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OECD GUIDELINE FOR THE TESTING OF CHEMICALS

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 material [as defined by the United Nations (UN) Globally Harmonized System of Classification and Labelling of Chemicals (GHS)] (1). This Test Guideline provides an *in vitro* procedure by which the assessment of corrosivity is not carried out in live animals.

2. A number of *in vitro* test methods have been proposed as alternatives for the standard *in vivo* rabbit skin procedure (OECD TG 404)(2) used to identify corrosive substances. This Test Guideline is for an *in vitro* membrane barrier test method that can be used to identify corrosive substances. The test method utilizes an artificial membrane designed to respond to corrosive substances in a manner similar to animal skin *in situ*.

3. Skin corrosivity has traditionally been assessed by applying the test substance to the skin of living animals and assessing the extent of tissue damage after a fixed period of time (2)(3). The UN GHS tiered testing and evaluation strategy for the assessment and classification of skin corrosivity allows for the use of validated and accepted *in vitro* test methods (1). In this tiered strategy, positive results from *in vitro* test methods can be used to classify a substance as corrosive without the need for animal testing, thus reducing and refining the use of animals in testing. Substances that are negative undergo additional testing in accordance with the tiered testing strategy (1)(see supplement to Test Guideline 404(2)). The use of *in vitro* test methods to identify corrosive substances can therefore avoid the pain and distress that might occur when animals are used for this purpose.

4. Validation studies have been completed for an *in vitro* membrane barrier test method commercially available as Corrositex® (4)(5)(6). Based on its acknowledged validity, this validated reference test method has been recommended for use as part of a tiered testing strategy for assessing the dermal corrosion hazard potential of chemicals (5). 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 comparable to that of the validated reference test method (7)(8)(9)(10).

5. A limitation of the validated reference test method (5) that is the basis for this Test Guideline is that, based on the results of the initial compatibility test (see paragraph 13), 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 (5). 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 and chemical mixtures 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. The samples may be pure chemicals, dilutions, formulations, or waste. No prior treatment of the sample is required.

6. This Test Guideline provides a third *in vitro* method for skin corrosivity testing. Other test methods are based on the use of reconstituted human skin (OECD TG 431)(11) and isolated rat skin (OECD TG 430)(12). However, this Test Guideline also provides for subcategorisation of corrosive substances into the three GHS subcategories of corrosivity and the three UN Transport Packing Groups for corrosivity hazard.

DEFINITIONS

7. Definitions used are provided in Annex 1.

INITIAL CONSIDERATIONS

8. The test described in this Guideline allows the identification of corrosive chemical substances and mixtures and allows the subcategorisation of corrosive substances as permitted in the 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 (5)(13)(14). This Test Guideline describes a generic procedure similar to a validated reference test method (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

PRINCIPLE OF THE TEST

9. The test system is composed of two components, a synthetic macromolecular bio-barrier and a CDS; the basis of this test method 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 (5), presumably by the same mechanism(s) of corrosion that operate on living skin.

10. Penetration of the membrane barrier (or breakthrough) might be 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.

11. 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 substances, able to categorize the

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

corrosive properties of chemicals across the various subcategories of corrosivity (1). The classification assigned is based on the time it takes a substance to penetrate through the membrane barrier to the indicator solution.

PROCEDURE

12. The following is a generic description of the components and procedures of an artificial membrane barrier test method for corrosivity assessment (7)(15). The membrane barrier and the compatibility/indicator and categorisation solutions can be constructed, prepared or obtained commercially, *e.g.,* Corrositex®. A sample test method protocol for the validated reference test method can be obtained at [http://iccvam.niehs.nih.gov]. Testing should be performed at ambient temperature (17-25ºC) and the components should comply with the following.

Test Substance Compatibility Test

13. Prior to performing the membrane barrier test, a compatibility test is performed 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.

Test Substance Timescale Category Test

14. If appropriate for the test method, a test substance 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 alkalai reserve is detected. Two different breakthrough timescales should be used for determining corrosivity and GHS skin corrosivity subcategory, based on the acid or alkali reserve of the chemical.

Membrane Barrier Test Method Components

Membrane Barrier

15. The membrane barrier should consist 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 substance. The supporting membrane should prevent sagging or shifting of the gel and be readily permeable to all test substances.

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 substance. 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, responds to the presence of a test substance. 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 substance or other types of chemical or electrochemical reactions can be used. The measurement system can be visual or electronic.

19. Detection systems that are developed for detecting the passage of the test substance through the barrier membrane should be assessed for their relevance and reliability in order to demonstrate the range of substances 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 Substance

21. A suitable amount of the test substance, *e.g.,* 500 µL of a liquid or 500 mg of a finely powdered solid (5), is carefully layered onto the upper surface of the membrane barrier and evenly distributed. An appropriate number of replicates, *e.g.,* four (5), is prepared for each test substance and its corresponding controls. The time of applying the test substance to the membrane barrier is recorded. To ensure that short corrosion times are accurately recorded, the application times of the test substance 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 substance, 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 substance. When applicable, solvent (or vehicle) control should be tested concurrently with the test substance to demonstrate the compatibility of the solvent with the membrane barrier system.

24. A positive (corrosive) control chemical with intermediate corrosivity activity, *e.g.,* sodium hydroxide (GHS Corrosive subcategory 1B) (7), should be tested concurrently with the test substance 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 substance may be useful for evaluating the relative corrosivity potential of a corrosive test substance. Positive control(s) should be selected that are intermediate in their corrosivity (*e.g.,* subcategory 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 (GHS subcategory 1A) or non-corrosive chemicals are of limited utility. A corrosive GHS subcategory 1B substance would allow detection of a too rapid or too

slow breakthrough time. A weak substance (GHS subcategory 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 substances. 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 substances(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 substance, *e.g.,* 10% citric acid, 6% propionic acid (7), should also be tested concurrently with the test substance 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 GHS corrosivity subcategories, the time (in minutes) elapsed between application of a test substance to the membrane barrier and barrier penetration is used to predict the corrosivity of the test substance. 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. Prior to routine use of a test method that adheres to this Test Guideline, laboratories may wish to demonstrate technical proficiency, using the twelve chemicals 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 (16) the performance standards described in Annex 2 of this Test Guideline should be used to demonstrate the reliability and accuracy of the new test method prior to its use for regulatory testing.

Interpretation of Results and Corrosivity Classification of Test Substances

27. The time (in minutes) elapsed between application of the test substance to the membrane barrier and barrier penetration is used to classify the test substance in terms of corrosivity (1) and, if applicable, UN Packing Group (17). 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).

DATA AND REPORTING

Data

28. The time (in minutes) elapsed between application and barrier penetration for the test substance 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 and Control Substances:

– identification data and Chemical Abstracts Services Registry Number, if known;
– physical nature and purity (major impurities);

– physico-chemical properties relevant to the conduct of the study;
– treatment of the test/control substances prior to testing, if applicable, *e.g.*, warming, grinding;
– stability, if known.

Justification of the 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 and control substance amounts;
– number of replicates;
– description and justification for the timescale categorisation test;
– method of application;
– observation times.

Results:
– tabulation of individual raw data from individual test and control samples for each replicate;
– descriptions of other effects observed;
– description of the evaluation and classification criteria.

Discussion of the results

Conclusions.

LITERATURE

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Table 2: Proficiency Chemicals

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 twelve chemicals listed above contain three substances from each of the three GHS subcategories for corrosive substances and three non-corrosive substances, and are taken from the list of 40 reference chemicals 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 (see Annex 2)(5)(16). These chemicals are readily available from commercial suppliers, and the UN GHS subcategory is based on the results of high-quality *in vivo* testing.

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

NC; Non-corrosive.

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. (7)(8)(10).

Chemical Detection System (CDS): A visual or electronic measurement system with an indicator solution that responds to the presence of a test substance, *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 substance or by other types of chemical or electrochemical reactions.

False negative rate: The proportion of all positive substances falsely identified by a test method as negative Its one indicator of test method performance.

False positive rate: The proportion of all negative (non-active) substances that are falsely identified as positive. It is one indicator of test performance.

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).

Inter-laboratory reproducibility: A measure of the extent to which different qualified laboratories, using the same protocol and testing the same substances, can produce qualitatively and quantitatively similar results. Inter-laboratory reproducibility is determined during the prevalidation and validation processes, and indicates the extent to which a test can be successfully transferred between laboratories, also referred to as between-laboratory reproducibility.

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 (1) essential test method components; (2) a minimum list of reference chemicals selected from among the chemicals used to demonstrate the acceptable performance of the validated test method; and (3) the comparable levels of accuracy and reliability, 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.

Reference chemicals: Chemicals selected for use in the validation process, for which responses in the in vitro or in vivo reference test system or the species of interest are already known. These chemicals should be representative of the classes of chemicals for which the test method is expected to be used, and should represent the full range of responses that may be expected from the chemicals for which it may be used, from strong, to weak, to negative. Different sets of reference chemicals may be required for the different stages of the validation process, and for different test methods and test uses.

Relevance: Description of relationship of the test to the effect of interest and whether it is meaningful and useful for a particular purpose. It is the extent to which the test correctly measures or predicts the

biological effect of interest. Relevance incorporates consideration of the accuracy (concordance) of a test method. (7)(8)(10).

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 and intra-laboratory repeatability.
(7)(8)(10).

Sensitivity: The proportion of all positive/active substances that are correctly classified by the test. 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.

Specificity: The proportion of all negative/inactive substances that are correctly classified by the test. 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.

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).

ANNEX 2

ASSESSMENT OF THE PERFORMANCE CHARACTERISTICS OF PROPOSED IN VITRO MEMBRANE BARRIER TEST METHOD FOR SKIN CORROSION

INTRODUCTION

1. Test methods proposed for use under this Test Guideline should be evaluated to determine their reliability and accuracy using substances of known corrosivity and non-corrosivity. When evaluated using the recommended reference chemicals (Table 1), the proposed test methods should have reliability and accuracy that are comparable to that of the validated reference test method (1)(Table 2). The reliability and accuracy standards that should be achieved are provided in paragraphs 3 and 4. Non-corrosive and corrosive substances, ranging from strong to weak and representing relevant chemical classes are included so that the reliability and accuracy, *i.e.,* sensitivity, specificity, false negative rates, and false positive rates, of the proposed test method can be compared to that of the validated reference test method (1)(2). For purposes of transportation hazard classification, the list of corrosive substances also covers the range of UN Packing Group classifications/GHS skin corrosivity subcategories (3)(4). This will allow for the determination of whether the penetration times used to assign test substances to a UN Packing Group/GHS skin corrosivity subcategory are appropriate. The elapsed times associated with the assignment of each Packing Group/GHS skin corrosivity subcategories to a test substance should be determined for each composition of barrier, indicator, and categorization systems, and the particular test method used. The reliability of the test method, as well as its ability to over- and under-predict known corrosive substances, should be determined prior to its use for testing new substances. Where possible, the classes or types of substances that are consistently over - or under-predicted should be defined.

PERFORMANCE STANDARDS

2. Reference chemicals are used to determine if the reliability and accuracy of a proposed *in vitro* membrane barrier test method is comparable to that of the validated reference test method (2). The 40 reference chemicals listed in Table 1 include chemicals representing different chemical classes of interest and the range of corrosivity responses, *i.e.,* non-corrosive, UN Packing Group I (GHS 1A), II (GHS 1B), and III (GHS 1C) corrosives, obtained for the *in vivo* reference test method. The distribution of chemicals in this list by corrosivity and UN Packing Group classifications/GHS skin corrosivity subcategories are 12 non-corrosive and 28 corrosive chemicals. Among the 28 corrosive chemicals there are nine substances each in Packing Groups I (GHS 1A) and II (GHS 1B), and 10 substances in Packing Group III (GHS 1C). These reference chemicals represent the minimum number that should be used to evaluate the accuracy and reliability of a proposed membrane barrier test method for skin corrosion. In situations where a listed chemical is unavailable, other chemicals or products for which adequate *in vivo* reference data are available could be used. If desired, additional chemicals representing other chemical or product classes and for which adequate *in vivo* reference data are available can be added to the minimum list of reference chemicals to further evaluate the accuracy of the proposed test method.

3. The reliability of the proposed test method should be comparable to that of the validated reference test method. However, an assessment of inter-laboratory reproducibility is not essential if the proposed test method is to be used in one laboratory only. The inter-laboratory reproducibility for corrosive versus non-corrosive and UN Packing Group classification/GHS skin corrosivity subcategories should be at least 93% (1). In terms of membrane breakthrough times, the median coefficient of variation

(CV) should not exceed 30% for studies conducted in different laboratories and should not exceed 5% for replicate measurements within a study (1).

Table 1. Reference Chemicals for Determination of Accuracy and Reliability of *In Vitro* Membrane Corrosivity Test Methods

Chemical[1]	CASRN	Chemical[2] Class	Conc (%)[2]	UN *In Vivo* PG[3]	Validated Test Method PG	pH[2]
Fluorosulfonic acid	7789-21-1	inorganic acids	neat	I	I	0
Nitric acid	7697-37-2	inorganic acids	90	I	I	0
Phosphorus pentachloride	10026-13-8	precursors of inorganic acids	98	I	I	0
Selenic acid	7783-08-6	inorganic acids	95	I	I	0
Boron trifluoride dihydrate	13319-75-0	inorganic acids	96	I	I	0.4
Phosphorus tribromide	7789-60-8	precursors of inorganic acids	97	I	I	1.0
Sulfuric acid, 10% wt.	7664-93-9	inorganic acid	10	I	I	1.2
Benzyl chloroformate	501-53-1	acid chlorides	95	I	NC	2.5
1,2-Diaminopropane	78-90-0	aliphatic amines	NA	I	II	8.3
Phosphoric acid	7664-38-2	inorganic acids	85	II	II	0.4
Valeryl chloride	638-29-9	acid chlorides	98	II	II	0.5
Acetic acid	64-19-7	organic acids	99+	II	II	1.9
Caprylic acid	124-07-2	organic acids	95	II	NC	2.7
Capric:caprylic acid (45:55)	68937-75-7	organic acids	95	II	NC	3.0
Ammonium bifluoride	1341-49-7	organic ammonium salts	98	II	II	5.2
1-(2-Aminoethyl) piperazine	140-31-8	aliphatic amines	99	II	II	11.8
Ethanolamine	141-43-5	aliphatic amines	99+	II	II	11.8
Sodium hydroxide	1310-73-2	inorganic bases	100	II	II	13.8
Cyanuric chloride	108-77-0	substituted triazines	99	III	III	1.7
Benzenesulfonyl chloride	98-09-9	acid chlorides	neat	III	III	1.8
Crotonic acid	107-93-7	organic acids	99+	III	III	2.3
Butyric anhydride	106-31-0	anhydrides	99	III	III	3.1
Hydroxylamine sulfate	10039-54-0	organic ammonium salts	97+	III	III	3.6
2-Methylbutyric acid	600-07-7	organic acids	NA	III	III	3.6
Dicyclohexylamine	101-83-7	aliphatic amines	99	III	III	9.6
N,N-Dimethyl benzylamine	103-83-3	anilines	99	III	III	10.7
Tetraethylenepent-amine	112-57-2	aliphatic amines	neat	III	III	11.9

Chemical[1]	CASRN	Chemical[2] Class	Conc (%)[2]	UN *In Vivo* PG[3]	Validated Test Method PG	pH[2]
2-Ethylhexylamine	104-75-6	Aliphatic amines	98	III	III	12.0
Maleic acid	110-16-7	organic acids	99	NC	II	1.3
Copper(II) chloride	7447-39-4	inorganic salts	97	NC	II	3.0
Eugenol	97-53-0	phenols	NA	NC	NC	3.7
Chromium(III) fluoride	7788-97-8	inorganic salts	97	NC	NC	3.9
Cinnamaldehyde	14371-10-9	aldehydes	100	NC	NC	3.9
Ethyl triglycol methacrylate	39670-09-2	acrylates/methacrylates	neat	NC	NC	4.5
Nonyl acrylate	2664-55-3	acrylates/methacrylates	neat	NC	NC	6.9
Benzalkonium chloride	8001-54-5	organicammonium salts	100	NC	NC	7.6
Sodium bicarbonate	144-55-8	inorganic salts	100	NC	NC	8.3
Sodium undecylenate	3398-33-2	anionic surfactant	33	NC	NC	8.3
Sodium carbonate, 50% aqueous	497-19-8	inorganic salts	100	NC	II	11.7
Calcium carbonate	471-34-1	inorganic salts	neat	NC	NC	12.6

Abbreviations: CASRN = Chemical Abstracts Service Registry Number; Conc = concentration; NA = not available; NC = non-corrosive; PG = Packing Group; UN = United Nations.

[1] The 40 reference chemicals comprise a representative selection from the 163 reference chemicals that were originally used to validate the reference test method (Corrositex®); the complete list and the selection criteria are provided in (1).

[2] The chemical class, the concentration tested, and the pH values were obtained from the original sources as indicated in (1). The pH values are rounded to one decimal point.

[3] Within the UN Globally Harmonized System of Classification and Labelling of Chemicals (GHS), the PG classifications correspond as follows: PG I = 1A, PG II = 1B, PG III = 1C. These classifications are based on high quality *in vivo* testing results (1).

4. The accuracy (sensitivity, specificity, false negative rate, false positive rate, ability to correctly identify UN Packing Group classifications/GHS skin corrosivity subcategories) of the proposed test method should be at least comparable to that of the validated test method (1)(2)(Table 2).

Table 2. Accuracy of the Validated Reference Test Method for Skin Corrosion[1]

Source	No. of Chemicals	Sensitivity[2]	Specificity[2]	False Negative Rate[2]	False Positive Rate[2]	Packing Group Accuracy[3]
Reference Chemicals[4]	40	89% (25/28)	75% (9/12)	11% (3/28)	25% (3/12)	96% (24/25)

[1] Table 2 provides the accuracy of the validated reference test method in correctly identifying the corrosivity potential of the 40 reference chemicals (Table 1).

[2] In this analysis (1), a substance is first classified as positive or negative for corrosivity within each laboratory based on the majority of test results obtained (when replicate testing was conducted). Next, the substance is classified as positive or negative for corrosivity based on the majority of test results obtained in multiple laboratories (when multiple laboratory studies were conducted).

[3] Packing Group Accuracy reflects the frequency with which the validated reference test method correctly identified the UN Packing Group classification (or GHS skin corrosivity subcategories) assigned to a corrosive substance based on *in vivo* rabbit skin test method results. This calculation is limited to substances correctly identified as corrosive by Corrositex®.

[4] See Table 1.

LITERATURE

(1) 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://iccvam.niehs.nih.gov/docs/reports/corprrep.pdf], together with a Sample Protocol for the Corrositex® test kit at [http://iccvam.niehs.nih.gov/methods/corrdocs/sampprot.pdf]

(2) ICCVAM (2004). ICCVAM Recommended Performance Standards for *In Vitro* Test Methods for Skin Corrosion. NIEHS, NIH Publication No. 04-4509. Available: [http://iccvam.niehs.nih.gov/docs/guidelines/validate.pdf]

(3) United Nations (UN) (2003).Recommendations of the Transport of Dangerous Goods, Model Regulations, 13th revised edition, ST/SG/AC.10/1/Rev.13 (Vol.I), UN New York and Geneva, 2003.Available : http://www.unece.org/trans/danger/publi/unrec/rev13/13files_e.html.

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