Adopted:
28 July 2015

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

In Vitro Skin Corrosion: Transcutaneous Electrical Resistance Test Method (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 chemical [as defined by the United Nations (UN) Globally Harmonized System of Classification and Labelling of Chemicals (GHS)] (1). This updated Test Guideline 430 provides an *in vitro* procedure allowing the identification of non-corrosive and corrosive substances and mixtures in accordance with UN GHS (1).

2.   The assessment of skin corrosivity has typically involved the use of laboratory animals (OECD Test Guideline 404 (TG 404); originally adopted in 1981, and revised in 1992, 2002 and 2015) (2). In addition to the present TG 430, other *in vitro* test methods for testing of skin corrosion potential of chemicals have been validated and adopted as OECD Test Guidelines 431 (3) and 435 (4), that are also able to identify sub-categories of corrosive chemicals when required. Several validated *in vitro* test methods have been adopted as OECD TG 439 (5), to be used for the testing of skin irritation. A document on Integrated Approaches to Testing and Assessment (IATA) for Skin Corrosion and Irritation describes several modules which group various information sources and analysis tools and provides guidance on (i) how to integrate and use existing testing and non-testing data for the assessment of skin irritation and skin corrosion potentials of chemicals and (ii) proposes an approach when further testing is needed (6).

3.   This Test Guideline addresses the human health endpoint skin corrosion. It is based on the rat skin transcutaneous electrical resistance (TER) test method, which utilizes skin discs to identify corrosives by their ability to produce a loss of normal *stratum corneum* integrity and barrier function. This Test Guideline was originally adopted in 2004 and updated in 2015 to refer to the IATA guidance document.

4.   In order to evaluate *in vitro* skin corrosion testing for regulatory purposes, pre-validation studies (7) followed by a formal validation study of the rat skin TER test method for assessing skin corrosion were conducted (8) (9) (10) (11). The outcome of these studies led to the recommendation that the TER test method (designated the Validated Reference Method – VRM) could be used for regulatory purposes for the assessment of *in vivo* skin corrosivity (12) (13) (14).

5.   Before a proposed similar or modified *in vitro* TER test method for skin corrosion other than the VRM can be used for regulatory purposes, its reliability, relevance (accuracy), and limitations for its proposed use should be determined to ensure its similarity to the VRM, in accordance with the requirements of the Performance Standards (15). 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.

DEFINITIONS

6.   Definitions used are provided in Annex 1.

INITIAL CONSIDERATIONS

7.   A validation study (10) and other published studies (16) (17) have reported that the rat skin TER test method 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.

8.   This Test Guideline addresses *in vitro* skin corrosion. It allows the identification of non-corrosive and corrosive test chemicals in accordance with the UN GHS (1). A limitation of this Test Guideline, as demonstrated by the validation studies (8) (9) (10) (11), is that it does not allow the sub-categorization of corrosive substances and mixtures in accordance with the UN GHS (1). The regulatory framework in member countries will decide how this Test Guideline will be used. 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* (5). For a full evaluation of local skin effects after a single dermal exposure, the Guidance Document n. 203 on Integrated Approaches for Testing Assessment should be consulted (6).

9.   A wide range of chemicals representing mainly substances has been tested in the validation underlying this Test Guideline and the empirical database of the validation study amounted to 60 substances covering a wide range of chemical classes (8) (9). On the basis of the overall data available, the Test Guideline is applicable to a wide range of chemical classes and physical states including liquids, semi-solids, solids and waxes. However, since for specific physical states test items with suitable reference data are not readily available, it should be noted that a comparably small number of waxes and corrosive solids were assessed during validation. The liquids may be aqueous or non-aqueous; solids may be soluble or insoluble in water. In cases where evidence can be demonstrated on the non-applicability of the Test Guideline to a specific category of substances, the Test Guideline should not be used for that specific category of substances. In addition, this Test Guideline is assumed to be applicable to mixtures as an extension of its applicability to substances. However, due to the fact that mixtures cover a wide spectrum of categories and composition, and that only limited information is currently available on the testing of mixtures, in cases where evidence can be demonstrated on the non-applicability of the Test Guideline to a specific category of mixtures (*e.g.* following a strategy as proposed by Eskes *et al.*, 2012) (18), the Test Guideline should not be used for that specific category of mixtures. Before use of the Test Guideline on a mixture for generating data for an intended regulatory purpose, it should be considered whether, and if so why, it may provide adequate results for that purpose. Such considerations are not needed, when there is a regulatory requirement for testing of the mixture. Gases and aerosols have not been assessed yet in validation studies (8) (9). While it is conceivable that these can be tested using the TER test method, the current Test Guideline does not allow testing of gases and aerosols.

PRINCIPLE OF THE TEST

10.   The test chemical 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 chemicals 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 (16) (see paragraph 32). For rat skin TER, a cut-off value of 5kΩ has been selected based on extensive data for a wide range of substances where the vast majority of values were either clearly well above (often > 10 kΩ), or well below (often < 3 kΩ) this value (16). Generally, test chemicals that are non-corrosive in animals but are irritant or non-irritant 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.

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11.   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 utilizing rat skin has shown to be predictive of *in vivo* corrosivity in the rabbit assessed under OECD guideline 404 (2).

DEMONSTRATION OF PROFICIENCY

12.   Prior to routine use of the rat skin TER test method that adheres to this Test Guideline, laboratories should demonstrate technical proficiency by correctly classifying the twelve Proficiency Substances recommended in Table 1. 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 (16)) provided that the same selection criteria as described in Table 1 is applied.

Table 1: List of Proficiency Substances[1]

Substance	CASRN	Chemical Class[2]	UN GHS Cat. Based on *In Vivo* Results[3]	VRM Cat. Based on *In Vitro* Results	Physical State	pH[4]
In Vivo **Corrosives**						
N,N'-Dimethyl dipropylenetriamine	10563-29-8	organic base	1A	6 x C	L	8.3
1,2-Diaminopropane	78-90-0	organic base	1A	6 x C	L	8.3
Sulfuric acid (10%)	7664-93-9	inorganic acid	(1A/)1B/1C	5 x C 1x NC	L	1.2
Potassium hydroxide (10% aq.)	1310-58-3	inorganic base	(1A/)1B/1C	6 x C	L	13.2
Octanoic (Caprylic) acid	124-07-2	organic acid	1B/1C	4 x C 2 x NC	L	3.6
2-tert-Butylphenol	88-18-6	phenol	1B/1C	4 x C 2 x NC	L	3.9
In Vivo **Non-corrosives**						
Isostearic acid	2724-58-5	organic acid	NC	6 x NC	L	3.6
4-Amino-1,2,4-triazole	584-13-4	organic base	NC	6 x NC	S	5.5
Phenethyl bromide	103-63-9	electrophile	NC	6 x NC	L	3.6
4-(Methylthio)-benzaldehyde	3446-89-7	electrophile	NC	6 x NC	L	6.8
1,9-Decadiene	1647-16-1	neutral organic	NC	6 x NC	L	3.9
Tetrachloroethylene	127-18-4	neutral organic	NC	6 x NC	L	4.5

Abbreviations: aq = aqueous; CASRN = Chemical Abstracts Service Registry Number; UN GHS = United Nations Globally Harmonised System (1); VRM = Validated Reference Method; ND = Not Determined.

[1]The proficiency substances, sorted first by corrosives versus non-corrosives, then by corrosive subcategory and then by chemical class, were selected from the substances used in the ECVAM validation study of the rat skin TER test method (8) (9). Unless otherwise indicated, the substances were tested at the purity level obtained when purchased from a commercial source (8). The selection included, to the extent possible, substances that: (i) are representative of the range of corrosivity responses (*e.g.* non-corrosives; weak to strong corrosives) that the VRM is capable of measuring or predicting; (ii) are representative of the chemical classes used in the validation study; (iii) reflect the performance characteristics of the VRM; (iv) have chemical structures that are well-defined; (v) induce definitive results in the *in vivo* reference test method; (vi) are commercially available; and (vii) are not associated with prohibitive disposal costs.
[2]Chemical class assigned by Barratt *et al.* (8).
[3]The corresponding UN Packing groups are I, II and III, respectively, for the UN GHS 1A, 1B and 1C.
[4]The pH values were obtained from Fentem *et al.* (9) and Barratt *et al.* (8).

PROCEDURE

13.   Standard Operating Procedures (SOP) for the rat skin TER skin corrosion test method are available (19). The rat skin TER test methods covered by this Test Guideline should comply with the following conditions:

Animals

14.   Rats should be used because the sensitivity of their skin to substances in this test method has been previously demonstrated (12) and is the only skin source that has been formally validated (8) (9). 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.

15.   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

16.   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 discs are used where it is shown that positive and negative control data are equivalent to that obtained with fresh skin.

17.   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. 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. Tube and 'O' ring dimensions are shown in Figure 2.

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

Application of the test chemical and control substances

19.   Concurrent positive and negative controls should be used for each run (experiment) to ensure adequate performance of the experimental model. Skin discs from a single animal should be used in each run (experiment). The suggested positive and negative control test chemicals are 10M hydrochloric acid and distilled water, respectively.

20.   Liquid test chemicals (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 chemical, or ground to produce a granular material or powder.

21.   Three skin discs are used for each test and control chemical in each testing run (experiment). Test chemicals are applied for 24 hours at 20-23^0 C. The test chemical is removed by washing with a jet of tap water at up to room temperature until no further material can be removed.

TER measurements

22.   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, 2000 μF, and 2 MΩ, 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 100 Hz 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 3 mL $MgSO_4$ 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).

23.   If the measured resistance value is greater than 20 kΩ, this may be due to the remains of the test chemical 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 $MgSO_4$.

24.   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 Test Guideline. Different threshold and control values may apply if the test conditions are altered or a different apparatus is used. Therefore, it is necessary

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to calibrate the methodology and resistance threshold values by testing a series of Proficiency Substances chosen from the substances used in the validation study (8) (9), or from similar chemical classes to the substances being investigated. A set of suitable Proficiency Substances is identified in Table 1.

Dye Binding Methods

25.　　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 (9). For example, neutral organics and substances that have surface-active properties (including detergents, emulsifiers and other surfactants) can remove skin lipids making the barrier more permeable to ions. Thus, if TER values produced by such chemicals are less than or around 5 kΩ in the absence of visually perceptible damage of the skin discs, 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 (7) (9). 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 substances and is not affected by the extraction procedure described below.

Sulforhodamine B dye application and removal

26.　　Following TER assessment, the magnesium sulphate is discarded from the tube and the skin is carefully examined for obvious damage. If there is no obvious major damage (*e.g.* perforation), 150 μL of a 10% (w/v) dilution in distilled water of the dye sulforhodamine B (Acid Red 52; C.I. 45100; CAS number 3520-42-1), 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 (8 mL). 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.

27.　　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 565 nm.

Calculation of dye content

28.　　The sulforhodamine B dye content per disc is calculated from the OD values (9) (sulforhodamine B dye molar extinction coefficient at 565nm = 8.7 x 10^4; 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.

Acceptability Criteria

29.　　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:

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

30.   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 in the following table:

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

Interpretation of results

31.   The cut-off TER value distinguishing corrosive from non-corrosive test chemicals was established during test method optimization, tested during a pre-validation phase, and confirmed in a formal validation study.

32.   The prediction model for rat skin TER skin corrosion test method (9) (19), associated with the UN GHS (1) classification system, is given below:

The test chemical is considered to be non-corrosive to skin:
i)   if the mean TER value obtained for the test chemical is greater than (>) 5 kΩ, or
ii) the mean TER value obtained for the test chemical is less than or equal to (≤) 5 kΩ, and
- the skin discs show no obvious damage(*e.g.* perforation), and
- the mean disc dye content is less than (<) the mean disc dye content of the 10M HCl positive control obtained concurrently (see paragraph 30 for positive control values).

The test chemical is considered to be corrosive to skin:
if the mean TER value obtained for the test chemical is less than or equal to (≤) 5 kΩ and the skin discs are obviously damaged(*e.g.* perforated), or
the mean TER value obtained for the test chemical is less than or equal to (≤) 5 kΩ, and
- the skin discs show no obvious damage(*e.g.* perforation), 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 30 for positive control values).

33.   A testing run (experiment) composed of at least three replicate skin discs should be sufficient for a test chemical when the classification is unequivocal. However, in cases of borderline results, such as non-concordant replicate measurements and/or mean TER equal to 5 ± 0.5 kΩ, a second independent testing run (experiment) should be considered, as well as a third one in case of discordant results between the first two testing runs (experiments).

DATA AND REPORTING

Data

34. Resistance values (kΩ) and dye content values (µg/disc), where appropriate, for the test chemical, as well as for positive and negative controls should be reported in tabular form, including data for each individual replicate disc in each testing run (experiment) and mean values ± SD. All repeat experiments should be reported. Observed damage in the skin discs should be reported for each test chemical.

Test report

35. 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 physico-chemical properties of the constituents;
- Physical appearance, water solubility, and additional relevant physico-chemical 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.

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, band pass used for measuring OD values, and OD linearity range of measuring device (*e.g.* spectrophotometer), if appropriate;
- Details of the test procedure used for TER measurements;
- Details of the test procedure used for the dye binding assessment, if appropriate;
- Test doses used, duration of exposure period(s) and temperature(s) of exposure;
- Details on washing procedure used after the exposure period;
- Number of replicate skin discs used per test chemical and controls (positive and negative control);
- Description of any modification of the test procedure;
- Reference to historical data of the model. This should include, but is not limited to;
  - i) Acceptability of the positive and negative control TER values (in kΩ) with reference to positive and negative control resistance ranges

    ii) Acceptability of the positive and negative control dye content values (in µg/disc) with reference to positive and negative control dye content ranges

    iii) Acceptability of the test results with reference to historical variability between skin disc replicates

  – Description of decision criteria/prediction model applied.

Results:

  – Tabulation of data from the TER and dye binding assays (if appropriate) for individual test chemicals and controls, for each testing run (experiment) and each skin disc replicate (individual animals and individual skin samples), means, SDs and CVs;

  – Description of any effects observed;

  – The derived classification with reference to the prediction model/decision criteria used.
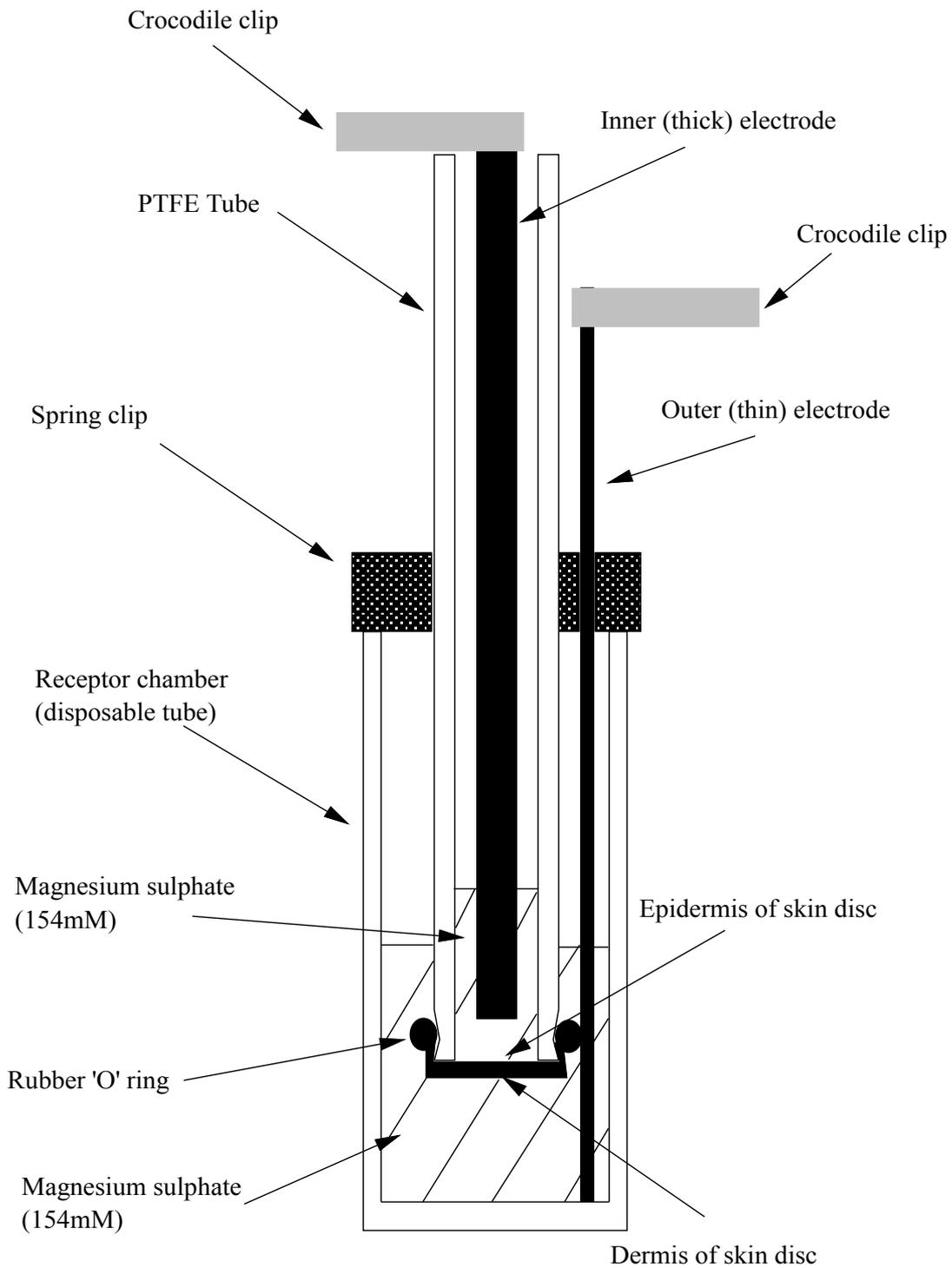
Discussion of the results

Conclusions

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Figure 1: Apparatus for the rat skin TER assay

Crocodile clip

Inner (thick) electrode

PTFE Tube

Crocodile clip

Spring clip

Outer (thin) electrode

Receptor chamber
(disposable tube)

Magnesium sulphate
(154mM)

Epidermis of skin disc

Rubber 'O' ring

Magnesium sulphate
(154mM)

Dermis of skin disc

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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 should not be 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 disc should be fixed well enough to the PTFE tube, such that the electrical resistance is a true measure of the skin properties.

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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 (20).

C: Corrosive.

Chemical: means a substance or a mixture.

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 (20).

GHS (Globally Harmonized System of Classification and Labelling of Chemicals (UN)): 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 as 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.

OD: Optical Density.

PC: Positive Control, a replicate containing all components of a test system and treated with a substance known to induce a positive response. To ensure that variability in the positive control response across time can be assessed, the magnitude of the positive response should not be excessive.

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;

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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.

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 (20).

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 (20).

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 (20).

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 (20).

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.

(Testing) run: A single test chemical concurrently tested in a minimum of three replicate skin discs.

Test chemical: means what is being tested.

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.

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