Adopted :
13 April 2004

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

DRAFT PROPOSAL FOR AN UPDATE OF TEST GUIDELINE 430

In Vitro Skin Corrosion: Transcutaneous Electrical Resistance Test (TER)

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 Harmonised System of Classification and Labelling of Chemicals (GHS)] (1). This Test Guideline provides an *in vitro* procedure allowing the identification of corrosive chemical substances and mixtures.

2. The assessment of skin corrosivity has typically involved the use of laboratory animals (OECD Test Guideline 404 (TG 404); adopted in 1981 and revised in 1992 and 2002)(2). In relation to animal welfare TG 404 was revised in 2002, allowing for the determination of skin corrosion by applying a tiered testing strategy, using validated *in vitro* or *ex vivo* test methods, thus avoiding pain and suffering of animals. In addition to TG 431 (originally adopted in 2004)(3), two other *in vitro* test methods for testing of corrosivity have been validated and adopted as OECD Test Guidelines 430 (4) and 435 (5).

3. This Test Guideline is based on the rat skin TER model, which utilizes skin discs to identify corrosives by identified by their ability to produce a loss of normal stratum corneum integrity and barrier function. This updated Test Guideline also includes a set of Performance Standards (PS)(Annex 1) for the assessment of similar and modified TER-based test methods (6), in accordance with the principles of Guidance Document No. 34 (7).

4. Prevalidation studies (8), followed by a formal validation study of *in vitro* methods for assessing skin corrosion (9)(10) have been conducted (11)(12). The outcome of these studies and other published literature (13) led to the recommendation that the following tests could be used for regulatory purposes for the assessment of *in vivo* skin corrosivity (14)(15)(16): the human skin model test (see Test Guideline 431) and the transcutaneous electrical resistance test (this Guideline).

5. Before a proposed similar or modified *in vitro* TER 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 TG 430, in accordance with the requirements of the PS set out in this Test Guideline (Annex 1).

DEFINITIONS

6. Definitions used are provided in the Annex 2.

INITIAL CONSIDERATIONS

7. A validation study and other published studies have reported that the rat skin transcutaneous

electrical resistance (TER) assay (17)(18) is able to discriminate between known skin corrosives and non-corrosives with an overall sensitivity of 94% (51/54) and specificity of 71% (48/68) for a database of 122 substances (10)(13).

8. The test described in this Test Guideline 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) (1)(2)(19)(20). It does not provide information on skin irritation, nor does it allow the sub-categorisation of corrosive substances as permitted in the Globally Harmonised Classification System (GHS) (1).

9. This Test Guideline also includes a set of PS (Annex 1) for determining the validation status of new and revised skin corrosion test methods that are structurally and mechanistically similar to the TER (6), in accordance with the principles of Guidance Document No. 34 (7). These performance standards include a list of 24 reference chemicals by which to evaluate assay performance, the essential test method components that should be included in the protocol for the test method to be considered structurally and mechanistically similar, and the minimum accuracy and reliability necessary for the test method to be considered comparable to the TER. Within the reference chemical list, a subset of 12 proficiency chemicals (Table 1) is provided that can be used by laboratories to demonstrate proficiency in using the TER.

10. For a full evaluation of local skin effects after a single dermal exposure, it is recommended to follow the sequential testing strategy as appended to TG 404 (2) and provided in the Globally Harmonised System (1). This testing strategy includes the conduct of *in vitro* tests for skin corrosion (as described in this guideline) and skin irritation before considering testing in live animals.

PRINCIPLE OF THE TEST

11. 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 (17). For rat TER, a cut-off value of 5kΩ 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 kΩ), or well below (often < 3 kΩ) this value (17). 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, use of other skin preparations or other equipment may alter the cut-off value, necessitating further validation.

12. A dye-binding step is incorporated into the test procedure for confirmation testing of positive results in the TER including values around 5 kΩ. The dye-binding step determines if the increase in ionic permeability is due to physical destruction of the stratum corneum. The TER method utilising rat skin has shown to be predictive of *in vivo* corrosivity in the rabbit assessed under OECD guideline 404 (2). It should be noted that the *in vivo* rabbit test is highly conservative with respect to skin corrosivity and skin irritation when compared with the human skin patch test (21).

DEMONSTRATION OF PROFICIENCY

13. Prior to routine use of any TER test method that adheres to this Test Guideline, laboratories

should demonstrate technical proficiency, using the twelve Proficiency Chemicals recommended in Table 1. For similar tests developed under this Test Guideline that are structurally and mechanistically similar to the rat skin TER test method, the PS requirements described in Annex 1 of this Test Guideline should be used to demonstrate similar reliability and accuracy of the test method prior to its use using the test method for regulatory testing.

14. As part of the proficiency exercise, it is recommended that the user verify the barrier properties of the tissues after receipt as specified by the test method RhE model manufacturer. Once a test method has been successfully established and proficiency in its use has been demonstrated, such verification will not be necessary on a routine basis. However, when using a test method routinely, it is recommended to continue to assess the barrier properties in regular intervals, *e.g.,* every six or twelve months.

Table 1. Proficiency Chemicals

Chemical	CASRN	UN *In Vivo* PG	pH[1]
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
Potassuim hydroxide (10%)	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

[1] The pH values were obtained from Fentem et al. (1998) and Barratt et al. (1998).

Most of the chemicals listed are taken from the list of chemicals selected for the ECVAM international validation study (9). Their selection is based on the following criteria:

i) equal number of corrosive and non-corrosive substances;

ii) commercially available substances covering most of the relevant chemical classes;

iii) inclusion of severely corrosive as well as less corrosive substances in order to enable discrimination based on corrosive potency;

iv) choice of chemicals that can be handled in a laboratory without posing **other serious** hazards than corrosivity.

PROCEDURE

Animals

15. Rats are the species of choice because the sensitivity of their skin to chemicals in this test has been previously demonstrated (14). The age (when the skin is collected) and strain of the rat is particularly important to ensure that the hair follicles are in the dormant phase before adult hair growth begins.

16. The dorsal and flank hair from young, approximately 22 day-old, male or female rats (Wistar-derived or a comparable strain), is carefully removed with small clippers. Then, the animals are washed by careful wiping, whilst submerging the clipped area in antibiotic solution (containing, for example, streptomycin, penicillin, chloramphenicol, and amphotericin, at concentrations effective in inhibiting bacterial growth). Animals are washed with antibiotics again on the third or fourth day after the first wash and are used within 3 days of the second wash, when the stratum corneum has recovered from the hair removal.

Preparation of the skin discs

17. Animals are humanely killed when 28-30 days old; this age is critical. The dorso-lateral skin of each animal is then removed and stripped of excess subcutaneous fat by carefully peeling it away from the skin. Skin discs, with a diameter of approximately 20-mm each, are removed. 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.

18. Each skin disc is placed over one of the ends of a PTFE (polytetrafluoroethylene) tube, ensuring that the epidermal surface is in contact with the tube. A rubber 'O' ring is press-fitted over the end of the tube to hold the skin in place and excess tissue is trimmed away. Tube and 'O' ring dimensions are shown in Figure 2. The rubber 'O' ring is then carefully sealed to the end of the PTFE tube with petroleum jelly. The tube is supported by a spring clip inside a receptor chamber containing $MgSO_4$ solution (154 mM) (Figure 1). The skin disc should be fully submerged in the $MgSO_4$ solution. As many as 10-15 skin discs can be obtained from a single rat skin.

19. Before testing begins, the electrical resistance of two skin discs is measured as a quality control procedure for each animal skin. Both discs should give resistance values greater than 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.

Application of the test and control substances

20. Concurrent positive and negative controls should be used for each study to ensure adequate performance of the experimental model. Skin discs from a single animal should be used. The suggested positive and negative control substances are 10M hydrochloric acid and distilled water, respectively.

21. Liquid test substances (150 µL) are applied uniformly to the epidermal surface inside the tube. When testing solid materials, a sufficient amount of the solid is applied evenly to the disc to ensure that the whole surface of the epidermis is covered. Deionised water (150 µL) is added on top of the solid and the tube is gently agitated. In order to achieve maximum contact with the skin, solids may need to be warmed to 30^0 C to melt or soften the test substance, or ground to produce a granular material or powder.

22. Three skin discs are used for each test and control substance. Test substances are applied for 24 hours at 20-23^0 C. The test substance is removed by washing with a jet of tap water at up to 30^0 C until no further material can be removed.

TER measurements

23. The skin impedance is measured as TER by using a low-voltage, alternating current Wheatstone bridge (18). General specifications of the bridge are 1-3 Volt operating voltage, a sinus or rectangular shaped alternating current of 50 – 1000 Hz, and a measuring range of at least 0.1 -30 kΩ. The databridge used in the validation study measured inductance, capacitance and resistance up to values of 2000H, 2000μF, and 2MΩ, respectively at frequencies of 100Hz or 1kHz, using series or parallel values. For the purposes of the TER corrosivity assay measurements are recorded in resistance, at a frequency of 100Hz and using series values. Prior to measuring the electrical resistance, the surface tension of the skin is reduced by adding a sufficient volume of 70% ethanol to cover the epidermis. After a few seconds, the ethanol is removed from the tube and the tissue is then hydrated by the addition of 3mL $MgSO_4$ solution (154mM). The databridge electrodes are placed on either side of the skin disc to measure the resistance in kΩ/skin disc (Figure 1). Electrode dimensions and the length of the electrode exposed below the crocodile clips are shown in Figure 2. The clip attached to the inner electrode is rested on the top of the PTFE tube during resistance measurement to ensure that a consistent length of electrode is submerged in the $MgSO_4$ solution. The outer electrode is positioned inside the receptor chamber so that it rests on the bottom of the chamber. The distance between the spring clip and the bottom of the PTFE tube is maintained as a constant (Figure 2), because this distance affects the resistance value obtained. Consequently, the distance between the inner electrode and the skin disc should be constant and minimal (1-2 mm).

24. If the measured resistance value is greater than 20 kΩ, this may be due to the remains of the test substance coating the epidermal surface of the skin disc. Further removal of this coating can be attempted, for example, by sealing the PTFE tube with a gloved thumb and shaking it for approximately 10 seconds; the $MgSO_4$ solution is discarded and the resistance measurement is repeated with fresh $MgSO_4$.

25. The properties and dimensions of the test apparatus and the experimental procedure used may influence the TER values obtained. The 5 kΩ corrosive threshold was developed from data obtained with the specific apparatus and procedure described in this Guideline. 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 proficiency chemicals chosen from the chemicals used in the validation study (9)(10), or from similar chemical classes to the chemicals being investigated. A set of suitable proficiency chemicals is identified in Table 1.

Dye Binding Methods

26. Exposure of certain non-corrosive materials can result in a reduction of resistance below the cut-off of 5 kΩ allowing the passage of ions through the stratum corneum, thereby reducing the electrical resistance (10). 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. Thus, if the TER values of test substances are less than or around 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 skin corrosion (8)(10). 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. This particular dye is stable to a wide range of chemicals and is not affected by the extraction procedure described below.

Sulforhodamine B dye application and removal

27. Following TER assessment, the magnesium sulfate is discarded from the tube and the skin is carefully examined for obvious damage. If there is no obvious major damage, sulforhodamine B dye (Acid Red 52; C.I. 45100; CAS number 3520-42-1), 150µL of a 10% (w/v) dilution 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 (e.g. a 20-mL glass scintillation vial) containing deionised water (8mL). 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 60^0 C.

28. After incubation, each skin disc is removed and discarded and the remaining solution is centrifuged for 8 minutes at 21^0 C (relative centrifugal force ~175 x g). A 1mL sample of the supernatant is diluted 1 in 5 (v/v) [i.e. 1mL + 4mL] with 30% (w/v) SDS in distilled water. The optical density (OD) of the solution is measured at 565nm.

Calculation of dye content

29. The sulforhodamine B dye content per disc is calculated from the OD values (10) (sulforhodamine B dye molar extinction coefficient at 565nm = 8.7 x $l0^4$; molecular weight = 580). The dye content is determined for each skin disc by the use of an appropriate calibration curve and mean dye content is then calculated for the replicates.

Interpretation of results

30. The mean TER results are accepted if the concurrent positive and negative control values fall within the acceptable ranges for the method in the testing laboratory. The acceptable resistance ranges for the methodology and apparatus described above are given in the following table:

Control	Substance	Resistance range (kΩ)
Positive	10M Hydrochloric acid	0.5 - 1.0
Negative	Distilled water	10 - 25

31. The mean dye binding results are accepted on condition that concurrent control values fall within the acceptable ranges for the method. Suggested acceptable dye content ranges for the control substances for the methodology and apparatus described above are given below:

Control	Substance	Dye content range (µg/disc)
Positive	10M Hydrochloric acid	40 - 100
Negative	Distilled water	15 - 35

32. The test substance is considered to be non-corrosive to skin:

i) if the mean TER value obtained for the test substance is greater than 5 kΩ, or

ii) the mean TER value is less than or equal to 5 kΩ, and

- the skin disc is showing no obvious damage, and
- the mean disc dye content is well below the mean disc dye content of the 10M HCl positive control obtained concurrently (see paragraph 26 for acceptable ranges).

33. The test substance is considered to be corrosive to skin:

i) if the mean TER value is less than or equal to 5 kΩ and the skin disk is obviously damaged, or

ii) the mean TER value is less than or equal to 5 kΩ, and
- the skin disc is showing no obvious damage, but
- the mean disc dye content is greater than or equal to the mean disc dye content of the 10M HCl positive control obtained concurrently (see paragraph 26 for positive control values).

DATA AND REPORTING

Data

34. Resistance values (kΩ) and mean dye content values (µg/disc), where appropriate, for the test material, as well as for positive and negative controls should be reported in tabular form (individual trial data and means ± S.D.), including data for replicates/repeat experiments, mean and individual values.

Test report

35. The test report should include the following information:

Test and Control Substances:
- Chemical Name(s) such as IUPAC or CAS name, and CAS number, if known;
- Purity and composition of the substance or preparation (in percentage(s) by weight) physical nature and purity;
- physico-chemical properties such as physical state, pH, stability, water solubility, 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.

Test Animals:
- strain and sex used;
- age of the animals when used as donor animals;
- source, housing condition, diet, etc.;
- details of the skin preparation.

Test Conditions:
- calibration curves for test apparatus;
- calibration curves for dye binding test performance;
- details of the test procedure used for TER measurements;

316 – details of the test procedure used for the dye binding assessment; if appropriate
317 – description of any modification of the test procedure;
318 – description of evaluation criteria used for considering studies as positive or
319 negative.
320
321 Results:
322 – tabulation of data from the TER and dye binding assay (if appropriate) for individual
323 animals and individual skin samples;
324 – description of any effects observed.
325
326 Discussion of the results.
327
328 Quality assurance statement for Good Laboratory Practice compliant studies:
329 – statement should indicate all inspections made during the study and the dates any
330 results were reported to the Study Director. The statement should also confirm that
331 the final report reflects the raw data.
332
333 Conclusions.
334
335

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428 **Figure 1: Apparatus for the rat skin TER assay**

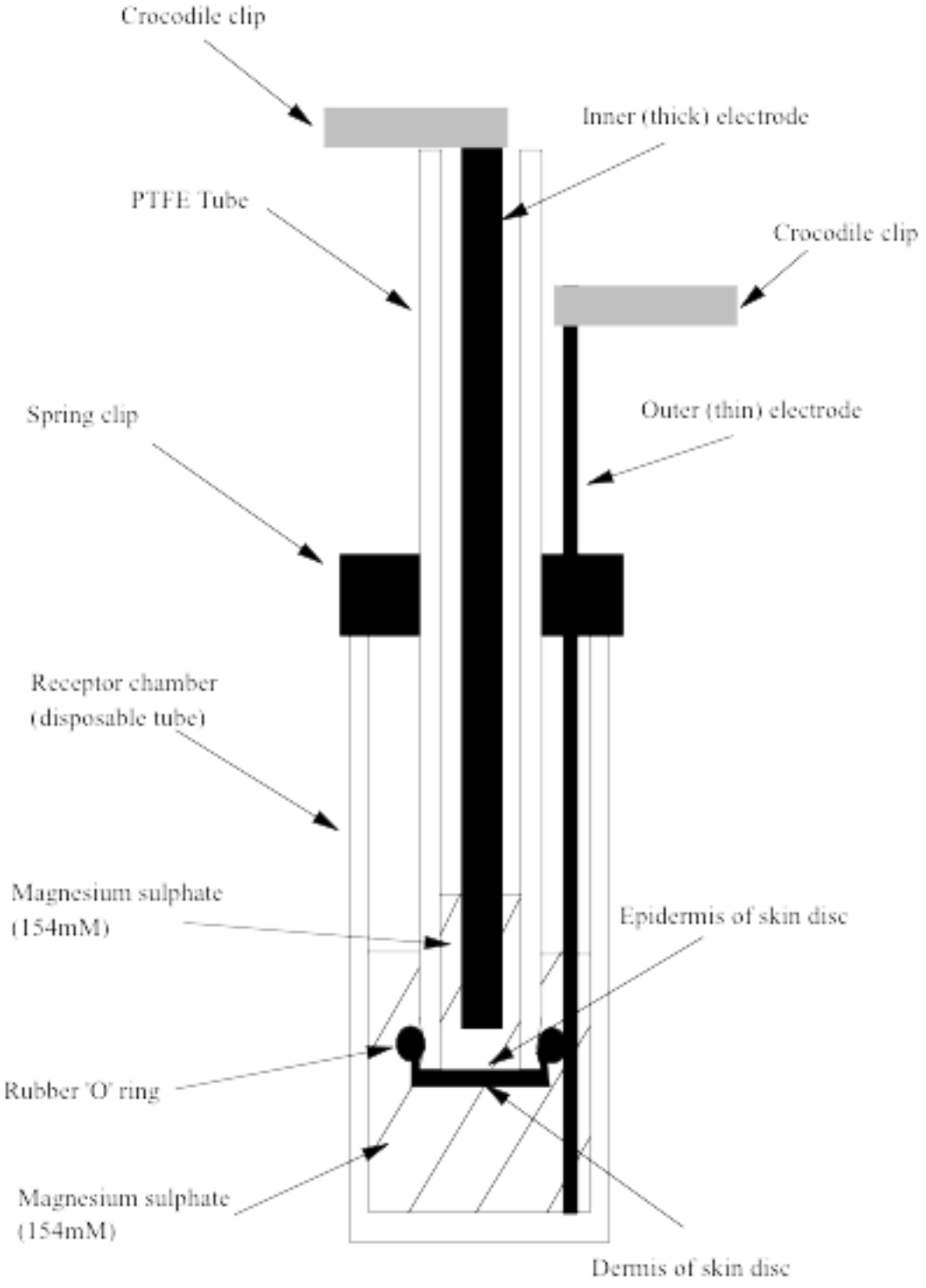

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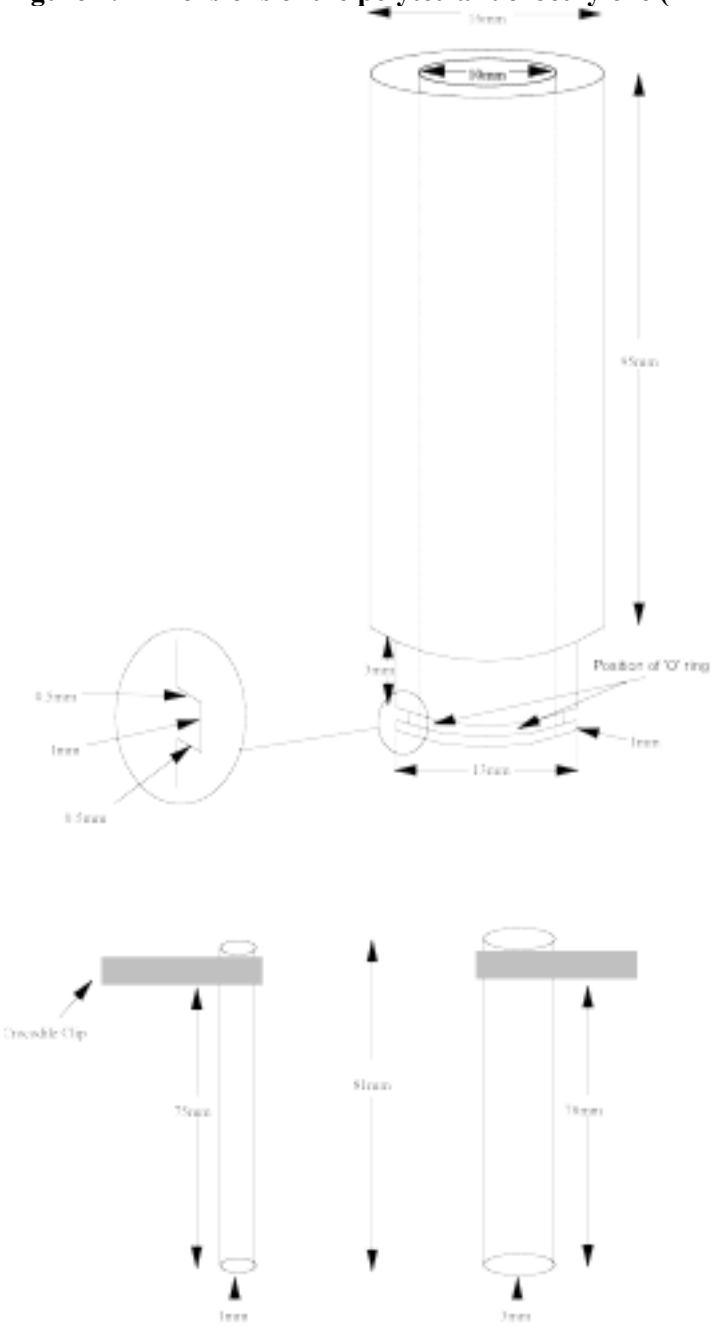
Figure 2: Dimensions of the polytetrafluoroethylene (PTFE) and receptor tubes and electrodes used



Critical factors of the apparatus shown above:

- the inner diameter of the PTFE tube,
- the length of the electrodes relative to the PTFE tube and receptor tube, such that the skin disc is not touched by the electrodes and that a standard length of electrode is in contact with the $MgSO_4$ solution,
- the amount of $MgSO_4$ solution in the receptor tube should give a depth of liquid, relative to the level in the PTFE tube, as shown in Figure 1,
- the skin disk should be fixed well enough to the PTFE tube, such that the electrical resistance is a true measure of the skin properties.

ANNEX 1

PERFORMANCE STANDARDS FOR EVALUATION OF THE VALIDATION STATUS OF PROPOSED NEW OR MODIFIED IN VITRO TEST METHODS THAT ARE STRUCTURALLY AND MECHANISTICALLY SIMILAR TO THE SKIN TRANSCUTANEOUS ELECTRICAL RESISTANCE (TER) TESTS FOR SKIN CORROSION

INTRODUCTION

1. The purpose of Performance Standards (PS) is to communicate the basis by which new test methods, both proprietary (*i.e.,* copyrighted, trademarked, registered) and non-proprietary can be determined to have sufficient accuracy and reliability for specific testing purposes. These PS, based on validated and accepted test methods, can be used to evaluate the reliability and accuracy of other analogous test methods (colloquially referred to as "me-too" tests) that are based on similar scientific principles and measure or predict the same biological or toxic effect (7).

2. Prior to adoption of modified test methods, *i.e.,* proposed potential improvements to an approved test method, there should be an evaluation to determine the effect of the proposed changes on the test's performance and the extent to which such changes affect the information available for the other components of the validation process. Depending on the number and nature of the proposed changes, the generated data and supporting documentation for those changes, they should either be subjected to the same validation process as described for a new test, or, if appropriate, to a limited assessment of reliability and relevance using established PS (7).

3. Similar (me-too) or modified test methods proposed for use under this Test Guideline should be evaluated to determine their reliability and accuracy using chemicals representing the full range of the TG 431 corrosivity scores.

4. These PS are based on the US-ICCVAM PS (6) for evaluating the validity of new or modified TER test methods. The PS consist of essential test method components, recommended reference substances, and standards for accuracy and reliability that the proposed test method should meet or exceed.

I) Essential test method components

5. To ensure that a modified TER test method is structurally and mechanistically similar to the rat skin TER and measures the same biological effect, the following components should be included in the test method protocol:

1. Procedures connected to the use of laboratory animals
2. The physical components of the test method including the apparatus for measuring skin impedance, the skin disc construct
3. Application of test substance
4. Criteria for appropriate control substances
5. Measurement of membrane barrier penetration
6. Dye binding procedures

If any of these criteria are not met, then these performance standards cannot be used for validation of the new or modified test method.

II) Minimum list of reference substances

6. ICCVAM identified 24 minimum required reference substances (12 noncorrosives, 12 corrosives) that are included in the skin corrosivity performance standards.

- The list of reference substances is representative of the 60 chemicals used in the ECVAM validation study of the rat skin TER assay (9)(10).
- The list of reference substances are representative of the range of corrosivity responses obtained for the *in vivo* rabbit skin reference test method.
- A subset of the 24 reference chemicals (12 total; 6 noncorrosives, 6 corrosives) serves as proficiency chemicals for the rat skin TER assay; the names of these chemicals are bolded.

Table 2. Recommended Chemicals for Validation of New or Modified *In Vitro* TER Corrosivity Test Methods

Chemical[1]	CASRN	Chemical Class[2]	UN *In Vivo* PG	pH[3]
***In Vivo* Corrosives**				
Phosphorus tribromide	7789-60-8	inorganic acid	II	1.0
Sulfuric acid (10%)	7664-93-9	inorganic acid	II/III	1.2
Boron trifluoride dihydrate	13319-75-0	inorganic acid	II	1.5
Glycol bromoacetate (85%)	3785-34-0	electrophile	II/III	2.0
Caprylic acid	124-07-2	organic acid	II/III	3.6
2-tert-Butylphenol	88-18-6	phenol	II/III	3.9
60/40 Caprylic/decanoic acids	68937-75-7	organic acid	II/III	3.9
Dimethyldipropylenetriamine	10563-29-8	inorganic base	I	8.3
Dimethylisopropylamine	996-35-0	organic base	II/III	8.3
1,2-Diaminopropane	78-90-0	organic base	I	8.3
n-Heptylamine	111-68-2	organic base	II/III	8.4
Potassium hydroxide (10% aq.)	1310-58-3	inorganic base	II	13.1
***In Vivo* Noncorrosives**				
Sulfamic acid	5329-14-6	inorganic acid	NC	1.5
Isostearic acid	30399-84-9	organic acid	NC	3.6
Phenethyl bromide	103-63-9	electrophile	NC	3.6
Eugenol	97-53-0	phenol	NC	3.7
1,9-Decadiene	1647-16-1	neutral organic	NC	3.9
Benzyl acetone	2550-26-7	neutral organic	NC	3.9
Sodium lauryl sulfate (20% aq.)	151-21-3	surfactant	NC	3.9
Tetrachloroethylene	127-18-4	neutral organic	NC	4.5
4-Amino-1,2,4-triazole	584-13-4	organic base	NC	5.5
4-(methylthio)-Benzaldehyde	3446-89-7	electrophile	NC	6.8
Sodium carbonate (50% aq.)	7664-93-9	inorganic base	NC	11.7
Dodecanoic acid (lauric acid)	143-07-7	organic acid	NC	ND

Abbreviations: aq = aqueous; CASRN = Chemical Abstracts Service Registry Number; PG = Packing Group; NC = Noncorrosive; ND = not determined (unable to measure); UN = United Nations. Recommended proficiency chemicals are indicated in bold type.

[1]These chemicals, sorted first by corrosives versus noncorrosives and then by pH, were selected from among the 60 chemicals used by ECVAM to validate TER (9)(10). Unless otherwise indicated, the chemicals were tested at the purity level obtained when purchased from a commercial source (9). The goal of the selection process is to include, to the extent possible, chemicals that: are representative of the range of corrosivity responses (e.g., noncorrosives; weak to strong corrosives) that the validated reference test method is capable of measuring or predicting; are representative of the chemical classes used during the validation process; reflect the overall performance characteristics of the validated reference test method; have chemical structures that are well-defined; induce reproducible results in the validated reference test method; induce definitive results in the *in vivo* reference test; are commercially available; and are not associated with prohibitive disposal costs.

[2]Chemical class assigned by Barratt et al. (1998).

505 [3]The pH values were obtained from Fentem et al. (1998) and Barratt et al. (1998).
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III) Standards for accuracy and reliability

7. When evaluated using the minimum list of recommended reference chemicals, the reliability and accuracy (i.e., sensitivity, specificity, false positive rates, and false negative rates) of the proposed *in vitro* skin TER assay should be at least comparable to that of the validated *in vitro* rat skin TER test method (17). Noncorrosive and corrosive chemicals, ranging in activity from strong to weak, and representing relevant chemical classes are included so that the performance of the proposed test method can be determined and compared to that of the validated reference test method.

8. An assessment of interlaboratory reproducibility is not essential if the test method is to be used in one laboratory only.

9. In terms of cell viability measurements, the median coefficient of variation (CV) should not exceed 35% for studies conducted in different laboratories (10)(17). The median CV for replicate studies conducted in the same laboratory should be less than median CV for studies conducted in different laboratories.

ANNEX 2

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.

Performance standards (PS): 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 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.

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.

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.

Sensitivity: The proportion of all positive/active chemicals 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 chemicals 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 *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.

Tiered testing strategy: Testing which uses test methods in a sequential manner; the test methods selected in each succeeding level are determined by the results in the previous level of testing.

Transcutaneous Electrical Resistance (TER): is a measure of the electrical impedance of the skin, as a resistance value in kilo Ohms. A simple and robust method of assessing barrier function by recording the passage of ions through the skin using a Wheatstone bridge apparatus.