For Official Use **ENV/JM/TG(2015)14**

Organisation de Coopération et de Développement Économiques
Organisation for Economic Co-operation and Development **20-Feb-2015**

English - Or. English

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

Test Guidelines Programme

DRAFT UPDATED TEST GUIDELINE 435 ON IN VITRO MEMBRANE BARRIER TEST METHOD FOR SKIN CORROSION

27th Meeting of the Working Group of National Coordinators of the Test Guidelines Programme (WNT)

14-17 April 2015, OECD Headquarters, Paris

Ms. Anne Gourmelon
Tel: +33 (0) 1 45 24 98 49; E-mail: anne.gourmelon@oecd.org

JT03370879

Complete document available on OLIS in its original format

ENV/JM/TG(2015)14
For Official Use

English - Or. English

Note from the Secretariat

This document contains the draft revised Test Guideline 435 on the *In Vitro* Membrane Barrier Test Method for Skin Corrosion. The revision of this Test Guideline is part of the project to revise all skin irritation and corrosion Test Guidelines, following the publication of the Guidance Document on Integrated Approaches to Testing and Assessment of Skin Irritation and Corrosion in 2014, and the WNT decision to separate the Performance Standards from the Test Guidelines themselves. This was also the opportunity to harmonise text of the various Guidelines where possible, bearing in mind though that they were developed at different times and each has a different history.

Each of the Test Guidelines was circulated twice to the WNT: July-August 2014 and December 2014-January 2015. For this Test Guideline (TG 435), there were relatively few changes beyond those mentioned above; information was added when requested, e.g. on the prediction model of Corrositex® test method. It should be noted that mixtures (simple mixtures and product formulations) were used during the validation of the test method; hence a disclaimer in relation to the utility of TG 435 for the testing of mixtures is probably not warranted in this case. A combined meeting of the Expert Groups on Skin and Eye Irritation was held in November 2014 at OECD [see ENV/JM/TG/M(2014)5] to address comments from the first WNT round. The compilation of comments and responses from the recent round is available in document [ENV/JM/TG/RD(2015)6].

ACTION REQUIRED ***The WNT is invited to review the draft updated Test Guideline 435 and approve it, amended as appropriate.***

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

DRAFT REVISED GUIDELINE 435:

In Vitro Membrane Barrier Test Method for Skin Corrosion

INTRODUCTION

1. Skin corrosion refers to 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 chemical as defined by the United Nations (UN) Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (1). This updated Test Guideline 435 provides an *in vitro* membrane barrier test method that can be used to identify corrosive chemicals. The test method utilizes an artificial membrane designed to respond to corrosive chemicals in a manner similar to animal skin *in situ*.

2. Skin corrosivity has traditionally been assessed by applying the test chemical to the skin of living animals and assessing the extent of tissue damage after a fixed period of time (2). Besides the present Test Guideline, a number of other *in vitro* test methods have been adopted as alternatives (3) (4) to the standard *in vivo* rabbit skin procedure (OECD TG 404) used to identify corrosive chemicals (2). The UN GHS tiered testing and evaluation strategy for the assessment and classification of skin corrosivity and the OECD Guidance document on Integrated Approaches to Testing and Assessment (IATA) for Skin Irritation/Corrosion recommend the use of validated and accepted *in vitro* test methods under modules 3 and 4 (1) (5). The IATA describes several modules which group information sources and analysis tools and provides guidance on (i) how to integrate and use existing the test and non-test data for the assessment of the skin irritation and skin corrosion potentials of chemicals and (ii) proposes an approach when further testing is needed, including when negative results are found (5). In this modular approach, positive results from *in vitro* test methods can be used to classify a chemical as corrosive without the need for animal testing, thus reducing and refining the use of animals in and avoiding the pain and distress that might occur if animals were used for this purpose.

3. Validation studies have been completed for the *in vitro* membrane barrier test method commercially available as Corrositex® (6)(7)(8), showing an overall accuracy to predict skin corrosivity of 79% (128/163), a sensitivity of 85% (76/89), and a specificity of 70% (52/74) for a database of 163 substances and mixtures (7). Based on its acknowledged validity, this validated reference test method (VRM) has been recommended for use as part of a tiered testing strategy for assessing the dermal corrosion hazard potential of chemicals (5) (7). Before an *in vitro* membrane barrier test method for skin corrosion can be used for regulatory purposes, its reliability, relevance (accuracy), and limitations for its proposed use should be determined to ensure that it is similar to that of the VRM (9), in accordance with the pre-defined performance standards (PS) (10). The Mutual Acceptance of Data will only be guaranteed after any proposed new or updated test method following the PS of this Test Guideline have been reviewed and included in this Test Guideline. Currently, only one *in vitro* test method is covered by this Test Guideline, the commercially available Corrositex® test method. .

4. Other test methods for skin corrosivity testing are based on the use of reconstituted human skin (OECD TG 431) (3) and isolated rat skin (OECD TG 430) (4). This Test Guideline also provides for

subcategorisation of corrosive chemicals into the three UN GHS Sub-categories of corrosivity and the three UN Transport Packing Groups for corrosivity hazard. This Test Guideline was originally adopted in 2006 and updated in 2015 to refer to the IATA guidance document and update the list of proficiency substances.

DEFINITIONS

5. Definitions used are provided in Annex 1.

INITIAL CONSIDERATIONS AND LIMITATIONS

6. The test described in this Guideline allows the identification of corrosive test chemicals and allows the sub-categorisation of corrosive test chemicals according to the UN GHS (Table 1) (1). In addition, such a test method may be used to make decisions on the corrosivity and non-corrosivity of specific classes of chemicals, *e.g.*, organic and inorganic acids, acid derivatives[1], and bases for certain transport testing purposes (7)(11)(12). This Test Guideline describes a generic procedure similar to the validated reference test method (7). While this Test Guideline does not provide adequate information on skin irritation, it should be noted that OECD TG 439 specifically addresses the health effect skin irritation *in vitro* (13). For a full evaluation of local skin effects after a single dermal exposure, the Guidance Document No. 203 on Integrated Approaches for Testing Assessment should be consulted (5).

Table 1. The UN GHS Skin Corrosive Category and Subcategories (1)

Corrosive Category (category 1) (applies to authorities not using subcategories)	Potential Corrosive Subcategories (only applies to some authorities)	Corrosive in ≥1 of 3 animals	
		Exposure	Observation
Corrosive	Corrosive subcategory 1A	≤3 minutes	≤1 hour
	Corrosive subcategory 1B	>3 minutes / ≤1 hour	≤14 days
	Corrosive subcategory 1C	>1 hour / ≤4 hours	≤14 days

7. A limitation of the validated reference test method (7) is that many non-corrosive chemicals and some corrosive chemicals may not qualify for testing, based on the results of the initial compatibility test (see paragraph 13). Aqueous chemicals 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 (7). The *in vitro* membrane barrier test methods may be used to test solids (soluble or insoluble in water), liquids (aqueous or non-aqueous), and emulsions. However, test chemicals not causing a detectable change in the compatibility test (*i.e.*, colour change in the Chemical Detection System (CDS) of the validated reference test method) cannot be tested with the membrane barrier test method and should be tested using other test methods.

[1] "Acid derivative" is a non-specific class designation and is broadly defined as an acid produced from a chemical either directly or by modification or partial substitution. This class includes anhydrides, halo acids, salts, and other types of chemicals.

PRINCIPLE OF THE TEST

8. The test system comprises two components: a synthetic macromolecular bio-barrier and a chemical detection system (CDS); this test method detects via the CDS membrane barrier damage caused by corrosive test chemicals after the application of the test chemical to the surface of the synthetic macromolecular membrane barrier (7), presumably by the same mechanism(s) of corrosion that operate on living skin.

9. Penetration of the membrane barrier (or breakthrough) might be measured by a number of procedures or CDS, including a change in the colour of a pH indicator dye or in some other property of the indicator solution below the barrier.

10. The membrane barrier should be determined 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 chemicals, able to categorize the corrosive properties of chemicals across the various UN GHS Sub-categories of corrosivity (1). The classification assigned is based on the time it takes a chemical to penetrate through the membrane barrier to the indicator solution.

DEMONSTRATION OF PROFICIENCY

11. Prior to routine use of the in vitro membrane barrier test method, adhering to this Test Guideline, laboratories should demonstrate technical proficiency by correctly classifying the twelve Proficiency Substances recommended in Table 2. In situations where a listed substance is unavailable or where justifiable, another substance for which adequate *in vivo* and *in vitro* reference data are available may be used (e.g. from the list of reference chemicals (10)) provided that the same selection criteria as described in Table 1 is applied.

Table 2: Proficiency Substances[1]

***Substance*[2]**	**CASRN**	**Chemical Class**	***In Vivo* UN GHS Sub-category[3]**	***In Vitro* UN GHS Sub-category[3]**
Boron trifluoride dihydrate	13319-75-0	Inorganic acids	1A	1A
Nitric acid	7697-37-2	Inorganic acids	1A	1A
Phosphorus pentachloride	10026-13-8	Precursors of inorganic acids	1A	1A
Valeryl chloride	638-29-9	Acid chlorides	1B	1B
Sodium Hydroxide	1310-73-2	Inorganic bases	1B	1B
1-(2-Aminoethyl) piperazine	140-31-8	Aliphatic amines	1B	1B
Benzenesulfonyl chloride	98-09-9	Acid chlorides	1C	1C
N,*N*-Dimethyl benzylamine	103-83-3	Anilines	1C	1C
Tetraethylenepentamine	112-57-2	Aliphatic amines	1C	1C
Eugenol	97-53-0	Phenols	NC	NC

Nonyl acrylate	2664-55-3	Acrylates/methacrylates	NC	NC
Sodium bicarbonate	144-55-8	Inorganic salts	NC	NC

[1]The twelve substances listed above contain three substances from each of the three UN GHS subcategories for corrosive substances and three non-corrosive substances, are readily available from commercial suppliers, and the UN GHS subcategory is based on the results of high-quality *in vivo* testing. These substances are taken from the list of 40 reference substances that are included in the minimum list of chemicals identified for demonstrating the accuracy and reliability of test methods that are structurally and functionally similar to the validated reference test method, and were selected from the 163 reference chemicals that were originally used to validate the reference test method (Corrositex®) (7) (10) (14). The goal of this selection process was to include, to the extent possible, chemicals that: were representative of the range of corrosivity responses (e.g., non-corrosives; UN Packing Groups I, II, and III corrosives) that the validated reference test method is capable of measuring or predicting; were representative of the chemical classes used during the validation process; have chemical structures that were well-defined; induced reproducible results in the validated reference test method; induced definitive results in the *in vivo* reference test; were commercially available; and were not associated with prohibitive disposal costs (14).

[2]Substances tested neat or with purity ≥ 90%

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

PROCEDURE

12. The following paragraphs describe the components and procedures of an artificial membrane barrier test method for corrosivity assessment (7) (15), based on the current VRM, i.e., the commercially available Corrositex®. The membrane barrier and the compatibility/indicator and categorisation solutions can be constructed, prepared or obtained commercially such as in the case of the VRM Corrositex®. A sample test method protocol for the validated reference test method is available (7). Testing should be performed at ambient temperature (17-25ºC) and the components should comply with the following conditions.

Test Chemical Compatibility Test

13. Prior to performing the membrane barrier test, a compatibility test is performed to determine if the test chemical is detectable by the CDS. If the CDS does not detect the test chemical, the membrane barrier test method is not suitable for evaluating the potential corrosivity of that particular test chemical 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.

Test Chemical Timescale Category Test

14. If appropriate for the test method, a test chemical that has been qualified by the compatibility test should be subjected to a timescale category test, *i.e.,* a screening test to distinguish between weak and strong acids or bases. For example, in the validated reference test method a timescale categorization test is used to indicate which of two timescales should be used based on whether significant acid or alkaline reserve is detected. Two different breakthrough timescales should be used for determining corrosivity and UN GHS skin corrosivity Sub-category, based on the acid or alkali reserve of the test chemical.

Membrane Barrier Test Method Components

Membrane Barrier

15. The membrane barrier consists of two components: a proteinaceous macromolecular aqueous gel and a permeable supporting membrane. The proteinaceous gel should be impervious to liquids and solids but can be corroded and made permeable. The fully constructed membrane barrier should be stored under pre-determined conditions shown to preclude deterioration of the gel, *e.g.*, drying, microbial growth, shifting, cracking, which would degrade its performance. The acceptable storage period should be determined and membrane barrier preparations not used after that period.

16. The permeable supporting membrane provides mechanical support to the proteinaceous gel during the gelling process and exposure to the test chemical. The supporting membrane should prevent sagging or shifting of the gel and be readily permeable to all test chemicals.

17. The proteinaceous gel, composed of protein, *e.g.*, keratin, collagen, or mixtures of proteins, forming a gel matrix, serves as the target for the test chemical. The proteinaceous material is placed on the surface of the supporting membrane and allowed to gel prior to placing the membrane barrier over the indicator solution. The proteinaceous gel should be of equal thickness and density throughout, and with no air bubbles or defects that could affect its functional integrity.

Chemical Detection System (CDS)

18. The indicator solution, which is the same solution used for the compatibility test, should respond to the presence of a test chemical. A pH indicator dye or combination of dyes, *e.g.*, cresol red and methyl orange that will show a colour change, in response to the presence of the test chemical, should be used. The measurement system can be visual or electronic.

19. Detection systems that are developed for detecting the passage of the test chemical through the barrier membrane should be assessed for their relevance and reliability in order to demonstrate the range of chemicals that can be detected and the quantitative limits of detection.

TEST PERFORMANCE

Assembly of the Test Method Components

20. The membrane barrier is positioned in a vial (or tube) containing the indicator solution so that the supporting membrane is in full contact with the indicator solution and with no air bubbles present. Care should be taken to ensure that barrier integrity is maintained.

Application of the Test Chemical

21. A suitable amount of the test chemical, *e.g.*, 500 μL of a liquid or 500 mg of a finely powdered solid (7), is carefully layered onto the upper surface of the membrane barrier and evenly distributed. An appropriate number of replicates, *e.g.*, four (7), is prepared for each test chemical and its corresponding controls (see paragraphs 23 to 25). The time of applying the test chemical to the membrane barrier is recorded. To ensure that short corrosion times are accurately recorded, the application times of the test chemical to the replicate vials are staggered.

Measurement of Membrane Barrier Penetrations

22. Each vial is appropriately monitored and the time of the first change in the indicator solution, *i.e.*, barrier penetration, is recorded, and the elapsed time between application and penetration of the membrane barrier determined.

Controls

23. In tests that involve the use of a vehicle or solvent with the test chemical, the vehicle or solvent should be compatible with the membrane barrier system, *i.e.*, not alter the integrity of the membrane barrier system, and should not alter the corrosivity of the test chemical. When applicable, solvent (or vehicle) control should be tested concurrently with the test chemical to demonstrate the compatibility of the solvent with the membrane barrier system.

24. A positive (corrosive) control with intermediate corrosivity activity, *e.g.*, 110 ± 15 mg sodium hydroxide (UN GHS Corrosive Sub-category 1B) (7), should be tested concurrently with the test chemical to assess if the test system is performing in an acceptable manner. A second positive control that is of the same chemical class as the test chemical may be useful for evaluating the relative corrosivity potential of a corrosive test chemical. Positive control(s) should be selected that are intermediate in their corrosivity (*e.g.*, UN GHS Sub-category 1B) in order to detect changes in the penetration time that may be unacceptably longer or shorter than the established reference value, thereby indicating that the test system is not functioning properly. For this purpose, extremely corrosive (UN GHS Sub-category 1A) or non-corrosive chemicals are of limited utility. A corrosive UN GHS Sub-category 1B chemical would allow detection of a too rapid or too slow breakthrough time. A weakly corrosive (UN GHS Sub-category 1C) might be employed as a positive control to measure the ability of the test method to consistently distinguish between weakly corrosive and non-corrosive chemicals. Regardless of the approach used, an acceptable positive control response range should be developed based on the historical range of breakthrough times for the positive control(s) employed, such as the mean ± 2-3 standard deviations. In each study, the exact breakthrough time should be determined for the positive control so that deviations outside the acceptable range can be detected.

25. A negative (non-corrosive) control, *e.g.*, 10% citric acid, 6% propionic acid (7), should also be tested concurrently with the test chemical as another quality control measure to demonstrate the functional integrity of the membrane barrier.

Study Acceptability Criteria

26. According to the established time parameters for each of the UN GHS corrosivity Sub-categories, the time (in minutes) elapsed between application of a test chemical to the membrane barrier and barrier penetration is used to predict the corrosivity of the test chemical. For a study to be considered acceptable, the concurrent positive control should give the expected penetration response time (e.g. 8-16 min breakthrough time for sodium hydroxide if used as a positive control), 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 chemical. Prior to routine use of a test method that adheres to this Test Guideline, laboratories should demonstrate technical proficiency, using the twelve substances recommended in Table 2. For new "me-too" test methods developed under this Test Guideline that are structurally and functionally similar to the validated reference test method (14) the pre-defined performance standards should be used to demonstrate the reliability and accuracy of the new test method prior to its use for regulatory testing (10).

Interpretation of Results and Corrosivity Classification of Test Chemicals

27. The time (in minutes) elapsed between application of the test chemical to the membrane barrier and barrier penetration is used to classify the test chemical in terms of UN GHS corrosive Sub-categories (1) and, if applicable, UN Packing Group (16). Cut-off time values for each of the three corrosive subcategories are established for each proposed test method. Final decisions on cut-off times should consider the need to minimize under-classification of corrosive hazard (*i.e.*, false negatives). In the present Test Guideline, the cut-off times of Corrositex® as described in table 3 should be used as it represents the only test method currently falling within the test guideline (7).

Table 3. Corrositex® prediction model

Mean b reakthrough time (min.) for CDS change		**UN GHS prediction**[3]
Category 1 test chemicals[1] (determined by the method's categorization colour assay)	**Category 2 test chemicals**[2] (determined by the method's categorization colour assay)	
0-3 min.	0-3 min.	**Corrosive optional Sub-category 1A**
> 3 to 60 min.	> 3 to 30 min.	**Corrosive optional Sub-category 1B**
> 60 to 240 min.	> 30 to 60 min.	**Corrosive optional Sub-category 1C**
> 240 min.	> 60 min.	**Non-corrosive**

[1] Test chemicals with high acid/alkaline reserve (6)

[2] Test chemicals with low acid/alkaline reserve (6)

[3] UN GHS Subcategories 1A, 1B and 1C correspond to UN packing groups I, II and III respectively

DATA AND REPORTING

Data

28. The time (in minutes) elapsed between application and barrier penetration for the test chemical and the positive control(s) should be reported in tabular form as individual replicate data, as well as means ± the standard deviation for each trial.

Test Report

29. The test report should include the following information:

Test Chemical and Control Substances:

- Mono-constituent substance: chemical identification, such as IUPAC or CAS name, CAS number, SMILES or InChI code, structural formula, purity, chemical identity of impurities as appropriate and practically feasible, etc;
- Multi-constituent substance, UVCB and mixture: characterised as far as possible

by chemical identity (see above), quantitative occurrence and relevant physicochemical properties of the constituents;
- Physical appearance, water solubility, and additional relevant physicochemical properties;
- Source, lot number if available;
- Treatment of the test chemical/control substance prior to testing, if applicable (*e.g.* warming, grinding);
- Stability of the test chemical, limit date for use, or date for re-analysis if known;
- Storage conditions.

Vehicle:
- Identification, concentration (where appropriate), volume used;
- Justification for choice of vehicle.

In vitro membrane barrier model and protocol used, including demonstrated accuracy and reliability

Test Conditions:
- Description of the apparatus and preparation procedures used;
- Source and composition of the *in vitro* membrane barrier used;
- Composition and properties of the indicator solution;
- Method of detection;
- Test chemical and control substance amounts;
- Number of replicates;
- Description and justification for the timescale categorisation test;
- Method of application;
- Observation times.
- Description of the evaluation and classification criteria applied;
- Demonstration of proficiency in performing the test method before routine use by testing of the proficiency chemicals.

Results:
- Tabulation of individual raw data from individual test and control samples for each replicate;
- Descriptions of other effects observed;
- The derived classification with reference to the prediction model/decision criteria used.

Discussion of the results

Conclusions

LITERATURE

(1) United Nations (UN) (2013). Globally Harmonized System of Classification and Labelling of Chemicals (GHS), First revised edition, UN New York and Geneva, 2013. Available at: http://www.unece.org/trans/danger/publi/ghs/ghs_rev05/05files_e.html.

(2) OECD (2015), OECD Guideline for Testing of Chemicals. No. 404: Acute Dermal Irritation, Corrosion, revised version, as adopted on xxxx, OECD, Paris. Available at: xxxxxxx. **DOI:** xxxxx.

(3) OECD (2015), OECD Guideline for the Testing of Chemicals No. 431. *In Vitro* Skin Model. Revised version as adopted on xxxx, OECD, Paris. Available at: xxxxxxx. **DOI:**xxxxx.

(4) OECD (2015), OECD Guideline for the Testing of Chemicals No. 430. *In Vitro* Skin Corrosion: Transcutaneous Electrical Resistance (TER). Revised version as adopted on xxxx, OECD, Paris. Available at: xxxxx . DOI: xxxxxxx.

(5) Guidance Document on Integrated Approaches to Testing and Assessment of Skin Irritation/Corrosion, Series on Testing and Assessment, No. 203, OECD, Paris.

(6) Fentem, J.H., Archer, G.E.B., Balls, M., Botham, P.A., Curren, R.D., Earl, L.K., Esdaile, D.J., Holzhutter, H.-G. and 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.

(7) ICCVAM (1999). Corrositex®. An *In Vitro* Test Method for Assessing Dermal Corrosivity Potential of Chemicals. The Results of an Independent Peer Review Evaluation Coordinated by ICCVAM, NTP and NICEATM. NIEHS, NIH Publication No. 99-4495. Available at: [http://ntp.niehs.nih.gov/pubhealth/evalatm/test-method-evaluations/dermal-corr-irrit/corrositex/index.html], together with a Sample Protocol for the Corrositex® test kit at: [http://ntp.niehs.nih.gov/iccvam/methods/dermal/corrositex/corrdocs/sampprot.pdf]

(8) Gordon, V.C., Harvell, J.D. and Maibach, H.I. (1994). Dermal Corrosion, the Corrositex® System: A DOT Accepted Method to Predict Corrosivity Potential of Test Materials. *In vitro* Skin Toxicology-Irritation, Phototoxicity, Sensitization. Alternative Methods in Toxicology 10, 37-45.

(9) OECD (2005), OECD Series on Testing and Assessment No. 34. Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment. Available at: [http://www.oecd.org/document/30/0,3343,en_2649_34377_1916638_1_1_1_1,00.html].

(10) Performance Standards for the assessment of proposed similar or modified *in vitro* membrane barrier test method for skin corrosion in relation to TG 435. Series on Testing and Assessment, No. XXX, OECD, Paris.

(11) ECVAM (2001). Statement on the Application of the CORROSITEX® Assay for Skin Corrosivity Testing. 15th Meeting of ECVAM Scientific Advisory Committee (ESAC), Ispra, Italy. ATLA 29, 96-97.

(12) U.S. DOT (2002). Exemption DOT-E-10904 (Fifth Revision). September 20, 2002. Washington, D.C., U.S. DOT. Available at: [http://hazmat.dot.gov/exemptions/E10904.pdf]

(13) OECD (2015), OECD Guideline for the Testing of Chemicals No. 439. *In vitro* skin irritation: reconstructed human epidermis test method. Revised version, as adopted on xxxx, OECD, Paris. Available at: xxxxxxx. **DOI:** xxxxx

(14) ICCVAM (2004). ICCVAM Recommended Performance Standards for *In Vitro* Test Methods for Skin Corrosion. NIEHS, NIH Publication No. 04-4510. Available at: [http://ntp.niehs.nih.gov/iccvam/docs/dermal_docs/ps/ps044510.pdf]

(15) U.S. EPA (1996). Method 1120, Dermal Corrosion. Available at: [http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/1120.pdf]

(16) United Nations (UN) (2013). UN Recommendations on the Transport of Dangerous Goods, Model Regulations, 18th revised edition (Part, chapter 2.8), UN, 2013. Available at: http://www.unece.org/fileadmin/DAM/trans/danger/publi/unrec/rev18/English/Rev18_Volume1_Part2.pdf.

(17) Eskes C. et al (2012). Regulatory assessment of in vitro skin corrosion and irritation data within the European framework: Workshop recommendations. Regul.Toxicol.Pharmacol. 62, 393-403)

ANNEX 1

DEFINITIONS

Accuracy: The closeness of agreement between test method results and accepted reference values. It is a measure of test method performance and one aspect of relevance. The term is often used interchangeably with "concordance" to mean the proportion of correct outcomes of a test method (9).

Chemical: means a substance or a mixture.

Chemical Detection System (CDS): A visual or electronic measurement system with an indicator solution that responds to the presence of a test chemical, *e.g.*, by a change in a pH indicator dye, or combination of dyes, that will show a colour change in response to the presence of the test chemical or by other types of chemical or electrochemical reactions.

Concordance: This is a measure of test method performance for test methods that give a categorical result, and is one aspect of relevance. The term is sometimes used interchangeably with accuracy, and is defined as the proportion of all chemicals tested that are correctly classified as positive or negative. Concordance is highly dependent on the prevalence of positives in the types of test chemical being examined (9).

GHS (Globally Harmonized System of Classification and Labelling of Chemicals): a system proposing the classification of chemicals (substances and mixtures) according to standardized types and levels of physical, health and environmental hazards, and addressing corresponding communication elements, such as pictograms, signal words, hazard statements, precautionary statements and safety data sheets, so that to convey information on their adverse effects with a view to protect people (including employers, workers, transporters, consumers and emergency responders) and the environment (1).

IATA: Integrated Approach on Testing and Assessment.

Mixture: means a mixture or solution composed of two or more substances in which they do not react.

Mono-constituent substance: A substance, defined by its quantitative composition, in which one main constituent is present to at least 80% (w/w).

Multi-constituent substance: A substance, defined by its quantitative composition, in which more than one main constituent is present in a concentration $\geq$ 10% (w/w) and < 80% (w/w). A multi-constituent substance is the result of a manufacturing process. The difference between mixture and multi-constituent substance is that a mixture is obtained by blending of two or more substances without chemical reaction. A multi-constituent substance is the result of a chemical reaction.

NC: Non corrosive.

Performance standards: Standards, based on a validated test method, that provide a basis for evaluating the comparability of a proposed test method that is mechanistically and functionally similar. Included are (i) essential test method components; (ii) a minimum list of Reference Chemicals selected from among the chemicals used to demonstrate the acceptable performance of the validated test method; and (iii) the similar levels of reliability and accuracy , based on what was obtained for the validated test method, that the proposed test method should demonstrate when evaluated using the minimum list of Reference Chemicals (9).

Relevance: Description of relationship of the test method to the effect of interest and whether it is meaningful and useful for a particular purpose. It is the extent to which the test method correctly measures or predicts the biological effect of interest. Relevance incorporates consideration of the accuracy (concordance) of a test method (9).

Reliability: Measures of the extent that a test method can be performed reproducibly within and between laboratories over time, when performed using the same protocol. It is assessed by calculating intra- and inter-laboratory reproducibility (9).

Sensitivity: The proportion of all positive/active chemicals that are correctly classified by the test method. It is a measure of accuracy for a test method that produces categorical results, and is an important consideration in assessing the relevance of a test method (9).

Skin corrosion *in vivo:* The production of irreversible damage of the skin; namely, visible necrosis through the *epidermis* and into the dermis, following the application of a test chemical 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.

Specificity: The proportion of all negative/inactive chemicals that are correctly classified by the test method. It is a measure of accuracy for a test method that produces categorical results and is an important consideration in assessing the relevance of a test method (9).

Substance: means chemical elements and their compounds in the natural state or obtained by any production process, including any additive necessary to preserve the stability of the product and any impurities deriving from the process used, but excluding any solvent which may be separated without affecting the stability of the substance or changing its composition.

Skin corrosion *in vivo*: The production of irreversible damage of the skin; namely, visible necrosis through the epidermis and into the dermis, following the application of a test chemical 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.

Test chemical: means what is being tested.

UVCB: substances of unknown or variable composition, complex reaction products or biological materials.