), or overnight at RT without shaking in dark and shake 15 min on plate shaker before using the extracts. Pierce the inserts with an injection needle and allow the extract to run into well (discard inserts). Pipette up and down 3x until the extractant solution is homogeneous. For each tissue, transfer2 x 200 µl in 96-well plate and read OD between 540 and 595, preferably at 570 nm without reference filter.	Transfer treated tissues in pre-filled wells with 300 µl of MTT solution (1 mg/ml in maintenance medium). Incubate 3 hours (± 5 min) at 37°C, 5% CO_2, 95% humidified atmosphere. Dry insert bottom of treated tissues and transfer inserts in pre-filled wells with 800µl isopropanol. Add 700 µl isopropanol to completely cover the tissue. Extract formazan for 2 hours (±5 min) at RT with gentle agitation (about 150 rpm). Pierce the inserts and the polycarbonate filter with a tip to get the extraction solution in the corresponding well. Homogenize the extraction solution by pipetting 3 times up and down. Transfer 3x 200 µl extraction solution per well in a 96 well plate. Read OD at 570 nm (eventually between 540 to 600 nm). Use isopropanol solution as blank.
Data interpretation	% viability = (OD treated tissue – mean OD blank) x100 / (mean (OD negative controls – mean OD blanks))	% viability = (OD treated tissues) x100 / (mean OD negative controls)	Tissue viability = (OD treated tissue – OD blank) *100/ (OD negative control – OD blank)
Acceptance Criteria	1. Negative control (PBS treated) absolute OD should be above historical established lower boundary of the confidence interval, i.e., OD value of the 3 tissues ≥ 0.6 and the SD of % viability is ≤ 18. 2. Positive control (5% aq. SDS) mean viability should be ≤ 40% (of negative control) and the SD ≤ 18. 3. Batch acceptance: samples from one batch are acceptable if both positive and negative controls fulfil the acceptance criteria 4. Chemical data acceptance: if SD > 18 the chemical is retested once. If 2 or 3 batches give SD > 18 the assay is not repeated (variability may be linked to the test material itself)	1. Negative control (DPBS treated) absolute mean OD_{570} should ≥ 1.0 and ≤ 2.5 2. Positive control (5% aq. SDS) mean viability should be ≤ 20% (of negative control) and within the 95±1% confidence interval of historical data. 3. Standard Deviation of % tissue viability from 3 replicates should be < 18%.	1. Negative control (PBS): mean OD_{570} value of 3 tissues ≥ 1.2, and SD ≤ 18%. 2. Positive control (5% aq. SDS): mean viability < 40% of the negative control, and SD ≤ 18%. 3. Batch acceptance: samples from one batch are acceptable if both positive and negative controls fulfil the acceptance criteria 4. Test substance data acceptance criteria: if SD > 18 the chemical is retested once. In this case all batches are used to calculate the final mean, except if technical problems are identified for a batch.
Prediction Model	See section 3.2.3.k.	See section 3.2.3.k.	See section 3.2.3.k.

* a visual description of the protocol is also available at Kandárová *et al.* (2009)

Table 13: Practical steps to be taken to detect and correct for non-specific MTT reduction and/or MTT interference with colouration of the test substance based on the SOPs of the RhE skin irritation models (see appendixes 7 - 9).

		EPISKIN™ SIT	**EpiDerm™ EPI-200-SIT**	**SkinEthic™ SIT-42bis**
Non-specific colour interference	Intrinsic test material colour or ability to become coloured	Add 10µl (liquids) or 10 mg (solids) to 90 µl water in transparent recipient. Mix 15 min, and check colour. If coloured solution is observed, the staining ability of the test chemical should be checked as described below.	Add 30µl (liquids) or 25 mg (solids) to 0.3 ml deionized water in transparent recipient. Incubate 60 min at 37°C, 5% CO_2, 95% relative humidity. Shake and evaluate presence and intensity of staining. If solution changes colour significantly, the potential to stain viable tissues should be checked as described below	Applicable to colouring test substances or dye test substances able to stain RHE tissues.
	Test material interference with the viability measurements	At least 1 chemical-treated tissue and 1 negative control per run should be carried out. Apply 10µl (liquids) or 10 mg (solids) of test chemicals onto viable tissue and run a PBS control in parallel. Follow normal test procedures, but replace the MTT incubation by incubation with the assay medium without MTT. Calculate the non-specific colour as follows: NSC (%) = (OD of treated tissues without MTT) *100/ (OD negative controls – mean OD blanks) If the NSC is > 5% and ≤ 30% the following corrections of OD values are needed: True relative viability (%) = (OD of treated tissues – OD of treated tissues without MTT) x100 / (OD negative control – mean OD blanks) Test chemicals with NSC ≤ 5% should be evaluated by using the common procedure.	Apply 30µl (liquids) or 25 mg (solids) of test material to one viable tissue. In parallel expose a tissue to DPBS (negative control). Follow testing procedures, but incubate the tissue for 3 hours in culture media without MTT instead of incubating in media with MTT. Rinse and extract the tissues using 2.0 ml isopropanol and measure OD at 570 nm. If the extract from the tissues treated with test material has an OD between 5 and 30% of the negative control tissue, the test material should be further tested on more tissues. The corrected OD is calculated as follows: Real OD = OD coloured tissue (MTT assay) – OD coloured tissue (no MTT assay) If OD of extract from treated tissues is < 5% of negative control and the tissue viability (determined in the MTT assay) is not close to the classification cut-off (50%), correction of the results is not necessary.	Use living RhE tissues, and follow SkinEthic steps up to post- treatment incubation step (see table 12). Incubate tissues in maintenance medium for 3 hours. Contact with MTT shall be avoided. Dry the insert bottom of treated tissues, transfer inserts in wells containing isopropanol and add 700µl isopropanol to completely cover each tissue. Incubate 2 h at RT with gentle agitation (about 150 rpm). Pierce tissue and polycarbonate filter with a tip to get extraction solution in corresponding well. Homogenize the extraction solution by pipetting 3 times up and down. Transfer 3x 200 µl extraction solution per well in a 96 well plate. Read OD at 570 nm. Use isopropanol as blank. Calculate the non-specific staining (NSS) as follows: NSS (%) = (OD of treated tissues without MTT) *100/ (OD negative control PBS without MTT) True relative viability (%) = (OD of treated tissues – OD of treated tissues without MTT) x100 / (OD negative control –OD blanks)
	Limitations	If the NSC is > 30%, additional steps must be taken or the chemical shall be considered as incompatible with the test.	If OD of extract from treated tissues is > 30% of negative control, additional steps and expert judgment must determine if test material may be considered incompatible with the test.	If the NSS is > 30% relative to the negative control, additional steps must be taken if possible, or the test material must be considered incompatible with the test.
Non-specific MTT reduction interference	Direct interaction of test material with MTT	2ml MTT (0.3 mg/ml) + 10µl (liquid) or 10 mg (solid) test material. Incubate 3 hours at 37°C protected from light. If MTT solution colour becomes blue or purple, the substance interacts with MTT. It is then necessary to determine the part of OD due to non specific reduction of the MTT as described below.	1ml MTT (1 mg/ml) + 30µl (liquid) or 25 mg (solid) test material. Incubate 1 hour at 37°C, 5% CO_2, 95% relative humidity. Untreated MTT medium is used as control. If MTT solution turns blue/purple, the test material reduces MTT and additional testing as described below must be performed.	300µl MTT (1mg/ml) + 16µl (liquid) or 16 mg (solid) test material. Mix and incubate 3 hours at 37°C protected from light. Use water as negative control. If treated MTT solution becomes blue or purple, the test material interacts with the MTT. It is then necessary to evaluate the part of OD due to non-specific reduction of MTT by using killed epidermis as described below.

Table 13 continued: Practical steps to be taken to detect and correct for non-specific MTT reduction and/or MTT interference with colouration of the test substance based on the SOPs of the RhE skin irritation models (see appendixes 7 - 9).

		EPISKIN™ SIT	**EpiDerm™ EPI-200-SIT**	**SkinEthic™ SIT-42bis**
	Test material interference with the viability measurements	Preparation of killed tissues Replace culture medium with 2 ml distilled water. Incubate 48h +/1h at 37°C, 5% CO_2, 95% humidity. Discard water and freeze dried epidermis at -18°C to 20°C (can be stored up to 6 months). Treatment of killed tissues Three killed issues and three killed negative control tissues should be used. All killed tissues must be from the same batch. These tissues follow the same treatment steps as the living tissues. De-freeze tissues at RT, 1h in 2ml maintenance medium. Use thawed tissues similar to living tissues (see table 12) Data correction The OD due to the non specific reduction needs to be substracted before calculating the true cell viability, as follows: True relative viability (%) = [OD of treated viable cells – (OD of treated killed tissues –OD untreated killed tissues)] x100 / (OD negative control – mean OD blanks)	Freeze-killed tissues are obtained from Mattek Corporation. Treatment of killed tissues Apply MTT reducing chemical in two freeze-killed tissues and use two untreated freeze-killed tissues as controls. Data correction If interference by the test material is < 30% of the negative control value, the following correction should be made: True viability = viability of treated tissue – interference from test material = OD of treated viable tissue – (mean OD of treated killed tissues – mean OD of untreated killed tissues)	Preparation of killed tissues Place living epidermis at -20°C (or -80°C) for at least 48 hours. Treatment of killed tissues Use 3 killed treated tissues for each MTT-interacting substance, and 3 killed untreated tissues as negative controls. Thaw killed tissues before use on 300 µl maintenance medium for 1 hour at RT. Follow the same protocol as the living tissues (see table 12). Data correction True relative viability (%) = [OD of treated viable cells – (OD of treated killed tissues –OD untreated killed tissues)] x100 / (OD negative control – mean OD blanks)
	Limitations	If the non specific MTT reduction (NSMTT) is > 30%, either additional steps must be taken if possible, or the chemical must be considered as non compatible with the assay. NSMTT (%) = (OD treated killed tissues –OD untreated killed tissues) x100 / (OD negative control – mean OD blanks)	If interference by the test material is greater than 30% of the negative control value, additional steps must be taken or the test material may be considered incompatible with the test system.	If the non specific MTT reduction (NSMTT) is > 30% relative to the negative control, additional steps must be taken if possible, or the test substance must be considered as incompatible with the test. NSMTT (%) = (OD treated killed tissues –OD untreated killed tissues) x100 / (OD negative control – mean OD blanks)
Non specific colour and MTT reduction combined together		Not described.	Not described.	If (NSS+NSMTT) is > 30% of the negative control, additional steps must be undertaken if possible, or the test substance must be considered incompatible with the test. Otherwise the following formula should be used: True relative viability (%) = [OD of treated viable cells – (true OD treated tissues without MTT) + (true OD treated killed tissues)] x100 / (OD negative control – mean OD blanks)

5.2.5. Proficiency testing and performance standards

Prior to routine use of a validated method that adheres to the guidelines, laboratories should demonstrate technical proficiency, using the proficiency substances as recommended in the EU B.46 test method and OECD draft guideline (Table 14).

In addition, normal human keratinocytes should be used to construct the epithelium. Multiple layers of viable epithelial cells (basal layer, stratum spinosum, stratum granulosum) should be present under a functional *stratum corneum*. *Stratum corneum* should be multilayered containing the essential lipid profile to produce a functional barrier with robustness to resist rapid penetration of cytotoxic marker substances, e.g. sodium dodecyl sulphate (SDS) or Triton X-100. The barrier function should be demonstrated either by determination of the concentration at which a marker substance reduces the viability of the tissues by 50 % (IC50) after a fixed exposure time, or by determination of the exposure time required to reduce cell viability by 50 % (ET50) upon application of the marker substance at a specified, fixed concentration. The containment properties of the model should prevent the passage of material around the *stratum corneum* to the viable tissue, which would lead to poor modelling of skin exposure. The skin model should be free of contamination by bacteria, viruses, mycoplasma, or fungi.

Table 14. Proficiency substances recommended in the EU B.46 and proposed OECD test guidelines

Substance	**CAS Number**	***In vivo* score**	**Physical state**	**GHS category***
naphthalene acetic acid	86-87-3	0	Solid	No Cat.
isopropanol	67-63-0	0,3	Liquid	No Cat.
methyl stearate	112-61-8	1	Solid	No Cat.
heptyl butyrate	5870-93-9	1,7	Liquid	No Cat. (Optional Cat. 3)
hexyl salicylate	6259-76-3	2	Liquid	No Cat. (Optional Cat. 3)
cyclamen aldehyde	103-95-7	2,3	Liquid	Cat. 2
1-bromohexane	111-25-1	2,7	Liquid	Cat. 2
butyl methacrylate (*potassium hydroxide 5% aq.)* **	97-88-1 (1310-58-3)	3 (3)	Liquid (Liquid)	Cat. 2 (Cat. 2)
1-methyl-3-phenyl-1-piperazine	5271-27-2	3,3	Solid	Cat. 2
Heptanal	111-71-7	4	Liquid	Cat. 2

* For the EU, the UN GHS optional category 3 is considered as no category.
** The EU guideline suggests butylmethacrylate, whereas the OECD draft proposed Test Guideline suggests “potassium hydroxide (5% aq.)”, information relative to potassium hydroxide 5% aq. is shown in brackets.

For novel similar (me-too) test methods developed under the official test guidelines that are structurally and functionally similar to the validated reference methods or for modifications of validated methods, pre-defined performance standards should be used to demonstrate comparable reliability and accuracy of the new test method prior to its use for regulatory testing. In that case, generally a dossier with the description of the new or modified test method and all relevant information and results are submitted to an international validation body, such as ECVAM or ICCVAM, which in their turn make an official statement on the validity of the test method for regulatory uses.

The performance standards of the *in vitro* RhE models for skin irritation were originally established based on the EU DSD classification system (using the R38 risk phrase versus non classification). It can be found in the appendix of the EU guideline B.46, and includes a list of 20 recommended reference substances. However, following the adoption of the EU CLP classification system in December 2008 (based on the UN GHS classification system), the cut-off scores to distinguish irritant

and non classified substances shifted from 2.0 to 2.3 (see section 1.4.5), so that substances with an *in vivo* score between 2.0 and 2.3 which were previously considered irritants will be considered non-irritants under the EU CLP classification system. As a consequence the performance standards were reviewed proposing a new list of reference chemicals with a balanced distribution according to the new EU CLP, as well as new predictive capacity and reproducibility standards to be m*et al*so based on the new EU CLP. The new performance standards for the EU CLP classification system can be found at ESAC (2009b) and at ECVAM (2009) and were taken up in the OECD draft proposal for a new test guideline on *in vitro* skin irritation (OECD, 2009c).

5.3. Known applicability and limitations

The reconstructed human epidermis tests falling under the EU B.46 guideline classify substances as skin irritants according to GHS Cat. 2. However, it does not allow classifying substances as mild irritants according to the optional GHS Cat. 3, nor does it provide adequate information on skin corrosion. Depending on member country or regional regulatory requirements, all non-category 2 chemicals may be considered non-classified (non category). Thus, regulatory requirements in member countries will decide if this test method will be used as a skin irritation replacement test (i.e., in the EU), as a screening test, or as part of a tiered testing strategy in a weight of evidence approach. In the EU, the RhE skin irritation test method allows the hazard identification of irritant substances as well as non-classified substances.

The reconstructed human epidermis model systems are expected to be generally applicable across a wide range of physico-chemical properties relevant to the current world of industrial commerce (Eskes *et al.*, 2007). The methods are applicable to test solids, liquids, semi-solids and waxes. However, gases and aerosols have not been assessed yet in a validation study. Chemicals classified as irritants based on persistent effects, i.e., showing effects below the threshold tissue scores present at d 14 in at least 2 out of 3 animals, could also not be included in the validation study. Out of the ~5'000 screened chemicals only one was found to be classified on the basis of persistence, and available from the supplier. However, it had insufficient information to allow its inclusion in the study (Eskes *et al.*, 2007). Furthermore non-corrosive acids, bases, salts, other inorganic substances, hydroperoxides, phenols and surfactants were not included or were only included to a limited extent in the validation study. However some of these categories may have been included in the 48 chemicals used in the optimisation study that led to the formal validation study of the EPISKIN™ model using the same SIT protocol as well as in a post-validation study which evaluated the skin irritancy of 184 cosmetic ingredients using the validated EPISKIN™ SIT protocol (Cotovio *et al.*, 2005, 2008).

Another limitation of the *in vitro* reconstructed human epidermis assay for skin irritation is in the case of a test substance interference with the formazan measurements, which is greater than 30% of the negative control value (due to colouration properties of the test substances or to MTT reduction by the test substance). In that case additional steps must be taken into account or the test substance may be considered as incompatible with the test system.

5.4. Comparison to the *in vivo* test method

Morphologically, the adopted *in vitro* reconstructed human epidermis methods are closer to the human epidermis as compared to the rabbit skin. Although these models do not present all functional complexity that exist *in vivo* (including the dermis and its components such as hair follicules, subaceous glands, nerve and immune cells, which could play a role in the mechanisms of skin irritation), *in vitro* reconstructed human epidermis models using multiple endpoint analyses seem to have good correlation with the results of the human patch test as shown by Welss *et al.* (2007).
The main endpoint considered in the EU B.46 and OECD proposed test guideline is cell viability, based on the principle that irritant substances are able to penetrate the *stratum corneum* by diffusion and are cytotoxic to the cells in the underlying layers. The *in vitro* test methods cover mainly the initial mechanisms of skin irritation occurring in the *in vivo* test (figure 9). The evaluation of the release of

Interleukin 1 alpha considered by ESAC as a useful adjunct to increase sensitivity of the assay without reducing specificity (ESAC, 2007), could give additional insight on the release of inflammatory mediators that may act in the subsequent mechanistic cascade of events occurring during skin irritation reactions.

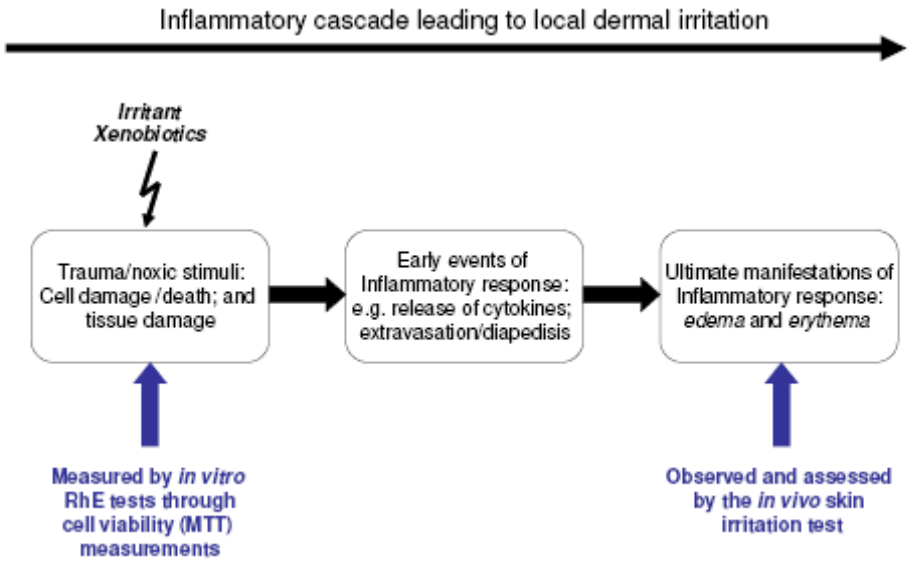

Figure 9. Extract from the Explanatory Background Document to the OECD Draft test guideline on *in vitro* skin irritation testing (Griesinger *et al*., 2009). Schematic representation of the inflammatory cascade leading to local acute dermal irritation.

A summary of the major components of the regulatory *in vivo* and *in vitro* reconstructed human epidermis methods for skin irritation is shown in Table 15. The exposure times used by the adopted *in vitro* RhE assays are in general shorter as compared to those used *in vivo* (15 - 60 min *in vitro* versus 4 hours *in vivo*). Similarly the post-treatment time is shorter *in vitro* with respect to the *in vivo* test (42 hours versus 14 days). These differences could be due to the more simple structure of the skin components involved in skin irritation reactions present in the *in vitro* models with respect to the *in vivo* situation. Moreover, the doses applied *in vitro* (26.3 to 47.6 μl or mg/cm^2) are smaller with respect to those applied *in vivo* (~83.3 μl or mg / cm^2).

Unlike the *in vivo* test, the *in vitro* assays make systematically use of positive and negative controls to check for the functionality of the test method. In addition, the EU B.46 and proposed OECD TG recommend also ensuring the technical proficiency of the assays, by the laboratory, prior to the routine use of the *in vitro* assays by testing a list of recommended proficiency chemicals.

Overall, the adopted *in vitro* assay for skin irritation is considered as a full replacement to the traditional *in vivo* assay for acute skin irritation, if only the two recommended GHS categories for irritancy are applied by the regulatory authorities of member countries. The assays are able to distinguish between irritants (GHS Cat. 2 or EU DSD R38) and non classified materials. However the assays do not allow classifying substances in the optional GHS Cat. 3 as mild irritants, nor does it provide adequate information on skin corrosion. Furthermore, the ability of the assays to detect irritants classified on the basis of persistence could not be assessed due to the poor availability of such test substances in around 5000 screened substances from the industrial commerce (see section 3.3). Due to such apparent scarce occurrence, the need to detect test substances classified based on persistence only may be questionable.

Finally, it is to be noted that the *in vitro* assays for skin irritation were validated for the purposes of testing chemicals, following internationally agreed principles of validation, which recommends that the tested chemicals are of the highest available purity (OECD, 2005). As a consequence their use was demonstrated to be relevant and reliable for testing chemicals within the framework of the EU

Dangerous Substance Directive. Up to day, their applicability to test formulation and/or dilutions was not evaluated in formal validation studies by e.g., ECVAM or ICCVAM, due to the scientific difficulties in including the large existing variety of possible formulations and/or dilutions in a comprehensive formal validation study. However, during the discussions of the OECD Expert Consultation Meeting in defining the new OECD Test Guideline for skin irritation, it was recommended that: "the RhE *in vitro* methods are empirical testing methods and directly address the initial step of the inflammatory cascade/mechanism of action (cell damage and tissue damage resulting in localized trauma). Therefore, there is no scientific reason to assume that these methodologies are not applicable to all substances and mixtures, unless there is specific information that provides evidence regarding such limitations" (Griesinger *et al*, 2009).

Table 15. Comparison of the principal method components of the regulatory accepted *in vivo and in vitro* tests for skin irritation

	***In vivo* test for skin irritation (OECD TG 404)**	***In vitro* human skin model (EU B.46 and draft OECD TG)**
Model used	Albino rabbit.	Three-dimensional reconstructed human epidermis, consisting of organized basal, spinous and granular layers, and a multilayered *stratum corneum.* Surface of tissue models: 0.38 cm^2 for Episkin™-SIT, 0.63 cm^2 for EpiDerm™ 200-SIT and 0.5 cm^2 for SkinEthic™ SIT.
Number of replicates	2 to 3 animals based on severity of effects.	3 replicates for each test material.
Dose and application of test substance	0.5 ml (liquids) or 0.5 g (solids) applied to ~ 6 cm^2 of skin and covered with a gauze patch (~ 83.3 µl or mg / cm^2). Solids might be moisten to ensure good skin contact.	Liquids: 10 to 30 µl (26.3 to 47.6 µl/cm^2 depending on model). Solids: 10-25 mg (26.3 to 39.7 mg / cm^2 depending on model). Tissues should be moisten prior to solid application to ensure good contact with the RhE.
Controls	Potential influence of the vehicle on irritation of the skin by the test substance should be minimal, if any.	Negative control: PBS Positive control: 5% aqueous SDS
Exposure time	4 hours	15 to 60 min. depending on the model (see table 12 for details).
Washing	At the end of exposure time to remove test substance	At the end of exposure time to remove test substance.
Post-treatment incubation time	If no corrosive effects seen, the animal is observed up to 14 days.	After washing, the exposure time is followed by a post-treatment incubation time of 42 hours to allow for recovery from weakly irritant effects and for appearance of clear cytotoxic effects.
Endpoint assessed	- Grading of skin reactions. - Other reactions such as: defatting of skin, clinical signs of toxicity and body weight, persistence of alopecia, hyperkeratosis, hyperplasia and scaling. - Histopathology may be carried out in case of equivocal responses.	Cell viability based on the premise that irritant substances are able to penetrate the *stratum corneum* by diffusion and are cytotoxic to the cells in the underlying layers. Inflammatory mediators such as Interleukin 1alpha was considered as a useful adjunct to increase sensitivity of the assay without reducing specificity.
Interpretation of results	Classification systems as shown in section 1.4. In the EU, two categories for skin irritation (irritants and no category). For GHS a third optional category for mild irritants.	Distinguishes GHS Cat. 2 from non category, and R38 from non labelled.
Limitations	- May overpredict human responses. - May be variable between laboratories. - Does not assess repetitive low-dose exposure. - Has the potential to cause discomfort or pain to laboratory animals.	- Not designed to distinguish the optional GHS Cat. 3 for mild irritants, corrosive substances, gases and aerosols. - Not applicable to test materials presenting non-specific interaction with MTT greater than 30% of negative control.

6 (Q)SARs for Skin Corrosion and Irritation

Several structure-activity relationships are reported for skin irritation and corrosion. For skin corrosion, the approach usually followed is to classify chemicals by using Principal Component Analyses. Amongst the different proposed structure-activity relationships for skin corrosion, the Skin Irritation Corrosion Rules Estimation Tool (SICRET) is one example. Despite its name, SICRET is not a computational tool, but a tiered assessment approach that uses physicochemical property limits, structural alerts and *in vitro* tests to classify chemicals. A detailed review of (Q)SARs for skin corrosion can be found at Gallegos-Saliner *et al.* (2008) and Zuang *et al.* (2005).

For skin irritation, commercially available expert systems are available such as DEREK for Windows, TOPKAT and HazardExpert. In addition, a publically available (Q)SAR model for the prediction of local irritation/corrosion potential has been developed by the German Federal Institute for Risk Assessment (BfR) based on data compiled from the EU chemicals notification procedures (Gerner *et al.*, 2004). A Decision Support System (DSS) for the prediction of skin and/or eye lesion potential was built from the information extracted from this database. The DSS combines SARs defining reactive chemical substructures relevant for local lesions to be classified, and QSARs for the prediction of the absence of such a potential (Gerner *et al.*, 2004). The BfR-DSS has been designed to predict EU risk phrases and has been evaluated for its validity by the European Chemicals Bureau as described below (Rorije and Hulzebos, 2005). Besides the BfR-DSS, a handful of other (Q)SAR models are available in the public literature. However, these have been developed for specific chemical classes such as organic acids, bases, phenols, alcohols and esters, and there is still a need to fully characterize the most promising models (for review see Gallegos-Saliner *et al.*, 2008 and Zuang *et al.*, 2005).

In 2007, a guidance document on the validation of (Q)SAR models has been developed by the OECD (OECD GD 69, 2007). It was agreed that "*the validation of (Q)SAR models for regulatory purposes are best carried out by the regulatory authorities of the member countries*" and that "*to facilitate the consideration of a (Q)SAR model for regulatory purposes, it should be associated with the following information (for details see OECD GD69, 2007):*
1. a defined endpoint;
2. an unambiguous algorithm;
3. a defined domain of applicability;
4. appropriate measures of goodness-of-fit, robustness and predictivity;
5. a mechanistic interpretation, if possible."

Up to date, the BfR-DSS model for skin irritation appears to be the only model that has undergone transparent evaluation and external validation (Rorije and, Hulzebos, 2005). The rule-base appeared to be useful for regulatory purposes, as almost all OECD principles on (Q)SARs were met and the (external) predictivity for skin irritation was shown to be good. The BfR-DSS was applicable to 146 out of 201 tested substances, and resulted in the correct prediction of 145 out of 145 non corrosives, 85 out of 85 non irritants. However, it led to the misclassification of 1 out of 1 skin corrosive and 3 out of 3 skin irritants as non-classified. Recommendations were made to improve the shortcomings of the existing exclusion rules, i.e., for setting the cut-off values of the rules at a consequent "safe" level (not allowing for any exception to the rule in the training set) and for including a consistently calculated safety margin. Overall, the rules were considered straightforward, easy to interpret, easily accessible and based on (measured) physico-chemical data that is available for every substance that has to be notified in the EU. Moreover, it was concluded that its use in combination with the OECD guideline 404 had the potential for saving animal lives, time and money (Rorije and, Hulzebos, 2005). It is to be noted nevertheless that the external validation appeared to be carried with a vast majority of non classified substances versus classified ones (142 versus 4), resulting in an unbalanced data-set which might compromise the statistical significance of the estimated sensitivity of the assay.

Other publically available (Q)SAR models for skin irritation, appear to concur reasonably with respect to the OECD principles, the mechanistic interpretability of the descriptors is strong, training sets are provided and algorithms are in many cases well defined. However, there remains a need to

characterize the most promising models further. In that sense, a considerable effort to promote the availability of valid (Q)SARs has been undertaken by the European Chemicals Bureau (Gallegos-Saliner *et al.*, 2008). In addition there is a need to extend the scope of the available models by developing new models covering other chemical classes as appropriate, as well as exploring the feasibility of combining various models together on the basis of common mechanisms of action (Gallegos-Saliner *et al.*, 2008).

The applicability of (Q)SARs for predicting skin irritation and/or corrosion have been also assessed in a few studies available in literature. Mombelli (2008) has shown that TOPKAT for skin irritation was applicable to the majority of the chemicals tested (81 out of 90), and presented good sensitivity and specificity, with 74% (29 out of 39) of skin irritants being correctly identified, and 66% (25 out of 38) of non irritants being correctly identified. On the other hand, DEREK and HAZARDEXPERT for skin irritation were applicable only to a few substances (7 out of 116 chemicals for DEREK, and 17 out of 116 for HAZARDEXPERT). Moreover, the few non irritant substances to which both models could be applied were all misclassified as false-positives (2 out of 2 for DEREK, and 4 out of 4 for HAZARDEXPERT).

Similarly, Hoffmann *et al.* (2008) have shown that TOPKAT for skin irritation had the larger applicability, where predictions could be derived for 70 out of 98 chemicals, and that DEREK for windows for skin irritation was applicable to only 4 our of 100 tested chemicals. The BfR-DSS for skin irritation was found to have a somehow intermediate applicability, being able to predict the irritancy or non-irritancy of 31 out of 98 tested chemicals. The authors have then performed a feasibility study to investigate how a combination of *in silico*, *in vitro* and *in vivo* information could be applied in the assessment of skin irritation hazard. They used a database of 100 existing and new chemicals and have assessed a number of strategies, both animal-free and inclusive of animal testing. The best animal-free test strategy was shown to be a combination of TOPKAT, BfR-DSS and the EPISKIN™ *in vitro* model. However such combination resulted in predictive capacity values almost identical as the EPISKIN™ *in vitro* model as a stand-alone test. The difference in costs was also considered marginal by the authors since the number of chemicals to be tested in EPISKIN™ was reduced only by eight when taking into account the expert system information (Hoffmann *et al.*, 2008).

Overall, the QSAR models available appear quite promising, however further development, validation and documentation of *in silico* systems for local toxicity to the skin and eye are necessary. In addition, many existing models have not been developed for current regulatory purposes, so they would need to be investigated for their regulatory applicability, and possibly refined accordingly.

(Q)SAR systems might not permit full replacement by themselves as stand-alone methods for regulatory purposes, but they are generally recognized as a valuable tool for screening and for prioritization. The application of QSAR models is recognized to be restrictive in size due to e.g., limited purity, chemical classes and sufficient similar structure available in their training sets. *In vitro* data sources may prove helpful in extending the scope of existing models and in developing new models to cover other chemical classes (Gallegos-Saliner *et al.*, 2008).

As a consequence, the most effective approach for using QSAR predictions might be to integrate all appropriate information to make a weight-of-evidence-based assessment of the chemical hazard and risk. Integrated Testing Strategies combining all possible sources of information from (Q)SARs, expert systems, read-across and other grouping approaches, and test methods (especially *in vitro* tests) is generally recognized as the most likely use of *in silico* tools in the mid term (Gallegos-Saliner *et al.*, 2008; Hartung and Hoffmann, 2009). However, the use of (Q)SAR models may still be considered on case-by-case basis, after the evaluation of its scientific validity and taking into account the applicability of the models.

7 On–going research programs and validation

ECVAM has been recently involved in the evaluation and formal validation of the performance under UN GHS of three *in vitro* reconstructed human epidermis models for skin irritation that were previously validated based on the EU DSD classification system (ESAC, 2009b). Moreover it has been involved in the adaptation of the reference chemicals and defined accuracy values of the ECVAM performance standards for skin irritation based on the updated performances of the validated *in vitro* assays under UN GHS (ESAC, 2009b; ECVAM, 2009). ECVAM was also involved in the regulatory acceptance of the test method B.46 on *in vitro* skin irritation testing. Currently, ECVAM is working in supporting the acceptance of such guideline at the OECD level, and has drafted the proposed OECD test guideline on *in vitro* skin irritation which is currently in consultation (OECD, 2009c).

On the other hand, ICCVAM plans to determine how corrosive substances that have produced false negative results in the *in vitro* corrosivity test methods will act in the *in vitro* dermal irritation test method protocols. ICCVAM also plans to evaluate the combination (or battery) of *in vitro* test methods for evaluating skin corrosivity and irritation, and to evaluate the usefulness and limitations of alternative dermal irritation test methods for U.S. regulatory testing (ICCVAM-NICEATM, 2009).

To overcome the current limitations of the validated *in vitro* assays isolated activities are taking place to enlarge the applicability of *in vitro* methods to those test materials that present non specific formazan interactions higher than 30% of the negative control. Tornier *et al.* (2009), using the SkinEthic^{TM} SIT^{-42bis} test method for skin irritation, have shown that histological analyses may be useful to provide evidence of tissue damage and identify skin irritancy potential for these currently incompatible test substances. Furthermore, McNamee *et al.* (2009), using the EpiOcular model for eye irritation, have investigated the use of HPLC/UPLC to detect formazan separately from intrinsically coloured test materials. The authors showed that such technique may allow extension of the applicability of the assays to intrinsically coloured materials that interfered with formazan when using the standard photometry. They also concluded that such results may be applicable to other reconstructed tissue models using viability measurement via the MTT assay, such as the RhE for skin irritation.

New reconstructed human epidermis models have also been developed and challenged with the set of reference compounds recommended in the ECVAM performance standards. These encompass:

- The EST-1000 model from CellSystems which is in prevalidation phase to addresses the new UN-GHS classification for skin irritation.
- The RhE LabCyte EPI-MODEL for skin irritation which seems to have met the ECVAM performance standards (Katoh *et al.*, 2009).
- The Leiden human epidermal (LHE) model that has been evaluated for determining skin corrosion and irritation, and with which corrosion classifications were obtained in concordance with those reported for the validated skin models EPISKIN^{TM} and EpiDerm^{TM} based on the OECD TG 431 recommended 12 reference chemicals (El Ghalbzouri *et al.*, 2008).

However none of these assays have been yet formally evaluated by an international validation body such as ECVAM or ICCVAM.

Otherwise relevant research activities which have recently taken place include:

- The development of reconstructed epidermis models which are able to determine the skin irritant potency of chemicals and not only to distinguish irritants from non irritants (Spiekstra *et al.*, 2009).
- The development of an innervated *in vitro* model of human skin including sensory neurons derived from embryonic rat dorsal root ganglion as neural components (Khammo *et al.*, 2007). The aim is to integrate the sensory neuronal components which are usually present in the skin and may play a role *in vivo* in the production of neurogenic inflammation leading to sensory irritation and pain (Garle and Fry, 2003).

- The development of an organotypic model based on rat epidermal keratinocytes for skin irritation testing, using cell membrane integrity (determined by the LDH assay) and pro-inflammatory effects (determined by IL-1alpha release) as endpoints (Pappinen and coworkers, 2005)
- The investigation of the usefulness of toxicogenomics for predicting acute skin irritation on *in vitro* reconstructed human epidermis. Borlon and coworkers (2007) have shown that about 16 genes out of 240 were found to be significantly and differentially expressed between tissues exposed to irritant and non-irritant chemicals, in the same way whatever the irritant compound applied. The authors concluded that the differential gene expressions might represent new or additional endpoints useful for the mechanistic understanding and perhaps also the hazard assessment of the skin irritation potential of chemicals and formulations.

8 Future prospects and recommendations for achieving animal replacement

It can be considered that today in Europe, validated and adopted *in vitro* methods allow for the full replacement of the animal test for identifying and classifying compounds as skin corrosives, skin irritants, and non irritants.

However the applicability of such validated assays in the regulatory assessment still requires some consideration. Indeed, if the validated assays allow all to distinguish corrosives from non-corrosives, the majority of the validated and adopted *in vitro* assays for skin corrosion do not allow for the sub-categorisation of corrosive substances into the optional UN GHS Cat. 1A, 1B and 1C as introduced in the new EU CLP classification scheme. Only the Corrositex® membrane barrier test described in the OECD TG 435 was considered valid to distinguish the three UN packaging groups which are similar to the three GSH subcategories, however only for the limited applicability of acids, bases and their derivatives which meet the technical requirements of the assay. In the EU, there is a need to clarify whether the three GHS subcategories may be systematically required or whether the most severe category could be applied (i.e., category 1A) since most of the adopted assays are not capable of distinguishing the three GHS optional subcategories.

With regard to skin irritation, *in vitro* test models were validated and adopted as full replacements to distinguish between irritants (GHS Cat. 2 or EU DSD R38) and non classified test substances. The assays were not designed to identify and classify substances as mild irritants according to the optional GHS Cat. 3. Depending on the member country or regional regulatory requirements, all non-category 2 chemicals may be considered non-classified (non category). Thus, regulatory requirements in member countries other than in the EU will decide if this test method will be used as a skin irritation replacement test (i.e., in the EU), as a screening test, or as part of a tiered testing strategy in a weight of evidence approach.

In addition, the development of harmonised guidelines to assess *in vitro* data submitted for regulatory purposes would favour harmonisation and standardisation in the regulatory assessment and definition of hazard properties of test materials. In particular, the development of guidelines for the assessment of the *in vitro* methods applied to test preparations and/or dilutions of test materials might be useful as the passed validation studies on skin corrosion and irritation mainly focused on test substances.

In particular, it may be helpful to clarify what is meant in the OECD guidelines by ‘mixtures’. Furthermore, it may be useful to further characterise the suitability of the currently validated and adopted alternative methods to estimate Derived No-Effect Levels (DNELs) on skin irritation/corrosion for a given test material. The DNEL is the level of exposure above which humans should not be exposed. DNELs for irritation/corrosion can only be derived if dose-response information is available. Acute, sub-acute or sub-chronic toxicity studies in animals by the dermal route may be able to provide this information, provided that symptoms of irritation/corrosion are recorded and reported in relevant studies. Also human data, primarily from reliable evidence of symptoms caused by occupational exposures, may have dose-response information. However, similar to the acute skin irritation / corrosion test, validated and accepted *in vitro* methods to replace this endpoint can give information on the hazard of the tested material. However, these methods were not designed to derive information such as DNEL (ECHA, 2008c). A dose-response assessment is therefore difficult to make for irritation and corrosion simply because these guidelines require data produced with undiluted chemicals. From a risk characterisation perspective it is therefore advisable to use the outcome of the classification procedure, i.e., a substance that is classified is assumed to be sufficiently characterised. However, a complete risk assessment requires both hazard, as well as dose-response data. Consequently, if the latter are available, they must be taken into account.

Finally, the combination of the adopted *in vitro* test methods with other recommended test methods in e.g., intelligent testing strategies might require further investigation in order to address issues such as:

- the identification of the most suitable strategies based on its performance and on the combination of the individual alternative methods currently available (e.g., TOPKAT, BfR-DSS, HAZARDEXPERT, DEREK, SICRET, EPISKIN™, EPIDERM™, SkinEthic™, EST-1000, TER, Corrositex®, EPISKIN™- SIT, EpiDerm™ EPI-200-SIT, SkinEthic™ SIT^{42bis});
- the definition of harmonised decision criteria for proceeding or not proceeding to the next step of a test strategy;
- the definition of transparent and harmonised criteria to perform weigh-of-evidence evaluation of existing data;
- the definition of clear guidelines on how to validate intelligent testing strategies, and/or evaluate the scientific relevance and reliability of such strategies;
- the evaluation of the usefulness of the *in vivo* test as a last step and if relevant the update and/or deletion of the OECD TG 404 accordingly.

9 Appendixes: available on request

Appendix 1: Invittox 118: EPISKIN™ Skin Corrosivity Test

Appendix 2: Invittox 119: EPIDERM™ Skin Corrosivity Test

Appendix 3: SOP *In vitro* Skin Corrosion: SkinEthic™ human skin model test

Appendix 4: SOP: Study on *In vitro* Skin Corrosion using Epidermal Skin Test (EST-1000)

Appendix 5: Invittox 115: Rat Skin Transcutaneous Electrical Resistance test

Appendix 6: Invittox 116: Corrositex® Continuous Time Monitor Assay

Appendix 7: SOP ECVAM Skin Irritation Validation Study: *in vitro* human epidermis model EPISKIN™

Appendix 8: Protocol for *in vitro* EpiDerm™ Skin Irritation Test (EPI-200-SIT)

Appendix 9: SOP SkinEthic™ Skin Irritation Test-42bis

10 References

Borlon C, Godard P, Eskes C, Hartung T, Zuang V, Toussaint O (2007). The usefulness of toxicogenomics for predicting acute skin irritation on *in vitro* reconstructed human epidermis. *Toxicology* **30**, 157-166

Botham PA, Chamberlain M, Barratt MD, Curren RD, Esdaile DJ, Gardiner JR, Gordon VC, Hildebrand B, Lewis RW, Liebsch M, Logemann P, Osborne R, Ponec M, Régnier J-F, Steiling W, Walker AP & Balls M (1995) A prevalidation study on *in vitro* skin corrosivity testing. The report and recommendations of ECVAM workshop 6. *ATLA* **23**, 219-255.

Botham PA, Earl LK, Fentem JH, Roquet R, Van de Sandt JJM (1998). Alternative methods for skin irritation testing: the current status. ECVAM Skin Irritation Task Force Report 1. *ATLA* **26**, 195-211.

Conseil Fédéral Suisse - CFS (2009). Ordonnance n. 813.11 sur la protection contre les substances et preparations dangereuses (Ordonance sur les produite chimiques, OChim), du 18 mai 2005, état le 1er février 2009.

Cotovio J., Grandidier M.-H., Portes P., Roguet R., Rubinstenn G. (2005). The *in vitro* acute skin irritation of chemicals : optimisation of the EPISKIN prediction model within the framework of the ECVAM validation process. *ATLA* **33**, 329-349.

Cotovio J., Lelièvre D., Grandidier M.-H., Roguet R., Tinois-Tessoneaud E., Leclaire J. (2008). *In vitro* acute skin irritancy of 184 cosmetic ingredients using the validated EPISKIN™ protocol. Poster presented at the 15th Congress on Alternatives to Animal Experimentation, Linz, Austria. Available at: http://www.altex.ch/resources/cotovio2.pdf. Accessed on 22.01.2010.

Draize, J.H., Woodard, G. & Calvery, H.O. (1944). Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *Journal of Pharmacology and Experimental Therapeutics* **82**, 377-390.

EC (1986). Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes. *Official Journal* **L358**, 1-28.

EC (1999). Directive 1999/45/EC of the European Parliament and of the Council of 31 May 1999 concerning the approximation of laws, regulations and administrative provisions of the Member States relating to the classification, packaging and labelling of dangerous preparations. *Official Journal of the European Communities* **L 200**, 1-68.

EC (2000). Annex I to Commission Directive 2000/33/EC adapting to technical progress for the 27th time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. *Official Journal of the European Communities* **L136**, 91-97.

EC (2001). Commission Directive 2001/59/EC of 6 August 2001 adapting to technical progress for the 28th time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. *Official Journal of the European Communities* **L 225**, 1-333.

EC (2006). Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council

Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. Official Journal of the European Union **L 396**, 1-849.

EC (2008a). Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). *Official Journal of the European Union* **L142**, 1-739.

EC (2008b). Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on Classification, Labelling and Packaging of substances and mixtures , amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. *Official Journal of the European Union* **L353**, 1-1355.

EC (2009). Annex III. Method B.46. *In vitro* Skin Irritation: Reconstructed Human Epidermis Model Test. pp. 24-35. In: Commission Regulation (EC) No 761/2009 of 23 July 2009 amending for the purpose of its adaptation to technical progress, Regulation No. 440/2008 laying down test methods pursuant to Regulation (EC) No. 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). *Official Journal of the European Union* **L220**, 1-94.

ECHA (2008a). R.7.2. Skin- and eye irritation/corrosion and respiratory irritation. In: Guidance on information requirements and chemical safety assessment. Chapter R.7a: Endpoint Specific Guidance, p. 199-255. Available at: http://guidance.echa.europa.eu/docs/guidance_document/information_requirements_r7a_en.pdf?vers=20_08_08. Accessed on 17.09.2009.

ECHA (2008b): Guidance on Information Requirements and Chemical Safety Assessment. Available at: http://guidance.echa.europa.eu/docs/guidance_document/information_requirements_en.htm?time= 1232447649. Accessed on 5.10.2009.

ECHA (2008c): Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.8: Characterisation of dose (concentration)-response for human health. Available at: http://guidance.echa.europa.eu/docs/guidance_document/information_requirements_r8_en.pdf?vers=20_08_08. Accessed on 22.01.2010.

ECHA (2009). Guidance on the Application of the CLP criteria. Guidance to regulation EC N. 1272/2008 on classification, labeling and packagind (CLP) of substances and mixtures. 528 pp. Available at: http://guidance.echa.europa.eu/docs/guidance_document/clp_en.pdf . Accessed on 22.12.2009.

ECVAM (2009). Updated performance standards for *In-Vitro* skin irritation test methods based on Reconstructed Human Epidermis (RhE). Available from internet at http://ecvam.jrc.ec.europa.eu/ under Validation Study Documents. Accessed on 17.09.2009.

El Ghalbzouri A, Siamari R, Willemze R, Ponec M. (2008). Leiden reconstructed human epidermal model as a tool for the evaluation of the skin corrosion and irritation potential according to the ECVAM guidelines. *Toxicology In vitro* **22**, 1311-1320.

ESAC (1998a). Statement on the scientific validity of the EPISKIN™ test (an *in vitro* test for skin corrosivity). *ATLA* **26**, 277-280.

ESAC (1998b). Statement on the scientific validity of the rat skin transcutaneous electrical resistance (TER) test (an *in vitro* test for skin corrosivity). *ATLA* **26**, 275-277.

ESAC (2000). Statement on the application of the Epiderm™ human skin model for skin corrosivity testing. *ATLA* **28**, 365-366.

ESAC (2001). Statement on the application of the CORROSITEX® assay for skin corrosivity testing. *ATLA* **29**, 96-97.

ESAC (2006). Statement on the application of the SkinEthic™ human skin model for skin corrosivity testing. Available at: http://ecvam.jrc.ec.europa.eu/ under "Publications", "ESAC statements". Accessed on 18.09.2009.

ESAC (2007) Statement on the validity of *in vitro* tests for skin irritation. Available at: http://ecvam.jrc.ec.europa.eu/ under "Publications", "ESAC statements". Accessed on 18.09.2009.

ESAC (2008). Statement on the Scientific Validity of *In vitro* tests for Skin Irriation testing (SkinEthic and modified EpiDerm). Available at: http://ecvam.jrc.ec.europa.eu/ under "Publications", "ESAC statements", accessed on 18.09.2009.

ESAC (2009a). ESAC Statement on the scientific validity of an *in vitro* test method for skin corrosivity testing. Available at: http://ecvam.jrc.ec.europa.eu/ under "Publications", "ESAC statements". Accessed on 18.09.2009.

ESAC (2009b). Statement on the Performance under UN GHS of three *in vitro* assays for Skin Irritation testing and the Adaptation of the Reference chemicals and defined accuracy values of the ECVAM Skin Irritation Performance Standards. Available at: http://ecvam.jrc.ec.europa.eu/ under "Publications", "ESAC statements". Accessed on 18.09.2009.

Eskes C, Cole T, Hoffmann S, Worth A, Cockshott A, Gerner I, Zuang V (2007). The ECVAM international validation study on *in vitro* tests for acute skin irritation: selection of test chemicals. *ATLA* **35**, 603-619.

Fentem, J. H., Archer, G. E. B., Balls, M., Botham, P. A., Curren, R. D., Earl, L. K., Esdaile, D. J., Holzhütter, H. G. & Liebsch, M. (1998). The ECVAM international validation study on *in vitro* tests for skin corrosivity. 2. Results and evaluation by the Management Team. *Toxicology in vitro* **12**, 483-524.

Fentem J.H., Briggs D., Chesné C., Elliott G.R., Harbell J.W., Heylings J.R., Portes P., Roguet R., Van De Sandt J.J.M., Botham P.A. (2001). A prevalidation study on *in vitro* tests for acute skin irritation: results and evaluation by the Management Team. *Toxicoloy in vitro* **15**, 57-93.

Gallegos Saliner A, Patlewicz G & Worth AP (2008). A Review of (Q)SAR Models for Skin and Eye Irritation and Corrosion. *QSAR & Combinatorial Science* **27**, 49-59.

Garle MJ, Fry JR. (2003). Sensory nerves, neurogenic inflammation and pain: missing components of alternative irritation strategies? A review and a potential strategy. *ATLA* **31**, 295-316.

Gerner I, Spielmann H, Hoefer T, Liebsch M, Herzler M.(2004). Regulatory use of (Q)SARs in toxicological hazard assessment strategies. *SAR QSAR Environ Res* **15**, 359-66.

Griesinger C., Barroso J., Zuang V., Cole T., Genschow E, Liebsch M. (2009). Explanatory background doucment to the OECD draft test guideline on *in vitro* skin irritation testing. Available at: http://ecvam.jrc.it/ under “Validation Study Documents” – “ Skin Irritation” – “3. Performance of the three ECVAM validated *in vitro* skin irritation methods under CLP (UN GHS)”. Accessed on 20.01.2010.

Grindon C., Combes R., Cronin M.T.D., Roberts D.W., Garrod J.F. (2008) Integrated decision-tree testing strategies for skin corrosion and irritation with respect to the requirements of the EU REACH legislation. *ATLA* **36** S1, 65-74.

Hartung T., Hoffmann S. (2009). Food for Thought… on *In silico* Methods in Toxicology. *ALTEX* **26**, 155-166.

Heylings J.R., Diot S., Esdaile D.J., Fasano W.J., Manning L.A., Owen H.M. (2003). A prevalidation study on the *in vitro* irritation function test (SIFT) for prediction of acute skin irritation *in vivo*: results and evaluation of ECVAM phase III. *Toxicology In vitro* **17**, 123-138.

Hoffmann S., Cole T., Hartung T. (2005). Skin irritation: prevalence, variability, and regulatory classification of existing *in vivo* data from industrial chemicals. *Regulatory Toxicology and Pharmacology* **41**, 159-166.

Hoffmann S, Saliner AG, Patlewicz G, Eskes C, Zuang V, Worth AP (2008). A feasibility study developing an integrated testing strategy assessing skin irritation potential of chemicals. *Toxicol Lett.* **180**, 9-20

ICCVAM-NICEATM (2009). Draft implementation plan for the 2008-2012 NICEATM-ICCVAM five year plan. Available at: http://iccvam.niehs.nih.gov/docs/5YRImplement/Draft_5-Year_Implementation_Plan_June-2009.pdf. Accessed on 13.10.2009.

Kandárová H., Liebsch M., Genschow E., Gerner I., Traue D., Slawik B., Spielmann H. (2004). Optimisation of the EpiDerm test protocol for the upcoming ECVAM validation study on *in vitro* skin irritation tests. *ALTEX* **21**, 107-114.

Kandárová H., Liebsch M., Gerner I., Schmidt E., Genschow E., Traue D., Spielmann H. (2005). The EpiDerm test protocol for the upcoming ECVAM validation study on *in vitro* skin irritation tests – an assessment of the performance of the optimized test. *ATLA* **33**, 1-17.

Kandárová, H., Liebsch, M., Spielmann, H., Genschow, E., Schmidt, E., Guest, R., Whittingham, A., Warren, N., Gamer, A., Remmele, M., Kaufmann, T., Wittmer, E., De Wever, B., Rosdy, M., (2006). Assessment of the SkinEthic Reconstituted Human Epidermis for skin corrosion testing according to OECD guideline 431. *Toxicology in vitro* **20**, 547–559.

Kandárová H, Hayden P, Klausner M, Kubilus J, Sheasgreen J. (2009). An *in vitro* skin irritation test (SIT) using the EpiDerm reconstructed human epidermal (RHE) model. *J Vis Exp*. **13**, pii: 1366. doi: 10.3791/1366.

Katoh M, Hamajima F, Ogasawara T, Hata K. (2009). Assessment of human epidermal model LabCyte EPI-MODEL for *in vitro* skin irritation testing according to European Centre for the Validation of Alternative Methods (ECVAM)-validated protocol. *J Toxicol Sci*. **34**,327-34.

Khammo N, Ogilvie J, Clothier RH (2007). Development of an innervated model of human skin. *ATLA* **35**, 487-491.

Liebsch, M., Traue, D., Barrabas, C., Spielmann, H., Uphill, P., Wilkins, S., Wiemann, C., Kaufmann, T., Remmele, M. & Holzhütter, H. G. (2000). The ECVAM prevalidation study on the use of EpiDerm for skin corrosivity testing. *ATLA* **28**, 371-401.

Louekari K., Sihvonen K., Kuittinen M., Somnes V. (2006). *In vitro* tests within the REACH information strategies. *ATLA* **34**, 377-389.

Macfarlane M., Jones P., Goebel C., Dufour E., Rowland J., Araki D., Costabel-Farkas M., Hewitt N.J., Hibatallah J., Kirst A., McNamee P., Shellauf F., Scheel J. (2009). A tiered approach to the use of alternatives to animal testing for the safety assessment of cosmetics: Skin irritation. *Regulatory Toxicology and Pharmacology* **54**, 188-196.

McNammee P., Faller C., Goebel C., Pfuhler S. Sieber T. (2009). Use of HPLC/UPLC instead of photometry for evaluation of MTT in *in vitro* irritation assays based on human reconstructed tissue for assessment of coloured test materials. Poster n. 155. VIIth World Congress on Alternatives and Animal Use in the Life Sciences, Rome, Italy.

Mombelli E. (2008). An evaluation of the predictive ability of the QSAR software packages, DEREK, HAZARDEXPERT and TOPKAT, to describe chemically-induced skin irritation. *ATLA* 36, 15-34.

OECD (2002). *OECD Guidelines for the Testing of Chemicals No. 404: Acute Dermal Irritation/Corrosion.* Paris, France: Organisation for Economic Cooperation and Development, 13pp.

OECD (2004a) *OECD Guidelines for the Testing of Chemicals No. 431. In vitro Skin Corrosion: Human Skin Model Test.* Paris, France: Organisation for Economic Cooperation and Development. 8p.

OECD (2004b). *OECD Guidelines for the Testing of Chemicals No. 430: In vitro Skin Corrosion: Transcutaneous Electrical Resistance Test (TER).* Paris, France: Organisation for Economic Cooperation and Development. 12pp.

OECD (2005) Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment. Environmental Health and Safety Monograph Series on Testing and Assessment No. 34. Available at: http://www.olis.oecd.org/olis/2005doc.nsf/LinkTo/NT00002EAE/$FILE/JT00188291.PDF. Accessed on 12.10.2009.

OECD (2006) *OECD Guidelines for the Testing of Chemicals No. 435. In vitro Membrane Barrier Test Method for Skin Corrosion.* Paris, France: Organisation for Economic Cooperation and Development. 15 pp.

OECD (2007). Guidance document on the validation of (Quantitiative) Structure-Activity Relationships ((Q)SAR) models. OECD Environmental Health and Safety Monograph Series on Testing and Assessment No. 69. Available at: http://www.olis.oecd.org/olis/2007doc.nsf/LinkTo/NT00000D92/$FILE/JT03224782.PDF. Accessed on 13.10.2009.

OECD (2009a). *OECD Guidelines for the Testing of Chemicals No. 431. In vitro Skin Corrosion: Reconstructed Human Epidermis (RhE) Test Method.* Paris, France: Organisation for Economic Cooperation and Development. 15 pp. Website: http://www.oecd.org/dataoecd/63/6/43302385.pdf. Accessed on 21.09.2009.

OECD (2009b). *OECD Guidelines for the Testing of Chemicals No. 430. In vitro Skin Corrosion: Transcutaneous Electrical Resistance Test (TER).* Paris, France: Organisation for Economic Cooperation and Development. 18 pp. Website: http://www.oecd.org/dataoecd/14/2/43295353.pdf. Accessed on 12.10.2009.

OECD (2009c). Draft Proposal for a new guideline. *In vitro* Skin Irritation: Reconstructed Human Epidermis (RhE) Test Method. Available at: http://www.oecd.org/dataoecd/1/59/43664841.pdf . Accessed on 08.10.2009.

NIH (1999). *Corrositex®: an in vitro test method for assessing dermal corrosivity potential of chemicals*. NIH Publication No. 99-4495. Research Triangle Park, NC, USA: NIEHS.

Pappinen S, Pasonen-Seppänen S, Suhonen M, Tammi R, Urtti A. (2005). Rat epidermal keratinocyte organotypic culture (ROC) as a model for chemically induced skin irritation testing. *Toxicology and Applied Pharmacology* **208**, 233-241.

Patrick and Maiback H (1994). Chapter 21. Dermatology. *In* Principles and methods of toxicology. Third edition (Hayes A.W. ed.). Raven Press, New York, 767-803.

Portes P., Grandidier M.H., Cohen C., Roguet R. (2002). Refinement of the EPISKIN protocol for the assessment of acute skin irritation of chemicals: follow-up to the ECVAM prevalidation study. *Toxicology In vitro* **16**, 765-770

Robinson M.K., Osborne R., Perkins M.A. (2000). *In vitro* and human testing strategies for skin irritation. *Ann. NY Acad. Sci.* **919**, 192-204.

Robinson M.K., Cohen C., de Brugerolle de Fraissinette A., Ponec M., Whittle E., Fentem J.H. (2002). Non-animal testing strategies for assessment of the skin corrosion and skin irritation potential of ingredients and finished products. *Food and Chemical Toxicology* **40**, 573-592.

Rorije E., Hulzebos E. (2005). Evaluation of (Q)SARs for the prediction of skin irritation/corrosion potential. Physico-chemical exclusion rules. Available at: http://ecb.jrc.ec.europa.eu/qsar/information-sources/ under '(Q)SAR Documents' – 'Evaluationo of (Q)SARs for the prediction of eye/skin irritation/corrosion potential' – 'Download report (skin)'. Accessed on 13.10.2009.

Spiekstra SW, Dos Santos GG, Scheper RJ, Gibbs S. (2009). Potential method to determine irritant potency *in vitro* - Comparison of two reconstructed epidermal culture models with different barrier competency. *Toxicology In vitro* **23**, 349-355.

Spielmann H, Hoffmann S, Liebsch M, Botham P, Fentem JH, Eskes C, Roguet R, Cotovio J, Cole T, Worth A, Heylings J, Jones P, Robles C, Kandárová H, Gamer A, Remmele M, Curren R, Raabe H, Cockshott A, Gerner I, Zuang V (2007). The ECVAM international validation study on *in vitro* tests for acute skin irritation: report on the validity of the EPISKIN and EpiDerm assays and on the Skin Integrity Function Test. *ATLA* **35**, 559-601

Tornier C., Alépée N., Bertino B., Kon-Man P., Cotovio J., de Brugerolle de Fraissinette A. (2009) *In vitro* skin irritation assessment of coloring test substances using the validated SkinEthic™ RHE "42bis" test method. Poster n. 406. VIIth World Congress on Alternatives and Animal Use in the Life Sciences, Rome, Italy.

UN (2001). Part 2. Classification. In Recommendations on the Transport of Dangerous Goods - Model Regulations. Twelfth revised edition. Website: http://www.unece.org/trans/danger/publi/unrec/English/Part2.pdf . Accessed on 08.10.2009.

United Nations-Economic Commission for Europe (UN/ECE) (2003). *Globally Harmonised System of Classification and Labelling of Chemicals (GHS)*. Part 3 Health and Environmental Hazards – Chapter 3.3 Serious eye damage / eye irritation. New York, USA, and Geneva, Switzerland: United Nations. p.137-150.

United Nations-Economic Commission for Europe (UN/ECE) (2009). Globally Harmonised System of Classification and Labelling of Chemicals (GHS). Updated Part 3 Health and Environmental Hazards – Chapter 3.3 Serious eye damage / eye irritation. New York, USA, and Geneva, Switzerland: United Nations. p.133-144. Available at: http://www.unece.org/trans/danger/publi/ghs/ghs_rev03/English/03e_part3.pdf. Accessed on 26.02.2010.

Van de Sandt J., Roguet R., Cohen C., Esdaile D., Ponec M., Corsini E., Barker C., Fusenig N., Liebsch M., Benford D., de Brugerolle de Fraissinette A., Fartasch M. (1999). The Use of Human Keratinocytes and Human Skin Models for Predicting Skin Irritation. The Report and Recommendations of ECVAM Workshop 38. *ATLA* **27**, 723-743.

Weil C.S., Scala A. (1971). Study of intra- and inter- laboratory variability in the results of rabbit eye and skin irritation tests. *Toxicology and Applied Pharmacoogy* **19**, 276-360.

Welss T., Basketter D.A., Schroder K.R. (2004). *In vitro* skin irritation: facts and future. State of the art review of mechanisms and models. *Toxicology in vitro* **18**, 231-243.

Welss T, Matthies W, Schroeder KR. (2007). Compatibility testing *in vitro*: a comparison with *in vivo* patch test data. *Int J Cosmet Sci* **29**, 143.

Worth A.P., Cronin M.T.D. (2001a). The use of bootstrap resampling to assess the variability of Draize tissue scores. *ATLA* **29**, 557-573.

Worth, A.P. & Cronin, M.T.D. (2001b). The use of pH measurements to predict the potential of chemicals to cause acute dermal and ocular toxicity. *Toxicology* **169**, 119-131.

York M., Griffiths H.A., Whittle E. Basketter D.A. (1996). Evaluation of a human patch test for the identification and classification of skin irritation potential. *Contact Dermatitis* **34**, 204-212.

Zuang V., Balls M., Botham P.A., Coquette A., Corsini E., Curren R.D., Elliott G.R., Fentem J.H., Heylings J.R., Liebsch M., Medina J., Roguet R., van de Sandt H., Wiemann C., Worth A.P. (2002). Follow-up to the ECVAM Prevalidation Study on *In vitro* Tests for Acute Skin Irritation. ECVAM Skin Irritation Task Force Report 2. *ATLA* **30**, 109-129.

Zuang V., Alonso M.-A., Botham P.A., Eskes C., Fentem J., Liebsch M., van de Sandt J.J.M. (2005). Subchapter 3.2. Skin Irritation. In Alternative (non-animal) Methods for Cosmetics Testing: Current Status and Future Prospects (Eskes C., Zuang V. eds). *ATLA* **33-**S1, 35-46.