新規試験法提案書

経皮電気抵抗試験を用いた
皮膚腐食性試験代替法

平成29年10月

国立医薬品食品衛生研究所

新規試験法提案書

平成 29 年 10 月 1 日

No.　2017-03

経皮電気抵抗試験を用いた皮膚腐食性試験代替法に関する提案

平成 29 年 7 月 25 日に東京、国立医薬品食品衛生研究所にて開催された新規試験法評価会議（通称：JaCVAM 評価会議）において以下の提案がなされた。

提案内容： 経皮電気抵抗試験は、種々の化学物質に適用でき、腐食性および非腐食性を分類するための代替法として、行政的利用が可能であると考える。ただし、EU では動物実験を行った化粧品等の販売を禁止していることから、化粧品等の安全性評価への本試験方法の適用は問題が生じる懸念がある。

この提案書は、Organisation for Economic Co-operation and Development (OECD) Test Guideline (TG) 430, *In Vitro* Skin Corrosion: Transcutaneous Electrical Resistance Test Method (TER)をもとに、皮膚腐食性試験資料編纂委員会によりまとめられた文書を用いて、JaCVAM評価会議が評価および検討した結果、その有用性が確認されたことから作成された。

以上の理由により、行政当局の安全性評価方法として経皮電気抵抗試験を用いた皮膚腐食性試験代替法の使用を提案するものである。

大野泰雄

JaCVAM 評価会議　議長

西川秋佳

JaCVAM 運営委員会　委員長

JaCVAM 評価会議

大 野 泰 雄　（公益財団法人　木原記念横浜生命科学振興財団）：座長
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任期：平成 28 年 4 月 1 日～平成 30 年 3 月 31 日
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小 島　　肇 （国立医薬品食品衛生研究所　安全性生物試験研究センター　安全性予測評価部
第二室）：事務局

JaCVAM Statement on the *In Vitro* Skin Corrosion: Transcutaneous Electrical Resistance Test Method (TER)

At a meeting held on 25 July 2017 at the National Institute of Health Sciences (NIHS) in Tokyo, Japan, the Japanese Center for the Validation of Alternative Methods (JaCVAM) Regulatory Acceptance Board unanimously endorsed the following statement:

Proposal: We consider the Transcutaneous Electrical Resistance Test Method (TER) to be suitable for predicting the skin corrosion potential of a test chemical in a regulatory context. We are concerned, however, that the use of isolated rat skin in this test method for the safety evaluation of cosmetic ingredients and products could be problematic due to the prohibition on animal testing for cosmetics that are sold in the EU.

This statement was prepared following a review of the Organisation for Economic Co-operation and Development (OECD) Test Guideline (TG) 430 *In Vitro* Skin Corrosion: TER Test Method together with other materials prepared by the Skin Corrosion Testing JaCVAM Editorial Committee to acknowledge that the results of a review and study by the JaCVAM Regulatory Acceptance Board have confirmed the usefulness of this assay.
Based on the above, we propose the TER test method as a useful means for assessing skin corrosion potential during safety assessments by regulatory agencies.

Yasuo Ohno
Chairperson
JaCVAM Regulatory Acceptance Board

Akiyoshi Nishikawa
Chairperson
JaCVAM Steering Committee

October 1, 2017

The JaCVAM Regulatory Acceptance Board was established by the JaCVAM Steering Committee, and is composed of nominees from the industry and academia.

This statement was endorsed by the following members of the JaCVAM Regulatory Acceptance Board:

Mr. Yasuo Ohno (Kihara Memorial Yokohama Foundation for the Advancement of Life Sciences) : Chairperson
Mr. Naofumi Iizuka (Pharmaceuticals and Medical Devices Agency)*
Mr. Yoshiaki Ikarashi (National Institute of Health Sciences: NIHS)
Mr. Noriyasu Imai (Japanese Society for Alternatives to Animal Experiments)
Mr. Tomoaki Inoue (Japanese Society of Immunotoxicology)
Mr. Yuji Ishii (Biological Safety Research Center: BSRC, NIHS)
Ms. Yumiko Iwase (Japan Pharmaceutical Manufacturers Association)
Mr. Takeshi Morita (Japanese Environmental Mutagen Society)
Mr. Shunji Nakai (Japan Chemical Industry Association)
Ms. Ruriko Nakamura (National Institute of Technology and Evaluation)
Mr. Akiyoshi Nishikawa (BSRC, NIHS)
Ms. Maki Noguchi (Pharmaceuticals and Medical Devices Agency)**
Mr. Satoshi Numazawa (Japanese Society of Toxicology)
Mr. Kazutoshi Shinoda (Pharmaceuticals and Medical Devices Agency)
Ms. Mariko Sugiyama (Japan Cosmetic Industry Association)
Mr. Hiroo Yokozeki (Japanese Society for Dermatoallergology and Contact Dermatitis)

Term: From 1st April 2016 to 31st March 2018
*: From 1st April 2016 to 31st March 2017
**: From 1st April 2017 to 31st March 2018

This statement was endorsed by the following members of the JaCVAM Steering Committee after receiving the report from JaCVAM Regulatory Acceptance Board:

Mr. Akiyoshi Nishikawa (BSRC, NIHS): Chairperson
Mr. Toru Kawanishi (NIHS)
Mr. Manabu Fuchioka (Ministry of Health, Labour and Welfare)
Ms. Yoko Hirabayashi (Division of Toxicology, BSRC, NIHS)
Mr. Akihiko Hirose (Division of Risk Assessment, BSRC, NIHS)
Ms. Mitsue Hirota (Pharmaceutical & Medical Devices Agency)
Mr. Masamitsu Honma (Division of Genetics and Mutagenesis, BSRC, NIHS)
Mr. Yasunari Kanda (Division of Pharmacology, BSRC, NIHS)
Mr. Atsushi Kato (National Institute of Infectious Diseases)
Mr. Kouichirou Koike (Ministry of Health, Labour and Welfare)
Ms. Kumiko Ogawa (Division of Pathology, BSRC, NIHS)
Mr. Taku Oohara (Ministry of Health, Labour and Welfare)
Mr. Kazutoshi Shinoda (Pharmaceuticals and Medical Devices Agency)
Mr. Atsuya Takagi (Animal Management Section of the Division of Toxicology, BSRC, NIHS)
Mr. Masaaki Tsukano (Ministry of Health, Labour and Welfare)
Mr. Shinichi Watanabe (Ministry of Health, Labour and Welfare)
Mr. Hajime Kojima (Division of Risk Assessment, BSRC, NIHS): Secretary

経皮電気抵抗試験を用いた皮膚腐食性試験代替法

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評価会議報告書

経皮電気抵抗試験を用いた皮膚腐食性試験代替法

JaCVAM 評価会議

平成 29 年（2017 年）7 月 25 日

JaCVAM 評価会議

大 野 泰 雄 （公益財団法人　木原記念横浜生命科学振興財団）：座長
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ラット摘出皮膚を用いる経皮電気抵抗試験（TER: Transcutaneous Electrical Resistance）は、ウサギを用いる皮膚腐食性試験の代替として開発された試験法である。本試験法では、腐食性物質が角質層に吸収された後拡散し、下層の表皮細胞を傷害するという考えをもとに、被験物質曝露後の電気抵抗値を指標として皮膚腐食性を評価する。本試験法については、ECVAM（European Centre for the Validation of Alternative Methods：欧州代替法評価センター）でバリデーション研究が実施され、ESAC（ECVAM Scientific Advisory Committee : ECVAM 科学諮問会議）や[1,2,3] ICCVAM（Interagency Coordinating Committee on the Validation of Alternative Methods：米国代替法に関する省庁間連絡会議）[4,5]において本試験法の信頼性等が確認された後、OECD（Organisation for Economic Co-operation and Development：経済協力開発機構）にてテストガイドライン（TG）430 として 2004 年に承認されている。この TG は何度か改訂されており、現在は 2015 年版となっている[6]。

JaCVAM 評価会議は、皮膚腐食性試験資料編纂委員会により作成された「経皮電気抵抗試験を用いた皮膚腐食性試験代替法の評価報告書」[7]を用いて、本試験法の妥当性について検討した。

１．試験法の定義

名称：　経皮電気抵抗試験を用いた皮膚腐食性試験代替法

代替する対象毒性試験：　ウサギを用いる皮膚腐食性試験

試験法の概略：　本試験法では、ラット（28-30 日齢ほど）から切除した 20 mm 円盤状の皮膚を試験システムとして使用する。被験物質を表皮面に一定時間適用し、角質層に吸収された後に拡散し、下層の表皮細胞に対し傷害する程度を、経皮電気抵抗を指標として測定することにより皮膚腐食性を評価する。さらに、電気抵抗が 5 kΩ 以下の場合には、10%スルホローダミンＢ染色液にて染色し、皮膚に吸着された色素の量を吸光度から算出する。この結果、経皮電気抵抗が 5ｋΩより小さく、明らかに損傷のある場合（例えば穿孔）、または、明らかな損傷がなくても色素量が陽性対照物質（10M塩酸）の結果以上の場合、皮膚腐食性と判定する。

２．評価に用いた資料及び評価内容の科学的妥当性

本試験法は、ECVAM による広い範囲の被験物質を用いたバリデーション研究により、妥当性が検証され、ESAC により信頼性と再現性は高いと評価されている[1,2,3]。この結論は、ICCVAM においても確認されている。これらの結果は公表されており、透明で独立な科学的評価が行われていると考える[4,5]。本試験法は、OECD により試験法ガイドラインに採択された（TG 430）[6]。本邦においては、JaCVAM 皮膚腐食性試験資料編纂委員会が、これらの資料を用いて本試験法を評価している[7]。また、本試験法は、腐食性物質が角質層を透過・拡散したのちに表皮細胞を傷害し、皮膚のバリア機能を傷害するという皮

膚傷害機構を模倣しており、皮膚バリア機能の指標として経皮電気抵抗を評価しており、皮膚腐食性発現機序が考慮されている。

以上の点から、本試験法の評価に用いた資料ならびに評価内容については、皮膚腐食性を評価する方法として科学的妥当性があると考える。

３．本試験法の有用性と適用限界

本試験法に対する Botham[1,8]と Fentem[3]の予測性の検討結果および ICCVAM による集計結果を表 1 に示した。両者の結果を合わせた結果とそれぞれの論文の正確度、感度、特異度に大きな差はなく、正確度は約 81%、感度は約 94%、特異度は約 71%であった。この中で、偽陰性は 3 物質 (6%) について認められているものの、これら 3 物質の腐食性の UN GHS[1]細分類はいずれも強度が弱い側のレベルにある(UN GHS 区分 1A には相当しない)と推察されるため、「腐食性は強くないと判断できる [7]」という資料編纂委員会の判断は支持できる。

表 1． TER の予測性

	ICCVAM による集計結果	Botham et al. (1992 and 1996)	Fentem et al. (1998)
感度	94% (51/54)	96% (27/28)	93% (25/27)
特異度	71% (48/68)	70% (26/37)	71% (22/31)
正確度	81% (99/122)	82% (53/65)	81% (47/58)

OECD の *in vitro* 腐食性試験ガイドラインとして、他に「TG431　ヒト表皮モデル試験」および「TG435 *in vitro* 膜バリア試験」があり、ICCVAM では本試験法とこれらの試験法 (EpiSkin™, EpiDerm™および Corrositex®)について特異性、感度、正確性等のバリデーション研究が比較されている [5,9,10]。この結果、本試験法は他の試験法と同等の予測性を有すると評価されている。

以上の点から、本試験法は種々の化学物質に対して適用でき、すべてのタイプの腐食性および非腐食性を分類するための代替法として有用であると考える。ただし、プロトコルから類推して、難水溶性の固体や粘性の高い物質は適切に評価できない可能性がある。また、UN GHS による腐食性の細分類への適用には検討が不十分であり、有用性を評価できない。なお、本邦における行政的な受け入れにおいては、UN GHS 区分 1 の細区分を求められていない。

[1] 国連 化学品の分類および表示に関する世界調和システム（UN GHS：United Nations Globally Harmonized System of Classification and Labelling of Chemicals）

４．目的とする物質又は製品の毒性を評価する試験法としての、社会的受け入れ性及び行政上の利用の可能性

社会的受け入れ性：

本試験法は、ラットの摘出皮膚を用いているため完全な置き換え試験ではない。しかしながら、本試験法を用いることで動物数の削減が可能であり、また、腐食性物質による動物へのストレスを与えることは無いことから、動物実験代替法の３Rs の原則に適った試験法である。本試験法は、必要な技術が複雑ではなく、適切な訓練によって容易に習得できると考えられる。

以上の観点から、本試験法は社会的に受け入れられるものであると判断する。

行政上の利用性：

本試験法は、種々の化学物質に適用でき、腐食性および非腐食性を分類するための代替法として、行政的利用が可能であると考える。ただし、EU では動物実験を行った化粧品等の販売を禁止していることから、化粧品等の評価への本試験方法の適用は問題が生じる懸念がある。

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1) Botham, P.A. et al. (1996) A prevalidation study on *in vitro* skin corrosivity testing: The report and recommendations of ECVAM workshop 6. ATLA, 23, 219-255
2) Barratt, M.D. et al. (1998) The ECVAM international validation study on *in vitro* tests for skin corrosivity. 1. Selection and distribution of the test chemicals. Toxicology in Vitro, 12, 471-482
3) Fentem, J.H. et al. (1998) The ECVAM International Validation Study on *in vitro* tests for skin corrosivity. 2. Results and evaluation by the management team. Toxicology in Vitro, 12, 483-524
4) ICCVAM (1999) NIH Publication No.99-4495. Corrositex: An *in vitro* test method for assessing dermal corrosivity potential of chemicals.
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6) OECD (2015) Guideline for the testing of chemicals. 430, *In Vitro* Skin Corrosion: Transcutaneous Electrical Resistance Test Method (TER).
7) JaCVAM 皮膚腐食性資料編纂委員会：皮膚腐食性試験評価報告書　経皮電気抵抗試験を用いた皮膚腐食性試験代替法の評価報告書（2017 年 2 月 24 日）.
8) Botham, P.A. et al.（1992）The skin corrosivity test in vitro. Results of an inter-laboratory trial. Toxicol In Vitro. 6(3):191-4
9) ICCVAM (1999) NIH Publication No.99-4495. Corrositex: An *in vitro* test method for assessing dermal corrosivity potential of chemicals
10) JaCVAM 皮膚腐食性資料編纂委員会：皮膚腐食性試験評価報告書　ヒト表皮モデルを用いた皮膚腐食性試験代替法の評価報告書（2017 年 2 月 24 日）.

評価報告書

経皮電気抵抗試験を用いた皮膚腐食性試験代替法

皮膚腐食性試験資料編纂委員会

平成 29 年（2017 年）2 月 24 日

評価委員

髙橋　祐次	国立医薬品食品衛生研究所 毒性部
中村るりこ	独立行政法人 製品評価技術基盤機構　化学物質管理センター
須方　督夫	住友化学株式会社 レスポンシブルケア部（化学品安全グループ）／日本化学工業協会
小島　肇	国立医薬品食品衛生研究所 安全性予測評価部

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要旨

ウサギを用いる皮膚腐食性試験の動物実験代替法（代替法）として、経済協力開発機構（OECD : Organisation for Economic Co-operation and Development）にて試験ガイドライン（TG: Test Guideline）430として承認されたラット摘出皮膚を用いる経皮電気抵抗試験（TER: Transcutaneous Electrical Resistance）の有用性を評価した。信頼性と妥当性という視点において、TERを評価した結果、適用範囲に限界はあるものの、本試験法により腐食性の有無を評価できると考えられた。しかし、生きているラットから採取した皮膚を用いることから化粧品等の評価への応用には問題がある。

1. 試験法の科学的および規制面からの妥当性

皮膚腐食性試験は皮膚刺激性試験の一環として行われ、ガイドラインではDraizeらにより提唱されたウサギを用いる方法が推奨されている[1)]。この方法は被験物質の刺激性や腐食性を検出する試験法として長く用いられてきたものの、判定を肉眼で行うため客観性に乏しく実験間や施設間での再現性が乏しい、更に動物に激しい痛みとストレスを与えることが社会的に問題となり、以前より動物を使用しない代替法の開発が切望されていた。この動物実験代替法（以下、代替法と記す）として、経済協力開発機構（OECD：Organisation for Economic Co-operation and Development）にて承認された試験ガイドライン（TG: Test Guideline）430には、経皮電気抵抗試験（TER: Transcutaneous Electrical Resistance)が記載されている[2)]。この試験法は、腐食性物質が角質層に吸収された後拡散し、下層の表皮細胞を傷害することにより皮膚のバリアー機能を傷害するという考えをもとに、被験物質曝露後の電気抵抗値を指標に皮膚腐食性を評価している。本試験法は欧州代替法評価センター(ECVAM : European Centre for the Validation of Alternative Methods）によるバリデーション研究を経て、欧州では化学物質の皮膚腐食性評価を目的として承認され、化学物質のリスク表示や識別等に利用されている。特に昨今では国連化学品の分類および表示に関する世界調和システム（UN GHS : United Nations Globally Harmonized System of Classification and Labelling of Chemicals）分類に従って評価されるケースが増えている。

我が国で既存の化学物質を評価する場合、OECDで承認された試験方法による結果を利用することは可能であるが、現在まで代替法での結果をもとに腐食性を評価された例は多くない。安全性評価における代替法の普及が切望されている現状において、我が国でも積極的に受け入れることが必要となっている。

これらの状況に鑑み、本評価書では、OECD　TG430に掲載されたTERの腐食性評価における有用性を評価した[2)]。

2. 試験プロトコル構成の妥当性[2)]

1) 原理　　切除したラット皮膚を試験システムとして使用し、その電気抵抗の変化を皮膚傷害性の指標とする。

2）方法　　摘出した直径20 mm円盤状の皮膚（ラット、28-30日齢ほど）を図1に示すように、テフロン（ポリテトラフルオロエチレン：PTFE）チューブの下部に表皮面を上にしてあて、ゴム製のOリングではさむ。皮膚のついたPTFEチューブを硫酸マグネシウム（154 mM）溶液につけ、被験物質溶液150 μLを表皮面に、30℃で24時間適用する。Wheatstone電橋装置に低電圧をかけ、100 Hzにおける電気抵抗度を測定する。

さらに、電気抵抗が5 kΩ以下の場合には、10%スルホローダミンB染色液にて2時間染色し、皮膚に吸着された色素の量を吸光度から算出する。

電気抵抗度が5 kΩより小さく、明らかに損傷のある場合、または損傷がなくても色素量が陽性対照物質である10 M塩酸以上の場合、腐食性と判断する。

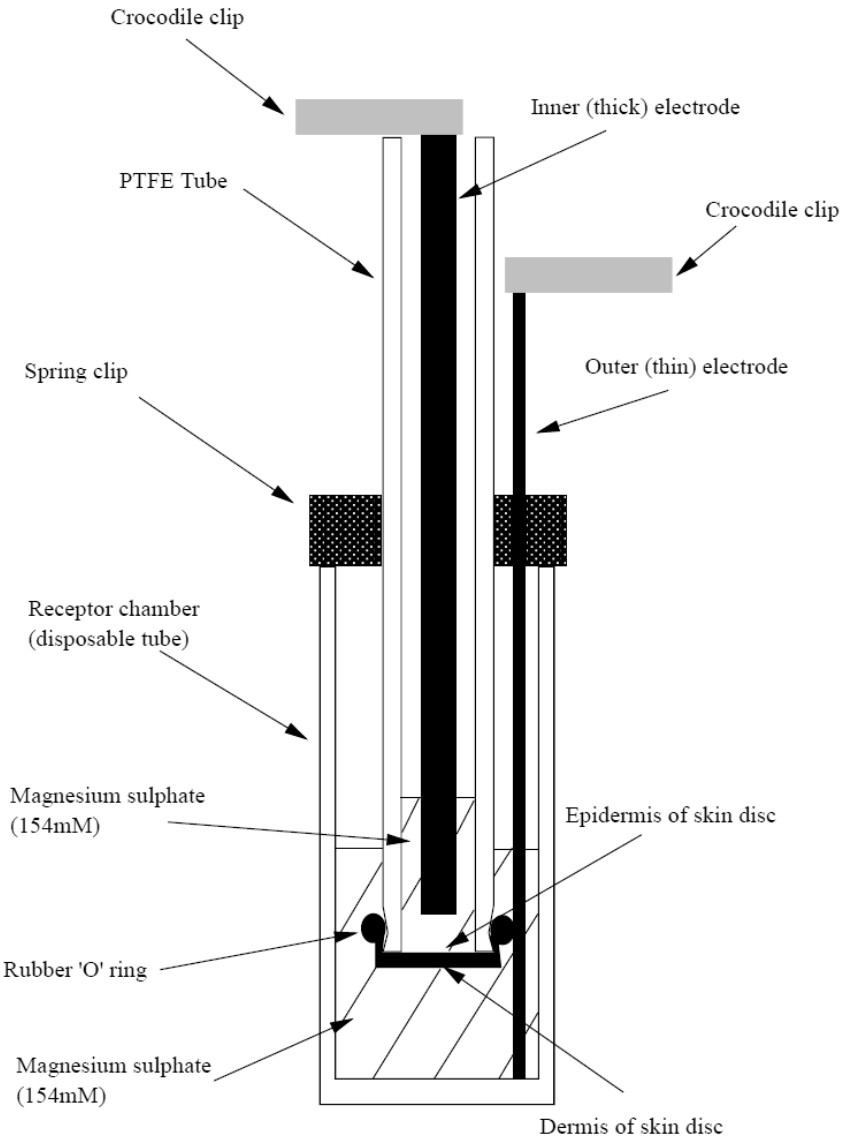

図 1． TER 概要図 [2)]

3. 開発および評価に使われた物質の分類、選択理由の妥当性

Botham らにより TER については、20 の被験物質により施設内再現性 [3)]、および 50 の被験物質により施設間再現性および予測性が調べられている [4)]。それらの物質リストとそれらの物理・化学学的性状を ANNEX 1 および 2 に示す。また Fentem らにより、60 物質を用いて、再現性および予測性が調べられている [5)]。それらの物質リストを腐食性や pH, 規制状況とともに ANNEX 3 に示す。それらでわかるように、本試験法は幅広い性状の化学物質で検討されている。

4. 試験法の正確性を評価するために用いられた物質の *in vitro* および参照データの有無

被験物質の多くは ECVAM の皮膚腐食性試験バリデーションで使用された物質であり [6)]、それらの腐食性に関する参照データが入手可能である

5. 試験法の正確性（再現性）

TER の施設内再現性は 20 の被験物質（6 腐食性物質および 14 非腐食性物質）を用い、3 施設

間で ANOVA 解析を用い、施設間に有意差がないとされている [3)]。

一方、施設間再現性では、2 施設が 50 の被験物質（25 腐食性物質および 25 非腐食性物質）を用い、92%（いずれの物質群も 23 物質での結果が一致した）であるとされている [4)]。以上の結果から、再現性は高いと判断されている。

60 物質を用いた 3 施設での 2 way の ANOVA 解析において、施設内および施設間の変動に有意な差はないと判断された [5)]。また、60 物質（27 腐食性物質および 33 非腐食性物質）のうち、37 物質は 3 施設とも施設内および施設間再現性が認められた。残る 23 物質のうち 10 物質で施設内再現性が悪かった。このように、いくつかの物質で再現性の結果が異なっていたが、ECVAM は本試験法の信頼性と再現性は高いと判断した。この結論は、ECVAM 科学諮問会議（ESAC: ECVAM Scientific Advisory Committee)[7)] および米国の代替法に関する省庁間連絡会議 (ICCVAM：Interagency Coordinating Committee on the Validation of Alternative Methods)[8)] での評価においても確認された。

6. 試験法の信頼性

Botham et al (1996)とFentem et al (1998)の検討結果およびそれらを合算したICCVAMの集計結果[6)]を表 1 に示す。その結果とそれぞれの論文の正確度、感度、特異度に大きな差はなく、正確度は約81%、感度は約94%、特異度は約71%であった。偽陰性は6%、3物質認められ、これらは Methacrolein、Glycol bromoacetate (85%)および1,2-Benzisothiazolin-3-one (33%) in aqueous propylene glycolであった。MethacroleinおよびGlycol bromoacetate (85%)はUNの腐食性の細分類でいずれも強くないもの(1B/1C)に属するものであり、1,2-Benzisothiazolin-3-one (19.3%)においては皮膚刺激性もないと化学物質等安全データシート (MSDS：Material Safety Data Sheet) に記載がある（未添付資料)。以上の結果から、上記偽陰性となった3物質の腐食性は強くないと判断できる。

種々の化学物質分類による差はなく、TER は腐食性および非腐食性を分類するための代替法として有用である。ただし、それ以上の細分類については検討が不十分であるとされている。

表 1． TER の予測性

	ICCVAM (1999) の総計 [6)]	Botham et al. (1992 and 1996)[3,4)]	Fentem et al. (1998) [5)]
正確度	81% (99/122)	82% (53/65)	81% (47/58)
感度	94%(51/54)	96%(27/28)	93%(25/27)
特異度	71%(48/68)	70%(26/37)	71%(22/31)

7. 他の科学的な報告との比較の有無

OECD の腐食性試験代替法ガイドラインとして、他に「TG431　ヒト表皮モデル試験」[9)]および「TG435　*in vitro* 膜バリア試験」[10)] が承認されている。これらはいずれもバリデーション研究が実施され、ICCVAM はこれらの試験法(Rat Skin TER, EpiSkin™, EpiDerm™および Corrositex®)の正確度、感度、特異度、について比較した結果を表 2 に示す。

試験物質の数量や選択物質の種類が異なっているため結果の判定だけをもって、単純に当該試験法の優越性を評価することは困難であるが、当該試験法は他の試験法と同等の予測性を有すると思われる。

表 2. 試験法の比較結果 [6,8)]

	TER	EpiSkin	EpiDerm	Corrositex
物質数	122	60	24	163
正確度	81% (99/122)	83% (50/60)	92% (22/24)	79% (128/163)
感度	94%(51/54)	82%(23/28)	92%(11/12)	85%(76/89)
特異度	71%(48/68)	84%(27/32)	83%(10/12)	72%(52/74)

8. ３Rs 原則への関与（動物福祉面からの妥当性）

本試験法は、ラットの摘出皮膚を用いているため完全な置き換え試験とはならない。しかし、安楽殺により皮膚を摘出すること、1 匹から得られる皮膚で複数の試験が実施可能である。したがって、動物数の削減が可能であり、また、腐食性物質による動物へのストレスを与えることは無く、動物実験代替法として３Rs の原則に適った試験法である。

9. 試験法の有用性と限界

本試験法は種々の種類の化学物質に対して適用できるが、プロトコルから類推して、難水溶性の固体や粘性の高い物質は適切に評価できない可能性がある。腐食性の細分類（UN GHS による）には使用できないと言われている [2)]。表 3 に示す習熟度確認物質を用いることにより専門技術の習熟について確認することができる [2)]。

10. その他（特許の有無など）

特許については示されていない。

11. 結論

信頼性と妥当性という視点において、ラット摘出皮膚を用いる経皮電気抵抗試験（TER）を評価した結果、本委員会は当該試験法を用いれば、腐食性の有無を評価できると考えた。

表 3　習熟度確認一覧表 [2)]

化学物質[1]	CASRN	化学物質クラス[2]	UN GHS 区分[3] *In vivo* 試験結果に基づく	VRM 区分[4] *In vitro* 試験結果に基づく	物理的状態	pH[5]
***In vivo* における腐食性物質**						
N,N'- ジメチルジプロピレントリアミン	10563-29-8	有機塩基	1A	6×腐食性	液体	8.3
1,2-ジアミノプロパン	78-90-0	有機塩基	1A	6×腐食性	液体	8.3
硫酸（10%）	7664-93-9	無機酸	(1A/)1B/1C	5×腐食性 1×非腐食性	液体	1.2
水酸化カリウム（10%aq）	1310-58-3	無機塩基	(1A/)1B/1C	6×腐食性	液体	13.2
オクタン酸（カプリル 酸）	124-07-2	有機酸	1B/1C	4×腐食性 2×非腐食性	液体	3.6
2-tert- ブチルフェノール	88-18-6	フェノール	1B/1C	4×腐食性 2×非腐食性	液体	3.9
***In vivo* における非腐食性物質**						
イソステアリン酸	2724-58-5	有機酸	非腐食性	6×非腐食性	液体	3.6
4- アミノ -1,2,4- トリアゾール	584-13-4	有機塩基	非腐食性	6×非腐食性	固体	5.5
臭化フェネチル	103-63-9	求電子物質	非腐食性	6×非腐食性	液体	3.6
4-（メチルチオ）-ベンズアルデヒド	3446-89-7	求電子物質	非腐食性	6×非腐食性	液体	6.8
1,9-デカジエン	1647-16-1	中性有機物 質	非腐食性	6×非腐食性	液体	3.9
テトラクロロエチレン	127-18-4	中性有機物 質	非腐食性	6×非腐食性	液体	4.5

略語：aq.=水溶液、CASRN=CAS 登録番号、UN GHS=国連化学品の分類および表示に関する世界調和システム(1)、 VRM=バリデーション済み参照試験法

1：これらの物質は、最初に腐食性物質と非腐食性物質、次に腐食性の細区分、さらに化学物質クラスの順で分類され、欧州代替法評価センター（ECVAM）によるラット TER 測定法のバリデーション試験に用いられる物質から選択されたものである（Barratt et al 1998[11)] , Fentem et al 1998[5)]）。特に断りがない限り、掲載した物質は市販品購入時の純度で試験を行った（Barratt et al 1998[11)]）。可能な限り、次の基準を満たす物質を選択した。(i)VRM による測定または予測を行える腐食性反応の範囲を代表する物質であること（例えば、非腐食性物質、腐食性物質のうち反応が弱い物質から強い物質まで）。(ii)バリデーション試験に用いられた化学物質クラスを代表する物質であること。(iii)VRM の性能特性を反映する物質であること。(iv)明確に定義された化学構造を持つ物質であること。(v) In vivo における参照試験法で明確な結果を導く物質であること。(vi)市販品であること。(vii)極端に高額な廃棄費用を伴わない物質であること。

2：Barratt et al（1998[11)]）により指定された化学物質クラスとした。

3：UN GHS の 1A、1B、1C に相当する国連容器等級はそれぞれ I、II、III である。

4：バリデーションにて施設間再現性結果を記載している。灰色部分は 6 実験の判定結果が異なること

を意味する。

5：pH 値は Fentem et al[5] および Barratt et al[11] の文献から得た。

12.文献

1) OECD (2015) Guideline for the testing of chemicals. 404, Acute Dermal Irritation/Corrosion.

2) OECD (2015) Guideline for the testing of chemicals. 430, in vitro Skin Corrosion: Transcutaneous electrical resistance test (TER).

3) Botham, P.A. et al.（1992）The skin corrosivity test in vitro. Results of an inter-laboratory trial. Toxicol In Vitro, 6:191-4

4) Botham, P.A. et al. (1996) A prevalidation study on in vitro skin corrosivity testing: The report and recommendations of ECVAM workshop 6. ATLA, 23, 219-255

5) Fentem, J.H. et al. (1998) The ECVAM International Validation Study on in vitro tests for skin corrosivity. 2. Results and evaluation by the management team. Toxicology in Vitro, 12, 483-524

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7) EC-ECVAM (1998) Statement on the Scientific Validity of the Rat Skin Transcutaneos Electrical Resistance (TER) Test (an *In Vitro* Test for Skin Corrosivity), Issued by the ECVAM Scientific Advisory Committee (ESAC10), 3 April 1998. Available at: [http://www.ecvam.jrc.ec.europa.eu.html].

8) ICCVAM (2002) NIH Publication No.02-4502. ICCVAM Evaluation of EPISKIN, and EpiDerm (EPI-200) and rat skin transcutaneous electrical resistance (TER) assay: in vitro test method for assessing dermal corrosivity potential of chemicals.

9) OECD (2016) Guideline for the testing of chemicals. 431, in vitro Skin Corrosion: Human skin model test.

10) OECD (2015) Guideline for the testing of chemicals. 435, in vitro membrane barrier test method for Skin Corrosion.

11) Barratt, M.D. et al. (1998) The ECVAM international validation study on in vitro tests for skin corrosivity. 1. Selection and distribution of the test chemicals. Toxicology in Vitro, 12, 471-482

ANNEX1:　Botham 1992 のバリデーションで使われた 20 物質

Table 1.

Code	Chemical name or substance type	pH	Physical form
1	Sodium silicate	>12	Solid
2	40% HCl	1–2	Liquid
3	40% H_2SO_4	1–2	Liquid
4	1 M-KOH	>12	Liquid
5	1,6-Hexane diamine	13.0	Solid
6	Surfactant-based material	10.0	Liquid
7	Surfactant-based material	7.3	Liquid
8	Surfactant-based material	5–6	Liquid
9	Surfactant-based material	5–6	Liquid
10	Lubricant (ethylene oxide + propylene oxide)	9.0	Liquid
11	Nickel molybdenum catalyst	5–6	Solid
12	Surfactant-based material	9.5	Liquid
13	Ink jet dye	7–8	Solid
14	Lubricant (ethylene oxide + propylene oxide)	9.0	Liquid
15	Ink jet dye	6–7	Solid
16	Dicyandiamine/formaldehyde condensate	6–7	Liquid
17	Tetrahydropyran	5–6	Liquid
18	Difluorohydroxy-based material	6–7	Solid
19	Amino-toluic acid	3–4	Solid
20	Deionized water	7–8	Liquid

Code No. 1～6 が腐食性物質、その他は非腐食性物質

ANNEX 2:　Botham 1996 のバリデーションで使われた 50 物質

Table I: Test chemicals

Trade name	Chemical name (if different)	Chemical class	Appearance
Corrosives			
Acetic acid (glacial)[a]		Organic acid	Clear liquid
Acrylic acid (99%)[a]		Organic acid	Clear liquid
Armeen CD[b]	Cocoamine	Organic base	Clear liquid
Armeen TD[b]	Tallowamine	Organic base	Opaque gel
Arquad 16-50[b]	Hexadecyltrimethyl-ammonium chloride, 50% in isopropanol	Cationic surfactant	Clear liquid
Arquad DMMCB-50[c]	Coco(C12)dimethylbenzyl-ammonium chloride, 50% in aqueous ethylene glycol	Cationic surfactant	Clear viscous liquid
Bromoacetic acid (8%)[a]		Organic acid	Clear liquid
Bromoacetic acid (55.6%)[a]		Organic acid	Clear liquid
Butylamine (40%)[a]		Organic base	Clear liquid
Capric/caprylic (45:55) acid[b]		Organic acid	Clear liquid
Caprylic acid[b]		Organic acid	Clear liquid
Cyclohexylamine (11.9%)[a]		Organic base	Clear liquid
1,4-Diaminobutane (30%)[a]		Organic base	Clear liquid
Dichloroacetic acid (36.1%)[a]		Organic acid	Clear liquid
Diethylamine (35%)[a]		Organic base	Yellow liquid
Duoquad T-50[b]	Pentamethyl-*N*-tallow-1,3-propanediammonium chloride, 50% in isopropanol	Cationic surfactant	Yellow liquid
Formic acid (33.9%)[a]		Organic acid	Clear liquid
Hexanoic acid[a]		Organic acid	Clear yellow liquid
Mercaptoacetic acid (15.1%)[a]		Organic acid	Clear liquid
Proxel BD[b] (biocide A)	1,2-Benzisothiazolin-3-one (33%) in aqueous propylene glycol	Neutral organic	Tan opaque liquid
Pyrrolidine (34.5%)[a]		Organic base	Yellow liquid
Sodium hydroxide (4.88%)[a]		Inorganic	Clear liquid
Sodium metasilicate[b]		Inorganic	Granular powder[c]
Sodium silicate A140[b]		Inorganic	Clear gel
Synprolam 35X2[b]	C13-15Alkyl-di(2-hydroxyethyl)amine	Organic base	Clear viscous liquid

[a] *Jacobs & Martens (12) classification from animal data.*

[b] *Original animal data.*

[c] *Prepared in distilled water at 1g/ml.*

Table I: continued

Trade name	Chemical name (if different)	Chemical class	Appearance
Non-corrosives			
Armeen 2C[d]	Dicocoamine	Organic base	Crystalline powder[c]
Aromox DMMCD-W[b]	Coco(C12)dimethylamine oxide (30%)	Amine oxide	Clear liquid
Arquad C-33-W[d]	Coco(C12)trimethyl-ammonium chloride, 33% in water	Cationic surfactant	Clear gel
Butylbenzene[a]		Neutral organic	Clear liquid
Dequest 2000[e]	Aminotris(methylphosphonic acid), 50% in water	Organic acid	Clear liquid
Dowanol PNB[f]	Propylene glycol *n*-butyl ether	Neutral organic	Clear liquid
Elfan OS 46[d]	C12-14α-Olefin sulphonate, sodium salt	Anionic surfactant	Yellow viscous liquid
Empicol LZPV/C[d]	Sodium dodecyl sulphate	Anionic surfactant	Dry pellets[c]
Empigen OB[d]	Coco(C12)dimethylamine oxide (30%)	Amine oxide	Clear liquid
Empilan CME[d]	Fatty acid monoethanolamide coco	Neutral organic	Dry chips[c]
Empilan KB2[d]	Fatty alkylethoxylate 2EO	Neutral organic	White opaque cream
Ethomeen T/25[b]	Polyoxyethylene(15)tallowamine	Organic base	Yellow viscous liquid
Genamin KDM-F[d]	Behenyl(C20-22)trimethyl-ammonium chloride, 80% in isopropanol	Cationic surfactant	Powdered flakes[c]
Genapol LRO[d]	Coco(C12)2EO sulphate, sodium salt (70%)	Anionic surfactant	Clear gel
n-Hexanol[a]		Neutral organic	Clear liquid

[a] *Jacobs & Martens (12) classification from animal data.*

[b] *Original animal data.*

[c] *Prepared in distilled water at 1g/ml.*

[d] *CESIO classification from animal data.*

[e] *Harmonised Electronic Dataset (HEDSET) data.*

[f] *Manufacturers' data sheet and summary of test data.*

Table I: continued

Trade name	Chemical name (if different)	Chemical class	Appearance
Hostaphat KLD[d]	Alkyl(4EO)phosphate ester	Neutral organic	Clear viscous liquid
Lauric acid[b]		Organic acid	Fine powder[c]
n-Nonanol[a]		Neutral organic	Clear liquid
Oleic/caprylic (80:20) acid[b]		Organic acid	Yellow liquid
Proxel AB[b] (biocide B)	1,2-Benzisothiazolin-3-one (33%), aqueous	Neutral organic	Opaque tan liquid
Sodium perborate[e]		Inorganic	Crystalline powder[c]
Sodium percarbonate[e]		Inorganic	Granular powder[c]
Sodium silicate H100[b]		Inorganic	Clear viscous liquid
Triethanolamine[a]		Organic base	Clear viscous liquid
n-Undecanol[a]		Neutral organic	Clear liquid

[a] *Jacobs & Martens (12) classification from animal data.*

[b] *Original animal data.*

[c] *Prepared in distilled water at 1g/ml.*

[d] *CESIO classification from animal data.*

[e] *Harmonised Electronic Dataset (HEDSET) data.*

ANNEX 3: Fentem 1998 のバリデーションで使われた 60 物質

Table 4. Test chemicals

No.	Chemical	C/NC	EU risk phrase	UN packing group	PII*
Organic acids					
1	Hexanoic acid	C	R34	II/III	—
29	65/35 Octanoic/decanoic (capric) acids	C	R34	II/III	NPC†
36	2-Methylbutyric acid	C	R34	II/III	>4
40	Octanoic (caprylic) acid	C	R34	II/III	4.44
47	60/40 Octanoic/decanoic acids	C	R34	II/III	NPC
50	55/45 Octanoic/decanoic acids	C	R34	II/III	5.11
7	3,3′-Dithiodipropionic acid	NC			0
12	Dodecanoic (lauric) acid	NC			0.44
26	Isostearic acid	NC			4.33
34	70/30 Oleine/octanoic acid	NC			3.78
58	10-Undecenoic acid	NC			2.42
Organic bases					
2	1,2-Diaminopropane	C	R35	I	—
15	Dimethyldipropylenetriamine	C	R35	I	NPC
38	Tallow amine	C	R35	II	NPC
55	1-(2-Aminoethyl)piperazine	C	R34	II	—
13	3-Methoxypropylamine	C	R34	II/III	6.67
17	Dimethylisopropylamine	C	R34	II/III	5.61
45	*n*-Heptylamine	C	R34	II/III	6.67
10	2,4-Xylidine (2,4-dimethylaniline)	NC			1.44
35	Hydrogenated tallow amine	NC			3.56
59	4-Amino-1,2,4-triazole	NC			0
Neutral organics					
8	Isopropanol	NC			0.78
11	2-Phenylethanol (phenylethylalcohol)	NC			0.92/2.22
16	Methyl trimethylacetate	NC			0
19	Tetrachloroethylene	NC			5.67
22	*n*-Butyl propionate	NC			1.08
27	Methyl palmitate	NC			4.56
44	Benzyl acetone	NC			1.21
51	Methyl laurate	NC			3.89
56	1,9-Decadiene	NC			3.0
Phenols					
3	Carvacrol	C	R34	II/III	>4
23	2-*tert*-Butylphenol	C	R34	II/III	5.67
9	*o*-Methoxyphenol (Guaiacol)	NC			2.38
30	4,4-Methylene-bis-(2,6-di-*tert*-butylphenol)	NC			0
49	Eugenol	NC			2.92
Inorganic acids					
4	Boron trifluoride dihydrate	C	R35	I	—
28	Phosphorus tribromide	C	R35	I	—
32	Phosphorus pentachloride	C	R35	I	—
25	Sulfuric acid (10% wt)	C	R34/R35‡	I/II/III	—
57	Phosphoric acid	C	R34	II	—
43	Hydrochloric acid (14.4% wt)	C	R34	II/III	—
53	Sulfamic acid	NC			—
Inorganic bases					
18	Potassium hydroxide (10%, aq.)	C	R34/R35‡	I/II/III	NPC
42	2-Mercaptoethanol, Na salt (45%, aq.)	C	R34	II/III	NPC
21	Potassium hydroxide (5%, aq.)	NC			5.22
24	Sodium carbonate (50%, aq.)	NC			2.33
Inorganic salts					
20	Iron (III) chloride	C	R34	II	—
52	Sodium bicarbonate	NC			0.11
54	Sodium bisulfite	NC			1.0
Electrophiles					
5	Methacrolein	C	R34	II/III	4.11
14	Allyl bromide	C	R34	II/III	7.17
48	Glycol bromoacetate (85%)	C	R34	II/III	7.67
6	Phenethyl bromide	NC			0
31	2-Bromobutane	NC			2.44
33	4-(Methylthio)-benzaldehyde	NC			0.89
39	2-Ethoxyethyl methacrylate	NC			1.56
46	Cinnamaldehyde	NC			3.71
Soaps/surfactants					
37	Sodium undecylenate (33%, aq.)	NC			1.67
41	20/80 Coconut/palm soap	NC			2.67
60	Sodium lauryl sulfate (20%, aq.)	NC			6.78

*PII = primary irritation index (Bagley *et al.*, 1996; ECETOC, 1995); †NPC = not possible to calculate; ‡ = the animal data and other supporting information are not sufficiently comprehensive to enable unequivocal classification as R34/II & III or R35/I; however, it is more probable that an R34/II & III label is appropriate, and this is the classification which has been used in the analysis of the results obtained in the validation study. The numbers are for the identification of individual chemicals (Barratt *et al.*, 1998).

OECD/OCDE 430

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.

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.

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

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 $l0^4$; 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.

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:

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

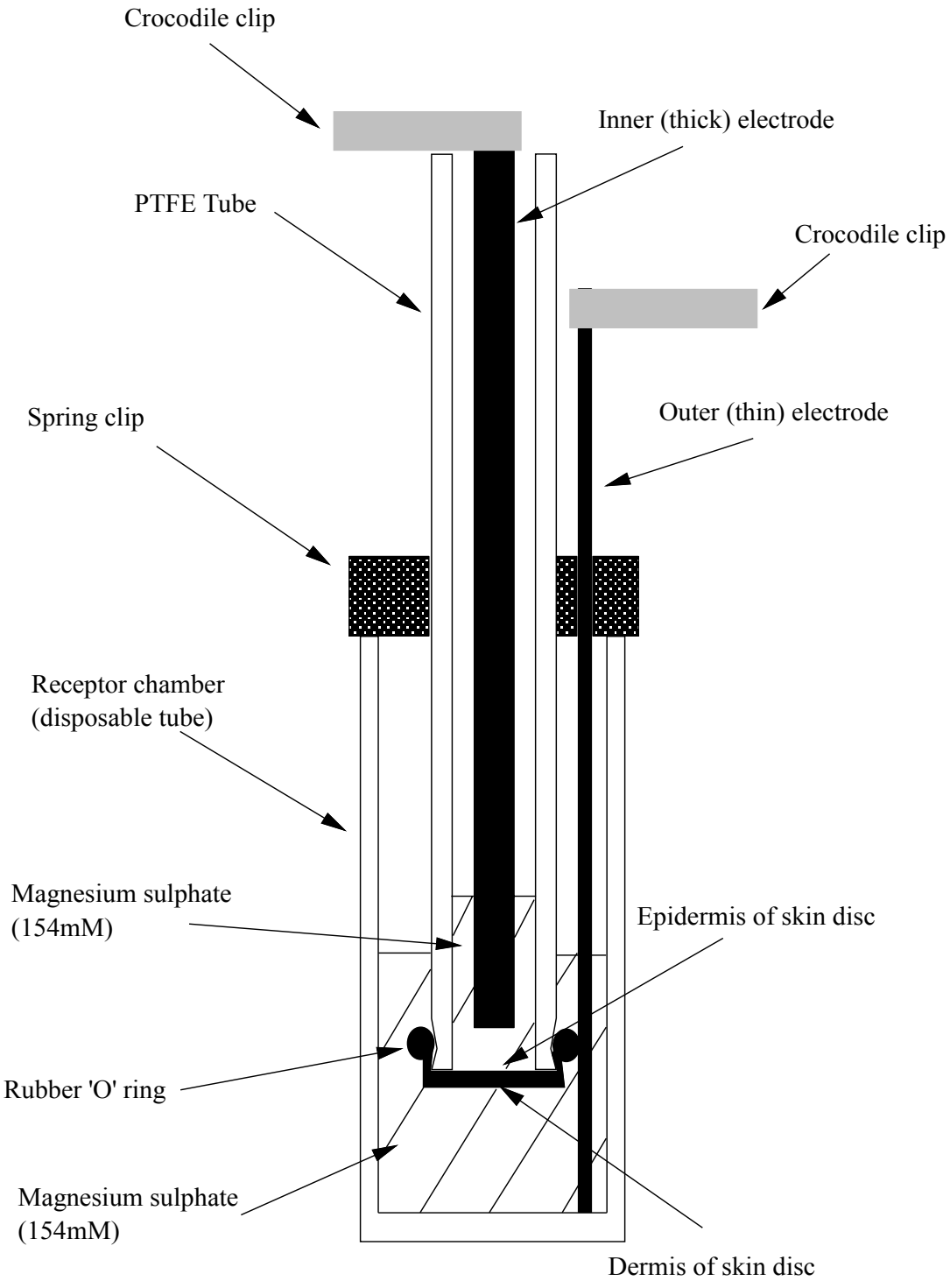
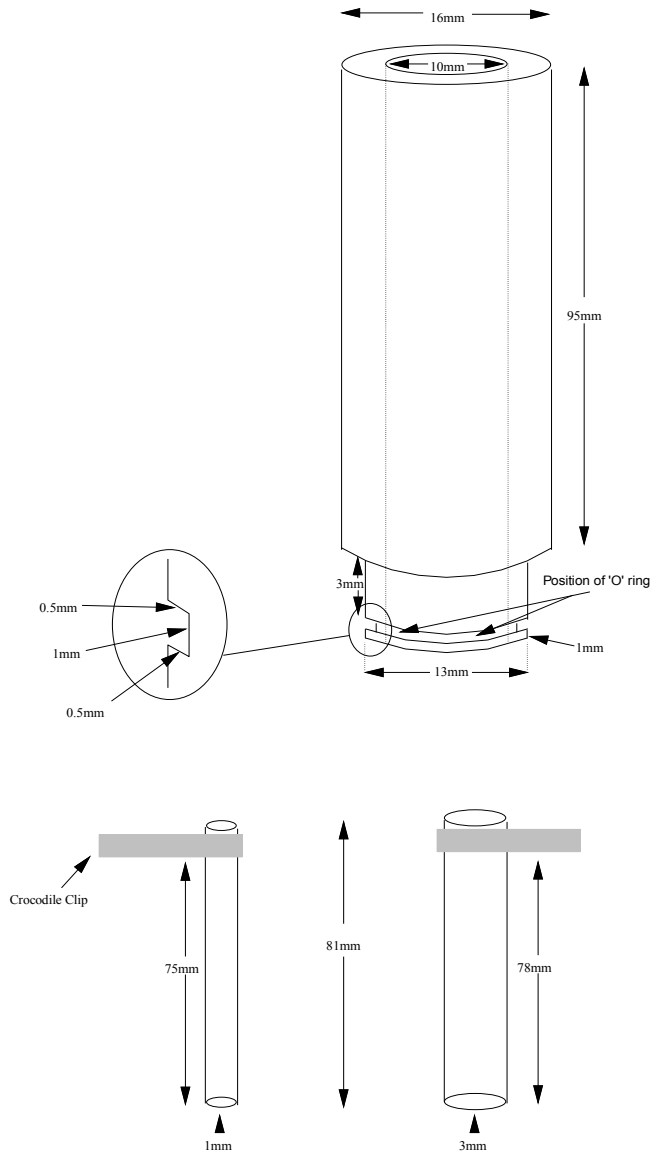


Figure 2: Dimensions of the polytetrafluoroethylene (PTFE) and receptor tubes and electrodes used



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.

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 ≥ 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;

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.