The ICCVAM Dermal Corrosivity and Irritation

Working Group Proposed

ICCVAM MINIMUM PERFORMANCE STANDARDS:
IN VITRO SKIN TRANSCUTANEOUS ELECTRICAL
RESISTANCE (TER) TESTS FOR SKIN CORROSION

June 23, 2003

<u>NOTICE</u>

These minimum performance standards (MPS) are being proposed by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Dermal Corrosivity and Irritation Working Group (DCIWG) for public review and comment. All public comments will be considered by the DCIWG and ICCVAM during development of the final ICCVAM MPS for this assay. Final ICCVAM MPS will be published as an addendum to the previously published ICCVAM report on this test method and will be forwarded to Federal agencies for their consideration.

The Dermal Corrosivity and Irritation Working Group of the
Interagency Coordinating Committee on the Validation of Alternative Methods

National Toxicology Program Interagency Center for the Evaluation of Alternative
Toxicological Methods

National Institute of Environmental Health Sciences
National Institutes of Health
U.S. Public Health Service
Department of Health and Human Service

*ICCVAM MINIMUM PERFORMANCE STANDARDS: IN VITRO TRANSCUTANEOUS
ELECTRICAL RESISTANCE (TER) TESTS FOR SKIN CORROSION*

1.0   PURPOSE AND BACKGROUND

This document describes the **minimum performance standards** (MPS) that should be met by *in vitro* skin TER tests proposed for testing the skin corrosion hazard potential of chemicals. These MPS were developed by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) in response to a request by the U.S. Environmental Protection Agency (EPA) to establish MPS for proprietary and nonproprietary *in vitro* skin corrosivity test methods previously evaluated and recommended by ICCVAM (1, 2). For future test methods evaluated by ICCVAM, MPS will be included in the recommendations forwarded to regulatory authorities.

2.0   INTRODUCTION

Prior to the acceptance of new test methods for regulatory testing applications, validation studies are conducted to assess reliability (i.e., the extent of intra- and inter-laboratory reproducibility) and accuracy (i.e., the ability of the test method to correctly predict or measure the biological effect of interest; also referred to as relevance) (1-5). The purpose of the proposed MPS are to communicate the basis on which new proprietary (e.g., copyrighted, trademarked, registered) and nonproprietary test methods have been determined to have sufficient accuracy and reliability for specific testing purposes. When a validated proprietary or nonproprietary test method is accepted for a regulatory testing application, U.S. regulatory authorities may provide MPS that can be used to evaluate the reliability and accuracy of other test methods, which are based on similar scientific principles and which measure or predict the same biological or toxic effect. The three elements of the proposed MPS are:

- Minimum procedural standards that identify essential structural, functional, and procedural components (e.g., procedural details, proper controls, morphologic structure and integrity of the test system, biological identity of key components, and expected biological responsiveness) of the validated test method. Adherence to the minimum procedural standards will help to assure that the proposed test method is based on the same concepts as the validated test method.

ICCVAM MINIMUM PERFORMANCE STANDARDS: IN VITRO TRANSCUTANEOUS
ELECTRICAL RESISTANCE (TER) TESTS FOR SKIN CORROSION

- A minimum list of recommended reference chemicals that can be used to assess the accuracy and reliability characteristics of the proposed test method. The list includes substances that are representative of the chemical and product classes for which the validated test method is considered applicable, as well as substances that are representative of the range of responses (e.g., negative, weak to strong positive) that the validated test method is capable of measuring or predicting.

- The **accuracy** and **reliability** that should be achieved by the proposed test method when evaluated using the minimum list of reference chemicals.

2.1 Regulatory Rationale for Use of *In Vitro* Test Methods to Assess Skin Corrosivity

Skin corrosion refers to the destruction of skin through the epidermis into the dermis following exposure of the skin to a chemical substance. Skin corrosivity has traditionally been assessed by applying the test substance to the skin of living animals and assessing the extent of tissue damage after a fixed period of time (6, 7). Some regulatory authorities require determination of corrosivity using three categories of responses, as provided in Table 1 (7-9).

Table 1   **Skin Corrosive Category and Subcategories**

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

ICCVAM MINIMUM PERFORMANCE STANDARDS: IN VITRO TRANSCUTANEOUS ELECTRICAL RESISTANCE (TER) TESTS FOR SKIN CORROSION

The EPA test guideline (10) and a globally-harmonized tiered testing strategy (11) for the assessment of skin corrosivity allow for the use of validated and accepted *in vitro* methods. In both the EPA guidelines and the tiered testing strategy, positive results from *in vitro* test methods can be used to classify a substance as corrosive without the need for animal testing. Substances that are negative *in vitro* might undergo additional testing in accordance with the tiered testing strategy. The use of *in vitro* methods to identify corrosive substances can therefore avoid the pain and distress that might occur when animals are used for this purpose.

A number of *in vitro* test methods have been proposed as alternatives for the standard *in vivo* rabbit skin procedure to identify corrosive substances. Generally, these test methods involve the use of a cultured mammalian cell membrane matrix, isolated rat skin, or a noncellular membrane barrier (12).

Prevalidation and validation studies have been completed for the rat skin TER assay (2, 12-17). Based on its scientific validity, this test method has been recommended for the testing of all classes of chemicals (2, 12, 18) and for inclusion in tiered testing strategies as part of a tiered or weight-of-evidence evaluation (2).

2.2   Principles of the *In Vitro* Skin TER Test for Skin Corrosion

The test material is applied for up to 24 hours to the epidermal surfaces of skin discs in a two compartment test system in which the skin discs function as the separation between the compartments. The skin discs are prepared from humanely killed, 28-30 day old rats. Corrosive materials are identified by their ability to produce a loss of normal stratum corneum integrity and barrier function, which is measured as a reduction in the TER below a threshold level (13). For rat skin TER, a cutoff value of 5 k has been selected based on extensive data for a wide range of chemicals where the vast majority of values were either clearly well above or well below this value (13). Generally, chemicals which are noncorrosive in animals but are irritating do not reduce the TER below this cutoff value. However, the use of other skin preparations or other equipment may alter the cutoff value, necessitating further validation. A dye-binding step is incorporated into the test procedure

ICCVAM MINIMUM PERFORMANCE STANDARDS: IN VITRO TRANSCUTANEOUS
ELECTRICAL RESISTANCE (TER) TESTS FOR SKIN CORROSION

for confirmation testing of positive results in the TER. The dye-binding step determines if the increase in ionic permeability is due to physical destruction of the stratum corneum.

Investigators using an *in vitro* skin TER corrosivity test must be able to demonstrate that the assay is valid for its intended use. This includes demonstrating that different preparations are consistent in barrier properties (i.e., capable of maintaining a barrier to noncorrosive substances, able to respond appropriately to weak and strong corrosive substances) and/or that any modification to the existing validated and accepted assay does not adversely affect its performance characteristics.

The *in vitro* TER test for skin corrosion may be used to test solids, liquids, and emulsions of any chemical or product class. The liquids can be aqueous or nonaqueous; solids can be soluble or insoluble in water. The samples may be pure chemicals, dilutions, formulations, or waste. Where appropriate, solids can be heated to 300°C to melt or soften the test material or ground to a powder before application; no other prior treatment of the sample is required. In some chemical classes, relatively few chemicals were included in the validation of the accepted *in vitro* rat skin TER test for skin corrosion (12). However, considering the limited mechanisms which result in corrosivity, this method is expected to be generally applicable across all chemical classes (2, 12, 18).

3.0   MINIMUM PROCEDURAL STANDARDS

The following is a description of the minimum procedural standards, including test method components, for an *in vitro* skin TER test for skin corrosivity, as provided in the Organisation for Economic Co-operation and Development (OECD) Test Guideline 430 (19).

3.1   Animals

All procedures involving the use of animals should be in compliance with relevant national. animal welfare act regulations and policies, and the studies should be approved by the Institutional Animal Care and Use Committee or its equivalent. Rats are the species of choice because the sensitivity of their skin to chemicals in this test has been previously

ICCVAM MINIMUM PERFORMANCE STANDARDS: IN VITRO TRANSCUTANEOUS ELECTRICAL RESISTANCE (TER) TESTS FOR SKIN CORROSION

demonstrated (13). 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. The use of skin from another species is possible as long as the test system is appropriately calibrated and the reliability and accuracy, using at the minimum, the provided list of reference chemicals (Table 2), is determined to be at least comparable to the performance characteristics of the validated test method.

If rat skin is used, 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, while 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 three days of the second wash, when the stratum corneum has recovered from the hair removal.

3.2   Preparation of Skin Discs

Animals are humanely killed when 28-30 days old; this age is critical to the performance of the assay. The dorsolateral 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 excised. The skin may be stored prior to use provided that positive and negative control data are equivalent to that obtained with fresh skin.

Each skin disc is placed over one of the ends of a polytetrafluoroethylene (PTFE) tube, ensuring that the epidermal surface is in contact with the tube. A rubber 'O' ring is press-fitted over the end of the tube to hold the skin in place and excess tissue is trimmed away. Tube and 'O' ring dimensions are provided in the OECD Test Guideline (19). 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) (19). 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.

ICCVAM MINIMUM PERFORMANCE STANDARDS: IN VITRO TRANSCUTANEOUS ELECTRICAL RESISTANCE (TER) TESTS FOR SKIN CORROSION

Before testing begins, the electrical resistance of two skin discs is measured as a quality control procedure for each animal skin pelt. If both discs have resistance values greater than 10 k then the remainder of the discs may be used for the test. If the resistance value is less than 10 k , the remaining discs from that skin pelt should be discarded.

3.3   Application of Test Substances

Liquid test substances (150 µL) are applied uniformly to the epidermal surface inside the tube. When testing solid materials, a sufficient amount of the solid is applied evenly to the disc to ensure that the whole surface of the epidermis is covered. In order to achieve maximum contact with the skin, solids may need to be warmed to 300°C to melt or soften the test substance, or ground to produce a granular material or powder. Deionized water (150 µL) is added on top of the solid and the tube is gently agitated.

Three skin discs are used for each test and control substance; skin discs from a single animal should be used. Test substances are applied for 24 hours at 20-23°C. The test substance is removed by washing with a jet of tap water at temperatures up to 30°C, until no further material can be removed.

3.4   Control Substances

3.4.1   Solvent Controls

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

3.4.2   Positive (Corrosive) Controls

A positive control (e.g., 10 M hydrochloric acid) should be tested concurrently with the test substance to demonstrate the suitability of the test system. The chemical selected as the

ICCVAM MINIMUM PERFORMANCE STANDARDS: IN VITRO TRANSCUTANEOUS ELECTRICAL RESISTANCE (TER) TESTS FOR SKIN CORROSION

positive control should generate a resistance value that is intermediate within the range of corrosive responses for the assay. An acceptable positive control response range must be developed based on historical positive control(s) data. In each assay, the positive control should be evaluated to determine if the value is within the acceptable positive control range.

3.4.3   Negative (Noncorrosive) Controls

A noncorrosive substance (e.g., distilled water) should also be tested concurrently with the test substance as another quality control measure to demonstrate the functional integrity of the skin membrane.

3.4.4   Benchmark Controls

Benchmark controls, which are known corrosive and noncorrosive chemicals of the same chemical class as the test chemical, may be useful as additional indicators of the relative corrosivity potential of the test chemical.

3.5   TER Measurements

The skin impedance is measured as TER by using a low-voltage, alternating current Wheatstone bridge (13). 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 k . For 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 (154 mM). The databridge electrodes are placed on either side of the skin disc to measure the resistance in k /skin disc (19). Electrode dimensions and the length of the electrode exposed below the crocodile clips are provided in the OECD Test Guideline (19). 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

7

ICCVAM MINIMUM PERFORMANCE STANDARDS: IN VITRO TRANSCUTANEOUS
ELECTRICAL RESISTANCE (TER) TESTS FOR SKIN CORROSION

between the spring clip and the bottom of the PTFE tube is maintained as a constant (18), 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).

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

The properties and dimensions of the test apparatus and the experimental procedure used may influence the TER values obtained. The 5 kΩ corrosive threshold was developed from data obtained with the specific apparatus and procedure described in this Guideline. Different threshold and control values may apply if the test conditions are altered or a different apparatus is used. Therefore, it is necessary to calibrate the methodology and resistance threshold values by testing a series of calibration chemicals (see Section 4.0).

3.6 Dye-Binding Methods

Exposure of certain noncorrosive materials can result in a reduction of resistance below the cutoff of 5 kΩ allowing the passage of ions through the stratum corneum, thereby reducing the electrical resistance (12). For example, neutral organics and chemicals that have surface-active properties (including detergents, emulsifiers, and other surfactants) can remove skin lipids making the barrier more permeable to ions. Thus, if the rat skin TER values of test substances are less than or around 5 kΩ in the absence of visual damage, an assessment of dye penetration should be carried out on the control and treated tissues to determine if the TER values obtained were the result of increased skin permeability or skin corrosion (12, 16). In the latter case where the stratum corneum is disrupted, the dye sulforhodamine B (Acid Red 52; Colour Index 45100; CAS RN 3520-42-1), when applied to the skin surface rapidly penetrates and stains the underlying tissue. This particular dye is stable to a wide range of chemicals and is not affected by the extraction procedure described below.

*ICCVAM MINIMUM PERFORMANCE STANDARDS: IN VITRO TRANSCUTANEOUS
ELECTRICAL RESISTANCE (TER) TESTS FOR SKIN CORROSION*

3.6.1   Sulforhodamine B Dye Application and Removal

Following TER assessment, the magnesium sulfate is discarded from the tube and the skin is carefully examined for obvious damage. If there is no obvious major damage, 150 µL of a 10% (w/v) dilution of sulforhodamine B in distilled water, is applied to the epidermal surface of each skin disc for two 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 deionized 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 5 mL of 30% (w/v) sodium dodecyl sulfate (SDS) in distilled water and are incubated overnight at 60°C.

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

3.6.2   Calculation of Dye Content

The sulforhodamine B dye content per disc is calculated from the OD values (12) (sulforhodamine B dye molar extinction coefficient at 565 nm = 8.7×10^4; molecular weight = 580). The dye content is determined for each skin disc by the use of an appropriate calibration curve and a mean dye content is then calculated for the replicates.

3.6.3   Interpretation of Results

The mean rat skin TER results are accepted if the concurrent positive and negative control values fall within the acceptable ranges for the testing laboratory. The acceptable resistance ranges for the rat skin TER methodology and apparatus described above are given in Table 2.

ICCVAM MINIMUM PERFORMANCE STANDARDS: IN VITRO TRANSCUTANEOUS ELECTRICAL RESISTANCE (TER) TESTS FOR SKIN CORROSION

Table 2   Acceptable Resistance Ranges for the Rat Skin TER Methodology and Apparatus

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

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 rat skin TER methodology and apparatus described above are provided in Table 3.

Table 3   Suggested Acceptable Dye Content Ranges for the Control Substances for the Rat Skin TER Methodology and Apparatus

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

The test substance is considered to be noncorrosive to skin:

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

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

- the skin disc is showing no obvious damage, and
- the mean disc dye content is well below the mean disc dye content of the 10 M HCl positive control obtained concurrently.

The test substance is considered to be corrosive to skin:

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

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

- the skin disc is showing no obvious damage, but

ICCVAM DCIWG Proposed MPS   *23 June 2003*

***ICCVAM MINIMUM PERFORMANCE STANDARDS: IN VITRO TRANSCUTANEOUS
ELECTRICAL RESISTANCE (TER) TESTS FOR SKIN CORROSION***

- the mean disc dye content is greater than or equal to the mean disc dye content of the 10 M HCl positive control obtained concurrently.

3.7   Reporting

<u>Test and Control Substances</u>

- Chemical name(s) such as Chemical Abstract Services (CAS) preferred name and Registry Number (RN), followed by other names, if known
- Purity and composition of the substance or preparation (in percentage(s) by weight)
- Physicochemical properties such as physical state, volatility, pH, stability, chemical class, water solubility relevant to the conduct of the study
- Treatment of the test/control substances prior to testing, if applicable (e.g., warming, grinding)
- Stability, if known

<u>Test Animals</u>

- Strain and sex used
- Age of the animals when used as donor animals
- Source, housing condition, diet, etc.
- Details of the skin preparation

<u>Justification of the Skin Model and Protocol Used</u>

<u>Test Conditions</u>

- Calibration curves for test apparatus
- Calibration curves for dye-binding test performance
- Details of the test procedure used for TER measurements
- Details of the test procedure used for the dye-binding assessment, if appropriate
- Description of any modification of the test procedures

***ICCVAM MINIMUM PERFORMANCE STANDARDS: IN VITRO TRANSCUTANEOUS
ELECTRICAL RESISTANCE (TER) TESTS FOR SKIN CORROSION***

- Description of evaluation criteria used

- Reference to historical data of the model

- Description of evaluation criteria used

Results

- Tabulation of data from the TER and dye-binding assay (if appropriate) for individual animals and individual skin samples for the test material, as well as for positive and negative controls (individual trial data and means ± S.D.), including data for replicates/repeat experiments, mean and individual values

- Description of any effects observed

- Tabulation of data from individual test samples (e.g., resistance values [k] and mean dye content values [μg/disc], where appropriate)

Description of Other Effects Observed

Discussion of the Results

Conclusion

4.0   CALIBRATION AND REFERENCE CHEMICALS

Calibration chemicals are used to demonstrate that the validated *in vitro* rat skin TER test method is performing as expected; reference chemicals are used to determine if the performance of a new or modified *in vitro* skin TER test for skin corrosion is comparable to that of the validated *in vitro* test method. The 24 reference chemicals (12 noncorrosives, 12 corrosives) listed in Table 4 provide a representative distribution of the 60 chemicals used in the European Centre for the Validation of Alternative Methods (ECVAM) validation study of the rat skin TER assay (12, 17) and the range of corrosivity responses obtained for the *in vivo* rabbit skin reference test method. Included in this list are five organic bases, four organic acids, four inorganic acids, three electrophiles, three neutral organics, two inorganic bases,

***ICCVAM MINIMUM PERFORMANCE STANDARDS: IN VITRO TRANSCUTANEOUS
ELECTRICAL RESISTANCE (TER) TESTS FOR SKIN CORROSION***

Table 4   Recommended Chemicals for Validation of New *In Vitro* TER

Corrosivity Test Methods

Chemical[1]	CASRN	Chemical Class[2]	UN *In Vivo* PG[3]	Validated Test Method Prediction	pH[4]
In Vivo **Corrosives**					
Phosphorus tribromide	7789-60-8	inorganic acid	I	C	1.0
Sulfuric acid (10%)	**7664-93-9**	**inorganic acid**	**II/III**	**C**	**1.2**
Boron trifluoride dihydrate	13319-75-0	inorganic acid	I	C	1.5
Glycol bromoacetate (85%)	3785-34-0	electrophile	II/III	NC	2.0
Caprylic acid	**124-07-02**	**organic acid**	**II/III**	**C**	**3.6**
2-tert-Butylphenol	**88-18-6**	**phenol**	**II/III**	**C**	**3.9**
60/40 Caprylic/decanoic acids	68937-75-7	organic acid	II/III	C	3.9
Dimethyldipropylenetriamine	**10563-29-8**	**inorganic base**	**I**	**C**	**8.3**
Dimethylisopropylamine	996-35-0	organic base	II/III	C	8.3
1,2-Diaminopropane	**78-90-0**	**organic base**	**I**	**C**	**8.3**
n-Heptylamine	111-68-2	organic base	II/III	C	8.4
Potassium hydroxide (10% aq.)	**1310-58-3**	**inorganic base**	**II**	**C**	**13.1**
In Vivo **Noncorrosives**					
Sulfamic acid	5329-14-6	inorganic acid	NC	C	1.5
Isostearic acid	**30399-84-9**	**organic acid**	**NC**	**NC**	**3.6**
Phenethyl bromide	**103-63-9**	**electrophile**	**NC**	**NC**	**3.6**
Eugenol	**97-53-0**	**phenol**	**NC**	**NC**	**3.7**
1,9-Decadiene	1647-16-1	neutral organic	NC	NC	3.9
Benzyl acetone	2550-26-7	neutral organic	NC	NC	3.9
Sodium lauryl sulfate (20% aq.)	151-21-3	surfactant	NC	C	3.9
Tetrachloroethylene	**127-18-4**	**neutral organic**	**NC**	**NC**	**4.5**
4-Amino-1,2,4-triazole	**584-13-4**	**organic base**	**NC**	**NC**	**5.5**
4-(methylthio)-Benzaldehyde	**3446-89-7**	**electrophile**	**NC**	**NC**	**6.8**
Sodium carbonate (50% aq.)	497-19-8	inorganic base	NC	C	11.7
Dodecanoic acid (lauric acid)	143-07-7	organic acid	NC	NC	ND

Abbreviations: aq = aqueous; C = corrosive; CASRN = Chemical Abstracts Service Registry Number; PG = Packing Group; NC = Non-corrosive; ND = not determined (unable to measure); UN = United Nations.

ICCVAM MINIMUM PERFORMANCE STANDARDS: IN VITRO TRANSCUTANEOUS ELECTRICAL RESISTANCE (TER) TESTS FOR SKIN CORROSION

[1]These chemicals, sorted first by corrosives versus noncorrosives and then by pH, were selected from among the 60 chemicals used by ECVAM to validate EPISKIN™ (12, 17). Unless otherwise indicated, the chemicals were tested at the purity level obtained when purchased from a commercial source (15). The goal of the selection process was to include, to the extent possible, chemicals that: were representative of the range of corrosivity responses (e.g., negative; weak to strong positives) that the validated *in vitro* test method is capable of measuring or predicting; were representative of the chemical classes used in the validation process; reflected the accuracy of the validated *in vitro* test method, as determined during the ICCVAM evaluation process; have a chemical structure that was well-defined; induced reproducible results in the validated *in vitro* test method; induced definitive results in the *in vivo* reference test; were commercially available; and were not associated with prohibitive disposal costs.

[2]Chemical class assigned by Barratt et al. (17).

[3]The assigned UN PG classification based on results of the *in vivo* rabbit skin test. Data from Barratt et al. (15).

[4]The pH values were obtained from Fentem et al. (12) and Barratt et al. (17).

two phenols, and one surfactant. The 12 calibration chemicals (6 noncorrosives, 6 corrosives), a subset of the 24 reference chemicals, are indicated in Table 4 using bolded print.

These 12 calibration and the 24 reference chemicals are the <u>minimum</u> number that should be used to calibrate the validated *in vitro* test method or to evaluate the performance of a new or modified *in vitro* skin TER test for skin corrosion, respectively. In situations where a listed chemical is unavailable, other chemicals or products for which adequate *in vivo* reference data are available could be used. If desired, additional chemicals for which adequate *in vivo* reference data are available can be added to the minimum list of reference chemicals to further evaluate the performance characteristics of the proposed *in vitro* test method. While not sufficient to allow for an assessment of the ability of an *in vitro* skin TER test to accurately predict the UN Packing Group classification for a test chemical, these chemicals are adequate to assess if a rat skin TER test is functioning appropriately and to assess the extent that a modified or new skin TER test can correctly identify corrosive and noncorrosive substances.

The goal of the reference chemical selection process was to include, to the extent possible, qualifying chemicals that:

ICCVAM MINIMUM PERFORMANCE STANDARDS: IN VITRO TRANSCUTANEOUS
ELECTRICAL RESISTANCE (TER) TESTS FOR SKIN CORROSION

- Were representative of the range of corrosivity responses (e.g., negative; weak to strong positives) that the validated *in vitro* test method is capable of measuring or predicting

- Were representative of the chemical classes used in the validation process

- Reflected the accuracy of the validated *in vitro* test method, as determined during the ICCVAM evaluation process

- Have a chemical structure that was well-defined

- Induced reproducible results in the validated *in vitro* test method

- Induced definitive results in the *in vivo* reference test

- Were commercially available

- Were not associated with prohibitive disposal costs

5.0   ACCURACY AND RELIABILITY

When calibrating the performance of the rat skin TER test, 100% concordance is required for the 12 calibration chemicals (6 corrosive, 6 noncorrosive) listed in Table 4.   With one exception, these 12 chemicals agree with those selected by the OECD as reference chemicals for Test Guideline 430 (*In vitro* skin corrosion: transcutaneous electrical resistance test [TER]) (19).   Acrylic acid, proposed by OECD as a severe corrosive, was not included because it was not one of the 60 chemicals used by ECVAM in its validation of the rat skin TER assay and the performance of this chemical in TER and the *in vivo* rabbit skin assay were not provided.

When evaluated using the minimum list of recommended reference chemicals in Table 4, the reliability and accuracy (i.e., sensitivity, specificity, false positive rates, and false negative rates) of the proposed *in vitro* skin TER assay should be at least comparable to that of the validated *in vitro* rat skin TER test method (2).   Noncorrosive and corrosive chemicals, ranging in activity from strong to weak, and representing relevant chemical classes are included so that the performance of the proposed test method can be determined and compared to that of the validated *in vitro* test method.

ICCVAM MINIMUM PERFORMANCE STANDARDS: IN VITRO TRANSCUTANEOUS ELECTRICAL RESISTANCE (TER) TESTS FOR SKIN CORROSION

The accuracy of the validated *in vitro* rat skin TER test method for the 24 reference chemicals, and the corresponding values obtained for the complete database considered by ICCVAM in its evaluation of this assay, are summarized in Table 5. The accuracy of the validated *in vitro* rat skin TER test method for the reference chemicals and the corresponding values obtained for the total database compiled during the ICCVAM evaluation process are not identical due to constraints associated with the chemical selection process.

The reliability of the proposed test method for the reference chemicals should be comparable to that of the validated *in vitro* rat skin TER test method. However, an assessment of interlaboratory reproducibility is not essential if the test method is to be used in one laboratory only. In terms of cell viability measurements, the median coefficient of variation (CV) should not exceed 35% for studies conducted in different laboratories (2, 12). The median CV for replicate studies conducted in the same laboratory should be appreciably less than median CV for studies conducted in different laboratories.

Table 5   Accuracy of the Validated *In Vitro* Skin TER Test for Skin Corrosion[1]

Source	# of Chemicals	Sensitivity[2]	Specificity[2]	False Negative Rate[2]	False Positive Rate[2]
MPS Reference Chemicals	24	92% (11/12)	75% (9/12)	8% (1/12)	25% (3/12)
Complete Validation Database	122	94% (51/54)	71% (48/68)	6% (3/54)	29% (20/68)

Definitions: Sensitivity is defined as the proportion of all positive chemicals that are correctly classified as positive in a test. Specificity is defined as the proportion of all negative chemicals that are correctly classified as negative in a test. False positive rate is defined as the proportion of all negative chemicals or chemical mixtures that are falsely identified as positive. False negative rate is defined as the proportion of all positive chemicals or chemical mixtures that are falsely identified as negative.

[1]Based on data in Fentem et al. (12). The accuracy of the validated *in vitro* rat skin TER test method for predicting the *in vivo* rabbit skin corrosivity potential of the 24 reference chemicals and the corresponding values obtained for the complete database reviewed during the ICCVAM evaluation process are not identical due to the constraints associated with selection of the reference chemicals. The goal of the selection process was to include, to the extent possible, chemicals that: were representative of the range of corrosivity responses (e.g., negative; weak to strong positives) that the

ICCVAM MINIMUM PERFORMANCE STANDARDS: IN VITRO TRANSCUTANEOUS ELECTRICAL RESISTANCE (TER) TESTS FOR SKIN CORROSION

validated *in vitro* test method is capable of measuring or predicting; were representative of the chemical classes used in the validation process; reflected the accuracy of the validated *in vitro* test method, as determined during the ICCVAM evaluation process; have a chemical structure that was well-defined; induced reproducible results in the validated *in vitro* test method; induced definitive results in the *in vivo* reference test; were commercially available; and were not associated with prohibitive disposal costs.

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

6.0   REFERENCES

(1)   ICCVAM. 1999. Corrositex®: An *In Vitro* Test Method for Assessing Dermal Corrosivity Potential of Chemicals. NIH Publication No. 99-4495. Research Triangle Park, NC: National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/docs/reports/corprrep.pdf [accessed 4 June 2003].

(2)   ICCVAM. 2002. ICCVAM Evaluation of EPISKIN, EpiDerm (EPI-200), and the Rat Skin Transcutaneous Electrical Resistance (TER) Assay: *In vitro* Test Methods for Assessing Dermal Corrosivity Potential of Chemicals. NIH Publication No. 02-4502. Research Triangle Park, NC. National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/methods/epiderm.htm [accessed 4 June 2003].

(3)   ICCVAM. 1997. Validation and Regulatory Acceptance of Toxicological Test Methods. A Report of the *ad hoc* Interagency Coordinating Committee on the Validation of Alternative Methods. NIH Publication No. 97-3981. Research Triangle Park, NC. National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/docs/guidelines/validate.pdf [accessed 4 June 2003].

(4)   OECD. 1996. Final Report of the OECD Workshop on Harmonisation of Validation and Acceptance Criteria for Alternative Toxicological Methods. (Solna Report) Paris: Organisation for Economic Co-operation and Development. Available: http://www.oecd.org [accessed 4 June 2003].

(5)   OECD. 2002. Report of the Stockholm Conference on Validation and Regulatory Acceptance of New and Updated Methods in Hazard Assessment. Paris: Organisation for Economic Co-operation and Development. Available: http://www.oecd.org [accessed 4 June 2003].

(6)   OECD. 2002. Test Guideline 404. OECD Guideline for Testing of Chemicals: Acute Dermal Irritation/Corrosion. Paris: Organisation for Economic Co-operation and Development. Available:http://www1.oecd.org/ehs/test/health.htm [accessed 4 June 2003].

(7)   U.S. EPA 1998. Health Effects Test Guidelines: Acute Dermal Irritation. OPPTS 870.2500. Washington, DC. U.S. Environmental Protection Agency. Available: http://www.epa.gov/docs/OPPTS_Harmonized/870_Health_Effects_Test_Guidelines/Series/870-2500.pdf [accessed 4 June 2003].

(8)   U.S. Department of Transportation (DOT). 2001. Shippers--General Requirements for Shipments and Packagings--Class 8 Definitions1. 49 CFR 173.136.   Available: http://www.access.gpo.gov/nara/cfr/cfr-retrieve.html#page1 [accessed 4 June 2003]

(9)   U.S. DOT. 2001. Shippers--General Requirements for Shipments and Packagings--Class 8 Assignment of Packing Group. 49 CFR 173.137.   Available: http://www.access.gpo.gov/nara/cfr/cfr-retrieve.html#page1 [accessed 4 June 2003]

(10)   U.S. EPA. 1996. Method 1120, Dermal Corrosion. Washington, DC: U.S. Environmental   Protection   Agency.   Available: http://www.epa.gov/epaoswer/hazwaste/test/pdfs/1120.pdf [accessed 4 June 2003].

(11)   UN Economic Commission for Europe (ECE). 2001. Globally Harmonized System of Classification   and   Labeling   of   Chemicals   (GHS).   Geneva.   Available: http://www.unece.org/trans/danger/publi/ghs/ghs.html [accessed 4 June 2003].

18

ICCVAM MINIMUM PERFORMANCE STANDARDS: IN VITRO TRANSCUTANEOUS
ELECTRICAL RESISTANCE (TER) TESTS FOR SKIN CORROSION

(12)   Fentem JH, Archer GEB, Balls M, Botham PA, Curren RD, Earl LK., et al. 1998. The ECVAM international validation study on *in vitro* tests for skin corrosivity. 2. Results and evaluation by the management team. Toxicol In Vitro 12: 483-524.

(13)   Oliver GJA, Pemberton MA, and Rhodes C. 1986. An *in vitro* skin corrosivity test-modifications and validation. Fd Chem Toxicol 24:507-512.

(14)   Oliver GJA, Pemberton MA, and Rhodes C. 1988. An *in vitro* model for identifying skin corrosive chemicals. I. Initial validation. Toxicol In Vitro 2: 7-17.

(15)   Botham PA., Hall TJ, Dennett R., McCall JC, Basketter DA, Whittle E., et al. 1992. The skin corrosivity test *in vitro*. Results of an inter-laboratory trial. Toxicol In Vitro 6:191-194.

(16)   Botham PA, Chamberlain M, Barratt MD, Curren RD, Esdaile DJ, Gardner JR., et al. 1995. A Prevalidation study on *in vitro* skin corrosivity testing: The Report and recommendations of ECVAM Workshop 6. ATLA 23:219-255.

(17)   Barratt MD, Brantom PG, Fentem JH, Gerner I, Walker AP, and Worth AP. 1998. The ECVAM international validation study on *in vitro* test for skin corrosivity. 1. Selection and Distribution of the Test Chemicals. Toxicol In Vitro 12:471-482.

(18)   Balls M, and Corcelle G. 1998. Statement on the scientific validity of the rat skin transcutaneous electrical resistance (TER) test (an *in vitro* test for skin corrosivity). ATLA 26: 275-277.

(19)   OECD. 2003. OECD Guideline for the Testing of Chemicals: Draft Proposal for a New Guideline 430, *In Vitro* Skin Corrosion: Transcutaneous Electrical Resistance Test

ICCVAM MINIMUM PERFORMANCE STANDARDS: IN VITRO TRANSCUTANEOUS
ELECTRICAL RESISTANCE (TER) TESTS FOR SKIN CORROSION

(TER). Paris: Organisation for Economic Co-operation and Development. Available: http://www.oecd.org [accessed 4 June 2003].