The ICCVAM Dermal Corrosivity and Irritation

Working Group Proposed

ICCVAM MINIMUM PERFORMANCE STANDARDS: *IN VITRO* HUMAN SKIN MODEL SYSTEMS FOR SKIN CORROSION

June 23, 2003

NOTICE

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.

ICCVAM MINIMUM PERFORMANCE STANDARDS:
IN VITRO HUMAN SKIN MODEL SYSTEMS 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 HUMAN SKIN MODEL SYSTEMS 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 HUMAN SKIN MODEL SYSTEMS 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).

Pre-validation and validation studies have been completed for an *in vitro* human skin model system commercially available as EPISKIN™ (2, 12-15). Based on its scientific validity, this test method has been recommended for the testing of all classes of chemicals (2, 12, 16) and for inclusion in tiered testing strategies as part of a tiered or weight-of-evidence evaluation (2). In addition to EPISKIN™, a related human skin model corrosivity test method marketed as EpiDerm™ has been validated and recommended as an alternative to EPISKIN™ (2, 17, 18).

2.2   Principles of *In Vitro* Human Skin Model Systems for Skin Corrosion

The test material is applied topically to a three-dimensional human skin model, comprised of at least a reconstructed epidermis with a functional stratum corneum. Corrosive materials are identified by their ability to induce a decrease in cell viability below defined threshold levels at specified exposure periods. The principle of the human skin model assay is based on the premise that corrosive chemicals are able to penetrate the stratum corneum by diffusion or erosion, and are cytotoxic to the cells in the underlying layers. The use of test systems that include human-derived cells or tissue should be accordance with applicable national and international laws, regulations, and policies.

DO NOT DISTRIBUTE OR ATTRIBUTE

ICCVAM MINIMUM PERFORMANCE STANDARDS:
IN VITRO HUMAN SKIN MODEL SYSTEMS FOR SKIN CORROSION

Investigators using an *in vitro* human skin model system for skin corrosion 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.

In vitro human skin model systems 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 should be 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* human skin model system for skin corrosion (12). However, taking into account the limited mechanisms which result in corrosivity, this method is expected to be generally applicable across all chemical classes (2, 12, 16).

3.0   MINIMUM PROCEDURAL STANDARDS

The following is a description of the minimum procedural standards, including test method components, for in vitro human skin model test methods for skin corrosivity , as provided in the Organisation for Economic Co-operation and Development (OECD) Test Guideline 431 (19). Human skin models can be obtained commercially (e.g., the EpiDerm™ model) (21) or they can be developed or constructed in the testing laboratory (221, 23).

3.1   *In Vitro* Human Skin Model Conditions

Human keratinocytes should be used to construct the epithelium. Multiple layers of viable epithelial cells should be present under a functional stratum corneum. The skin model may also have a stromal component layer. Stratum corneum should be multilayered with the necessary lipid profile to produce a functional barrier with robustness to resist rapid

ICCVAM MINIMUM PERFORMANCE STANDARDS:
IN VITRO HUMAN SKIN MODEL SYSTEMS FOR SKIN CORROSION

penetration of cytotoxic markers. The containment properties of the model should prevent the passage of material around the stratum corneum to the viable tissue, which would lead to poor modeling of the exposure to skin. The skin model should be free of contamination with bacteria, mycoplasma, or fungi.

The magnitude of viability is usually quantified by using MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, thiazolyl blue; CASRN 298-93-1) or other metabolically converted vital dyes (reviewed in 24). The negative control tissue should be stable in culture (provide similar viability measurements) for the duration of the test exposure period. The stratum corneum should be sufficiently robust to resist the rapid penetration of cytotoxic marker chemicals (e.g., 1% Triton X-100). This property can be estimated by the exposure time required to reduce cell viability by 50% (e.g., for the EpiDerm™ model this is >2 hours).

3.2   Application of the Test Substances

Two tissue replicates are used for each treatment, including controls. For liquid materials, sufficient test substance must be applied to uniformly cover the skin surface; a minimum of 25 $\mu L/cm^2$ should be used. For solid materials, sufficient test substance must be applied evenly to cover the skin, and it should be moistened with deionized or distilled water to ensure good contact with the skin. Where appropriate, solids should be ground to a powder before application. At the end of each exposure period (3 minutes to 1 or 4 hours), the test material must be carefully washed from the skin surface with an appropriate buffer or 0.9% NaCl.

ICCVAM MINIMUM PERFORMANCE STANDARDS:
IN VITRO HUMAN SKIN MODEL SYSTEMS FOR SKIN CORROSION

3.3   Control Substances

3.3.1   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.

3.3.2   Positive (Corrosive) Controls

A positive control (e.g., glacial acetic acid, 8N KOH) should be tested concurrently with the test substance to demonstrate that the human skin membrane barrier is functioning properly. The positive control should generate a response 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.

3.3.3   Negative (Noncorrosive) Controls

A noncorrosive substance (e.g., 0.9% sodium chloride, water) should also be tested concurrently with the test substance as another quality control measure to demonstrate the functional integrity of the human skin membrane barrier.

3.3.4   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. Cell

3.4   Viability Measurements

Only standardized, quantitative methods should be used to measure cell viability. Furthermore, the measure of viability must be compatible with use in a three-dimensional tissue construct. Non-specific dye binding must not interfere with the viability measurement.

DO NOT DISTRIBUTE OR ATTRIBUTE

ICCVAM MINIMUM PERFORMANCE STANDARDS:
IN VITRO HUMAN SKIN MODEL SYSTEMS FOR SKIN CORROSION

Protein binding dyes and those which do not undergo metabolic conversion (e.g., neutral red) are therefore not appropriate. The most frequently used assay is MTT reduction, which has been shown to give accurate and reproducible results (12) but others may be used.

Chemical action by the test material on the vital dye may mimic that of cellular metabolism leading to a false estimate of viability. This has been shown to happen when such a test material is not completely removed from the skin by rinsing (17). If the test material directly acts on the vital dye, additional controls should be used to detect and correct for test substance interference with the viability measurement (17, 25).

3.5 Interpretation of Results

The optical density (OD) values obtained for each test sample can be used to calculate a percentage viability relative to the negative control, which is arbitrarily set at 100%. The cell viability criteria used to distinguish between corrosive and noncorrosive test chemicals (or to discriminate between different corrosive classes), or the statistical procedure(s) used to evaluate the results and identify corrosive materials must be clearly defined and documented, and be shown to be appropriate. In general, such criteria are established during test optimization, tested during a prevalidation phase, and confirmed in a validation study. As an example, the prediction of corrosivity associated with the EpiDerm™ model is (17):

The test substance is considered to be corrosive to skin:
  i) if the viability after 3 minutes of exposure is less than 50%, or
  ii) if the viability after 3 minutes of exposure is greater than or equal to 50 % and the viability after 1 hour of exposure is less than 15%.

The test substance is considered to be noncorrosive to skin:
  i) if the viability after 3 minutes of exposure is greater than or equal to 50% and the viability after 1 hour of exposure is greater than or equal to 15%.

ICCVAM MINIMUM PERFORMANCE STANDARDS:
IN VITRO HUMAN SKIN MODEL SYSTEMS FOR SKIN CORROSION

3.6   Reporting

The test report should include the following information**:**

Test and Control Substances

- 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

Justification of the Skin Model and Protocol Used

Test Conditions

- Cell system used

- Calibration information for measuring device used for measuring cell viability (e.g., spectrophotometer)

- Complete supporting information for the specific skin model used including its validity

- Details of test procedure used

- Test doses used

- Description of any modifications of the test procedure

- Reference to historical data of the model

- Description of evaluation criteria used

8

ICCVAM MINIMUM PERFORMANCE STANDARDS:
IN VITRO HUMAN SKIN MODEL SYSTEMS FOR SKIN CORROSION

<u>Results</u>

- Tabulation of data from individual test samples (e.g., OD values and calculated percentage cell viability data for the test material, positive, negative, and benchmark controls, reported in tabular form, including data from replicate repeat experiments as appropriate, and means and ± the standard deviation for each trial)

<u>Description of Other Effects Observed</u>

<u>Discussion of the Results</u>

<u>Conclusion</u>

4.0   REFERENCE CHEMICALS

Reference chemicals are used to determine if the performance of a proposed *in vitro* human skin model system 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 2 provide a representative distribution of the 60 chemicals used in the European Centre for the Validation of Alternative Methods (ECVAM) validation study of EPISKIN™ (12, 15) 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 inorganic acids, three inorganic bases, three organic acids, three electrophiles, three phenols, two neutral organics, and one surfactant. The goal of the reference chemical selection process was to include, to the extent possible, qualifying 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

DO NOT DISTRIBUTE OR ATTRIBUTE

ICCVAM MINIMUM PERFORMANCE STANDARDS:
IN VITRO HUMAN SKIN MODEL SYSTEMS FOR SKIN CORROSION

Table 2   Chemicals Recommended for Validation of New *In Vitro* Human Skin Model 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	C	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
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	NC	8.4
2-Mercapoethanol, sodium salt (45% aq.)	37482-11-4	inorganic base	II/III	NC	12.0
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
o-Methoxyphenol	90-05-1	phenol	NC	C	3.9
Sodium lauryl sulfate (20% aq.)	151-21-3	surfactant	NC	NC	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	NC	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 HUMAN SKIN MODEL SYSTEMS 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, 15). 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. (15).
[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. (15).

- 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 evaluated using the minimum list of recommended reference chemicals (Table 2), the proposed test method should have reliability and performance (i.e., sensitivity, specificity, false positive rates, and false negative rates) characteristics that are at least comparable to the performance of the validated reference method (2, 12). 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. Eleven of these 24 reference chemicals agree with those selected by the OECD as reference chemicals for Test Guideline 431 (*In vitro* skin corrosion human skin model system)(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 EPISKIN™ and the performance of this chemical in this *in vitro* test method and the *in vivo* rabbit skin assay was not provided.

ICCVAM MINIMUM PERFORMANCE STANDARDS:
IN VITRO HUMAN SKIN MODEL SYSTEMS FOR SKIN CORROSION

The accuracy of the validated *in vitro* human skin model test system, EPISKIN™, for the 24 reference chemicals and the complete validation database considered by ICCVAM are provided in Table 3. 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 reference 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 3   Accuracy of the Validated *In Vitro* Human Skin Model System Test Method for Skin Corrosion[1]

Source	# of Chemicals	Sensitivity[2]	Specificity[2]	False Negative Rate[2]	False Positive Rate[2]
MPS Reference Chemicals	24	83% (10/12)	83% (10/12)	17% (2/12)	17% (2/12)
Complete Validation Database	60	82% (23/28)	84% (27/32)	18% (5/28)	16% (5/32)

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* human skin model system 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:

ICCVAM MINIMUM PERFORMANCE STANDARDS:
IN VITRO HUMAN SKIN MODEL SYSTEMS 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; 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/corrrep.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].

ICCVAM MINIMUM PERFORMANCE STANDARDS:
IN VITRO HUMAN SKIN MODEL SYSTEMS FOR SKIN CORROSION

(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 4June 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].

(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)   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.

(14)   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.

(15)   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.

(16)   Balls M, and Corcelle G. 1998. Statement on the scientific validity of the EPISKIN test (an *in vitro* test for skin corrosivity). ATLA 26: 278-280.

(17)   Liebsch, M, Traue, D, Barrabas, C, Spielmann, H, Uphill, P, Wilkins, S, et al. 2000. The ECVAM prevalidation study on the use of EpiDerm for skin corrosivity testing. ATLA 28:371-401.

(18)   Balls M, and Hellsten E. 2000. Statement on the Application of the EpiDerm human skin model for corrosivity testing. ATLA 28: 365-367.

ICCVAM MINIMUM PERFORMANCE STANDARDS:
IN VITRO HUMAN SKIN MODEL SYSTEMS FOR SKIN CORROSION

(19)   OECD. 2003. OECD Guideline for the Testing of Chemicals: Draft Proposal for a New Guideline 431, *In Vitro* Skin Corrosion Human Skin Model System. OECD ENV/JM/TG(2002)8. Paris: Organisation for Economic Co-operation and Development. Available: http://www.oecd.org [accessed 4 June 2003].

(20)   Cannon CL, Neal PJ, Southee JA, Kubilus J, and Klausner M. 1994. New epidermal model for dermal irritancy testing. Toxicol In Vitro 8: 889-891.

(21)   Ponec M, Boelsma E, Weerheim A, Mulder A, Bouwstra J, and Mommaas, M. 2000. Lipid and ultrastructural characterization of reconstructed skin models. Intl J Pharmaceutics 203:211-225.

(22)   Parenteau NL, Bilbo P, Nolte CJ, Mason VS, and Rosenberg M. 1992. The organotypic culture of human skin keratinocytes and fibroblasts to achieve form and function. Cytotechnology 9:163-171.

(23)   Wilkins LM, Watson SR, Prosky SJ, Meunier, SF, and Parenteau, NL. 1994. Development of a bilayered living skin construct for clinical applications. Biotechnology and Bioengineering 43:747-756.

(24)   Marshall, NJ, Goodwin, CJ, and Holt, SJ. 1995. A critical assessment of the use of microculture tetrazolium assays to measure cell growth and function. Growth Regulation 5:69-84.

(25)   Fentem JH, Briggs D, Chesné C, Elliot GR, Harbell JW, Heylings JR., et al, 2001. A prevalidation study on *in vitro* tests for acute skin irritation: results and evaluation by the Management Team. Toxicol In Vitro 15:57-93.

DO NOT DISTRIBUTE OR ATTRIBUTE