

**Summary Report of the
EpiDerm™ (EPI-200) *In Vitro* Assay
for Assessing Dermal Corrosivity**

Prepared for

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PURPOSE

This report focuses on the performance of EpiDerm (EPI-200) to determine the usefulness and limitations of the assay for the identification of potential human corrosive chemicals. This report discusses also how EpiDerm (EPI-200) compares to EPISKIN, a mechanistically related *in vitro* human skin model system, and to other validated *in vitro* corrosivity tests (Rat Skin Transcutaneous Electrical Resistance [TER] and Corrositex). The data and assessments reviewed for this report included the European Centre for the Validation of Alternative Methods (ECVAM) formal pre-validation/validation study on EpiDerm (EPI-200) (Liebsch et al., 2000) and additional information formally submitted by MatTek, the commercial source of the assay, to ICCVAM for consideration (see MatTek Submission to ICCVAM; Appendix F, September 13, 2000).

EVALUATION OF REGULATORY AND SCIENTIFIC RATIONALE

EpiDerm (EPI-200) is one of several *in vitro* corrosivity assays formally evaluated by ECVAM as alternatives to the *in vivo* rabbit corrosivity test (Fentem et al., 1998; Liebsch et al., 2000). The assay is a three-dimensional human skin model that uses cell viability as a measure of toxicity (i.e., corrosivity). Because EpiDerm (EPI-200) is a human skin model, it may be more relevant to assessing human skin corrosivity potential than a test based on skin from another species. Also, the mode of application (topical) of the test material mimics the route of human exposure.

EpiDerm (EPI-200) has been approved by the ECVAM Scientific Advisory Committee

for use in corrosivity testing in Europe (Balls and Hellsten, 2000). This method has also been adopted for regulatory use within the European Union (EU) by the European Commission (EU, 2000).

EVALUATION OF THE TEST METHOD

A standard kit contains media, reagents, and 24 tissues. The tissues provided in the test kit consist of normal, human epidermal keratinocytes cultured in a chemically defined medium to produce a stratified, highly differentiated, organotypic tissue model of the human epidermis. An EpiDerm (EPI-200) kit is equipped with sufficient amounts of medium, washing solutions, and sterile, disposable tissue culture plasticware to test four test materials and concurrent negative and positive controls. For use in corrosivity testing, the test material (liquids: 50 μ L; solids: 25 mg) is topically applied to a tissue for 3 and 60 minutes. Per test compound, replicate plates are used for each test period. Cell viability is assessed by measuring mitochondrial activity using the MTT (a tetrazolium salt) assay. A test chemical is classified as corrosive if it induces a 50% or greater decrease in relative cell viability at 3 minutes or an 85% or greater decrease in relative cell viability at 60 minutes. The scientific rationale for these decision criteria are based on a correlative analysis of the ability of a number of corrosive (C) and noncorrosive (NC) chemicals to induce histopathological necrosis and an associated reduction in cell viability (Perkins et al., 1996). EpiDerm (EPI-200) will complement EPISKIN, an ECVAM-validated *in vitro* corrosivity method, by providing an alternative and commercially available method.

Information on differences and similarities between EpiDerm (EPI-200) and EPISKIN are detailed in **Table 3.1**.

EVALUATION OF TEST METHOD DATA QUALITY

The performance of EpiDerm (EPI-200) was evaluated in three phases (Liebsch et al., 2000). Phase I was conducted by ZEBET (Centre for Documentation and Evaluation of Alternative Methods to Animal Experiments, Berlin, Germany), and involved protocol and prediction model refinement using 50 chemicals. Phase II involved the transfer of the protocol to a second laboratory (Huntington Life Sciences) and the reproducibility of the assay was assessed by the repeat testing of 11 chemicals. In addition, in Phase II, ZEBET tested those chemicals classified as false negative in Phase I, aiming to refine the protocol and prediction model by increasing test sensitivity. Phase III was a formal evaluation of the reliability and performance of the assay using three laboratories (ZEBET, Huntington Life Sciences, and BASF AG), in which a blind trial conducted with 24 test chemicals was performed using the refined final protocol. In designing the Phase III study, ECVAM based its validation process on experimental results demonstrating that the EpiDerm (EPI-200) and EPISKIN assays were mechanistically identical (Roguet et al., 1999). For Phase III, ECVAM selected a subset of 24 chemicals from the 60 chemicals tested in the EPISKIN ECVAM validation study (Fentem et al., 1998). The selection of the 60 chemicals in the original validation study was based on unequivocal animal data (Barratt et al., 1998). Care was taken to ensure a balanced representation of the chemical classes in this subset, as well as to minimize the number of chemicals previously in Phase I (there was an overlap

of 5 chemicals). The 24 chemicals selected included 12 corrosive tested and 12 noncorrosive chemicals -- four organic acids (2 C; 2 NC), six organic bases (4 C, 2 NC), four neutral organic bases (4 NC), two phenols (1 C, 1 NC), three inorganic acids (2 C; 1 NC), two inorganic bases (1 C; 1 NC), two electrophiles (2 C), and one surfactant (1 NC).

Table 3.1 General Protocol Comparison between EPISKIN™ and EpiDerm™ (EPI-200)

	EPISKIN	EpiDerm (EPI-200)
Assay	Reconstructed human epidermis and a functional stratum corneum (not an animal model). Tissue approximates the barrier of normal human skin.	
Known limits of use	No known restrictions except for chemicals that reduce MTT. Although a relatively small numbers of chemicals have been evaluated in some chemical classes (i.e., cleaners and detergents), classified by ECVAM as otherwise without limits.	
Tissue construct acceptability	QC measures are based on historical laboratory control data.	
Materials, equipment, and supplies needed	Similar	
Replicates	Single tissue (culture)/experiment (ECVAM, 2000b) or 3 replicates/experiments (OECD, 2001c)	Duplicate tissues/experiment, experiment replication if needed
Dosing procedures	Liquids: 50 µL applied neat Solids: 20 mg + saline	Liquids: 50 µL applied neat Solids: 25 mg + 25 µL H ₂ O
Exposure duration	3 minutes, 1 hour, 4 hours	3 minutes, 1 hour
Endpoint	Relative cell viability compared to concurrent negative control, based on MTT assay (measure of mitochondrial function); assay based on optical density.	
Negative and positive controls	No vehicle control (undiluted test material used) Negative control: saline Positive control: glacial acetic acid	No vehicle control (undiluted test material used) Negative control: water Positive control: 8.0 N KOH
Acceptable range of control responses	Negative control: 4-hour optical density at 545-595 nm = 0.113-0.309 for MTT incubations at 20-28°C. Positive control: viability at 4 hours must be 0-20%.	Negative control: 3-min and 1-hour optical density at 570 or 540 nm = 0.8. Positive control: viability at 3 min must be 30%.
Data analysis	Determination of relative viability at each exposure duration. No statistical analysis.	
Positive response	Relative cell viability <35% at any exposure duration (=packing group).	Relative cell viability <50% after 3 minutes and/or <15% after 60 minutes.
Criteria for accepting or rejecting a test	Acceptable control values Test repeated if inconsistent toxicity response pattern across exposure durations (i.e., less toxicity at a longer exposure duration) or if corrosivity classification is variable	Acceptable control values Test repeated if difference in viability between duplicate tissues >30% and the corrosivity classification is variable, or (recommended) if the resulting viability is near to a classification cut-off.

The tests were conducted in the "spirit" of GLP. Each chemical was tested twice using independent lots of tissue by each of three different laboratories. A formal audit of the ECVAM data by a Quality Assurance Unit was not conducted; however, it was stated that all data submitted by the participating laboratories were verified against the original data sheets by ECVAM staff.

EVALUATION OF TEST METHOD PERFORMANCE

For this summary report, an analysis was conducted, similar to the performance analysis conducted for the ICCVAM Peer Review of Corrositex ; the current analysis evaluated the performance characteristics of the EpiDerm (EPI-200) assay against the corresponding *in vivo* rabbit corrosivity data and the corresponding *in vitro* corrosivity data generated by EPISKIN . The database used in the evaluation of the performance characteristics of EpiDerm (EPI-200) consisted of data from the ECVAM pre-validation/validation study only (Liebsch et al., 2000); other data were not located.

For ease of comparison, chemicals evaluated in the EpiDerm (EPI-200) assay were classified into the same chemical and product class designations used in the Corrositex evaluation. A weight-of-evidence approach was used for classifying discordant results within or between laboratories; in instances where discordant results could not be resolved (i.e., there was an equal number of positive and negative calls), the chemical was eliminated from inclusion in the performance calculations.

Based on the database of 24 chemicals and chemical mixtures used in the validation study and using a weight-of-evidence

approach to classify the corrosivity results (**Tables 3.2** and **3.4**), EpiDerm (EPI-200) had an accuracy of 92% (22/24 chemicals or chemical mixtures), a sensitivity of 92% (11/12 chemicals or chemical mixtures), a specificity of 83% (10/12 chemicals or chemical mixtures), a false positive rate of 17% (2/12 chemicals or chemical mixtures), and a false negative rate of 8% (1/12 chemicals or chemical mixtures). From these data, which met pre-study acceptance criteria of no more than 20% false negatives and 20% false positives, the ECVAM concluded that EpiDerm (EPI-200) was valid for use as a replacement for the *in vivo* rabbit skin test for distinguishing between corrosive and noncorrosive chemicals for all of the chemical classes studied (Liebsch et al., 2000). As for EPISKIN , due to the relatively small numbers of chemicals evaluated in some chemical classes, definitive conclusions as to the adequacy of EPISKIN or EpiDerm (EPI-200) for some classes of chemicals were difficult to make with a high degree of confidence. However, taking into account the relative simplicity of the mechanism of action of corrosives, ECVAM concluded that the EpiDerm (EPI-200) method would be generally applicable across all chemical classes (Fentem et al., 1998; Liebsch et al., 2000). A comparison of the ability of EpiDerm (EPI-200) and EPISKIN to correctly identify corrosive and noncorrosive chemicals among the 24 chemicals tested in Phase III is provided in **Table 3.2**. Both assays are nearly identical in their performance (see also **Table 3.4**).

Table 3.2 Summary of Results for EpiDerm™ (EPI-200) and EPISKIN™ Compared to *In Vivo* Rabbit Results

Material	EPISKIN™	EpiDerm™ (EPI-200)
Corrosive	11/12	11/12
Noncorrosive	11/12	10/12

EVALUATION OF TEST METHOD RELIABILITY (REPEATABILITY/REPRODUCIBILITY)

The inter- and intra-laboratory reliability of EpiDerm (EPI-200) was evaluated in the ECVAM pre-validation/validation study (Liebsch et al., 2000). In Phase III, each chemical was tested twice using different tissue lots in each of three laboratories (i.e., 144 tests were conducted). Of 72 replicate tests, 5 (6.9%) did not replicate. Regarding inter-laboratory reproducibility, three of the 24 chemicals (12.5%) were not predicted by all three laboratories (i.e., the performance characteristics of the three laboratories were nearly identical). Intra- and inter-laboratory reliability was evaluated formally using a relative mean square diagram (determined using a two-way ANOVA with laboratory and experiments as factors), scatter diagrams to assess the possibility of divergence between results obtained in different laboratories, and range diagrams to summarize the overall performance of the tests. Based on the results obtained, ECVAM concluded that EpiDerm (EPI-200) provided excellent reliability (Liebsch et al., 2000). After reviewing the intra- and inter-laboratory evaluations conducted by ECVAM, it was concluded by NICEATM that the analyses were appropriate and that the conclusions were accurate.

OTHER SCIENTIFIC REVIEWS

In May 2001, a search of the open literature was conducted to locate additional EpiDerm (EPI-200) studies. Four databases (PubMed, Web of Science, Toxline, and Current Contents Connect) were searched using the key terms "EpiDerm", and "Epi" within one word of "derm". Additional references were obtained from the MatTek technical references section at www.mattek.com. The search found no additional relevant studies conducted with EpiDerm (EPI-200).

OTHER CONSIDERATIONS

Like EPISKIN, the EpiDerm (EPI-200) kit contains all of the necessary materials to conduct the test and does not require additional preparation. No animals are used in this test. The cost for conducting EpiDerm (EPI-200) is reported by MatTek (e-mail communication from Mitch Klausner, MatTek Corporation) to be approximately \$800 per kit or \$200 per test chemical (**Table 3.3**). This cost is less than the *in vivo* rabbit skin test and similar to that for the other validated *in vitro* corrosivity assays (Fentem et al., 1998). The time needed to conduct the EpiDerm (EPI-200) is similar to EPISKIN.

RELATED ISSUES

Refinement, Reduction, and Replacement

Since the method is designed as a replacement for animals, EpiDerm (EPI-200) would clearly reduce the requirement for animal testing for corrosivity. Therefore, it has the potential to eliminate the use of animals for the determination of corrosivity. If used in an integrated testing approach, EpiDerm (EPI-200) provides for reduction and refinement of animal use.

Comparison to Other *In Vitro* Assays

General comparative information on EpiDerm (EPI-200) compared to Rat Skin TER, EPISKIN , and Corrositex is provided in **Tables 3.3** through **3.6**. In contrast to Corrositex and EPISKIN , EpiDerm (EPI-200), like Rat Skin TER, cannot be used to identify packing group classifications.

Table 3.3 General Comparison of the Rat Skin TER, EPISKIN™, EpiDerm™ (EPI-200), and Corrositex® Assays

	Rat Skin TER	EPISKIN™ (prediction model B)	EpiDerm™ (EPI-200) (prediction model 2)	Corrositex®
Test Method Description	Acceptable	Acceptable	Acceptable	Acceptable
Adequacy/Completeness of Protocol	Acceptable	Acceptable	Acceptable	Acceptable
Usefulness for Assessing Corrosivity/Non-corrosivity	Acceptable (Botham et al., 1992; 1995; Fentem et al., 1998)	Acceptable (Fentem et al., 1998)	Acceptable (Liebsch et al., 2000)	Acceptable (ICCVAM, 1999)
Usefulness for Determining Packing Groups	Not Acceptable (Fentem et al., 1998)	Can group as UN packing group II/III or I (Fentem et al., 1998) ^a	Not Acceptable (Liebsch et al., 2000)	Acceptable (ICCVAM, 1999)
Repeatability and Reproducibility	Acceptable (Botham et al., 1992; 1995; Fentem et al., 1998)	Acceptable (Fentem et al., 1998)	Acceptable (Liebsch et al., 2000)	Acceptable (Fentem et al., 1998; ICCVAM, 1999)
Animal Use Refinement, Reduction, and Replacement Considerations	Refines and reduces animal use when used as a stand-alone test or in an integrated testing strategy.	Replaces animal use when used as a stand-alone test. Refines and reduces animal use when used in an integrated testing strategy.	Refines and reduces animal use when used in an integrated testing strategy.	Replaces animal use when used as a stand-alone test. Refines and reduces animal use when used in an integrated testing strategy.
Cost	~\$500-850/test	~\$450/test kit ^b	~\$200/test chemical	~\$300/test chemical
Study duration	2 work-days	1 work-day	1 work-day	4 hr/chemical

^a Since the performance of EPISKIN™ was not assessed for distinguishing between UN packing groups II and III, all R34 classifications would be conservatively classified as UN packing group II.

^b One to three chemicals may be tested per test kit; however, it is recommended by the supplier that each test chemical be assayed using 3 different skin batches/kits which equates to a total cost of ~\$430/ test chemical.

Table 3.4 General Comparison of the Rat Skin TER, EPISKIN™, EpiDerm™ (EPI-200), and Corrositex® Assays Based on a Weight-of-Evidence Approach^a by Chemical using Data from the ECVAM and other Validation Studies (Fentem et al., 1998; ICCVAM, 1999; Liebsch et al., 2000)

	Rat Skin TER	EPISKIN	EpiDerm™ (EPI-200) (prediction model 2)	Corrositex®
Number of Chemicals	122	60	24	163
	94% (51/54)	82% (23/28)	92% (11/12)	85% (76/89)
Overall Sensitivity^b	71% (48/68)	84% (27/32)	83% (10/12)	70% (52/74)
Overall Specificity^b	81% (99/122)	83% (50/60)	92% (22/24)	79% (128/163)
Overall Accuracy^b	29% (20/68)	16% (5/32)	17% (2/12)	30% (22/74)
False Positive Rate	6% (3/54)	18% (5/28)	8% (1/12)	15% (13/89)
False Negative Rate				
Test Chemical Inter-laboratory Coefficient of Variation	34.7 ^c 3.8-322 ^d 120 ^e	11.3 ^c 3.9-148.8 ^d 20 ^e	12.3 ^c 0.9-51.2 ^d 144 ^e	30.3 ^c 7.7-252.5 ^d 180 ^e

^a A chemical 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 chemical 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). In instances where discordant results could not be resolved (i.e., there was an equal number of positive and negative calls within or across laboratories), the chemical was eliminated from inclusion in the performance calculations.

^b 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. Accuracy (concordance) is defined as the proportion of correct outcomes of a method.

^c Median values

^d Range of values

^e The total number of independent values, which is calculated as the number of chemicals tested multiplied by the number of participating laboratories.

Table 3.5 General Comparison of the Rat Skin TER, EPISKIN™, and EpiDerm™ (EPI-200) Assays from Independent Test Results in the ECVAM Validation Studies (Fentem et al., 1998; Liebsch et al., 2000)

	TER	EPISKIN™ (prediction model B)	EpiDerm™ (EPI-200) (prediction)
Number of Chemicals Tested in ECVAM Validation Study	60 (Fentem et al., 1998)	60/24 ^a (Fentem et al., 1998)	24 (Liebsch et al., 2000)
Sensitivity^b	88% (140/159)	83% (201/243) / 88% (87/99)	88% (63/72)
Specificity^b	72% (142/196)	80% (237/297) / 79% (92/117)	86% (62/72)
Accuracy^b	79% (282/355) ^c	81% (438/540) / 83% (179/216)	87% (125/144)
False Positive Rate^b	28% (54/196)	20% (60/297) / 21% (25/117)	14% (10/72)
False Negative Rate^b	12% (19/159)	17% (42/243) / 12% (12/99)	13% (9/72)
Number of Trials^d	155	540 / 216	144
Test Chemical Inter-laboratory Coefficient of Variation	34.7 ^d 10-322 ^e 155 ^f	30.2 ^d 7.7-252.5 ^e 540 ^f	12.3 ^d 0.9-51.2 ^e 144 ^f

^a The first numbers for accuracy, sensitivity, specificity, false positive rate, and false negative rate correspond to the 60 chemicals tested in the ECVAM Skin Corrosivity Test using EPISKIN (Barratt et al., 1998; Fentem et al., 1998); the latter values correspond to a direct comparison of EpiDerm (EPI-200) and EPISKIN for the same 24 materials tested in both systems (Liebsch et al., 2000).

^b 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. Accuracy (concordance) is defined as the proportion of correct outcomes of a method. 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.

^c The percentages are based on the number of correct trials among the total number of trials (i.e., independent tests) provided in parenthesis.

^d Median values

^e Range of values

^f The total number of trials conducted in the validation study minus the non-qualified (NQ) results. This number is usually equal to the number of chemicals multiplied by the number of participating laboratories multiplied by the number of replicate tests.

Table 3.6 Classification Results from the ECVAM Validation Studies of Rat Skin TER, EPISKIN™, and EpiDerm™ (EPI-200) Assays as Compared to the *In Vivo* Classification (Fentem et al., 1998; Liebsch et al., 2000)

No. ^a	Chemical	Type	<i>In Vivo</i>	Rat Skin TER	EPISKIN™ ^b	EpiDerm™ (EPI-200)
1	Hexanoic acid	ORGAC	R34/II&III	R35	R35	N/A
29	65/35 Octanoic/decanoic acid	ORGAC	R34/II&III	R34	R35	N/A
36	2-Methylbutyric acid	ORGAC	R34/II&III	R35	R34	N/A
40	Octanoic acid (caprylic acid)	ORGAC	R34/II&III	R35	R34/C	C
47	60/40 Octanoic/decanoic acids	ORGAC	R34/II&III	R34	R34/C	C
50	55/45 Octanoic/decanoic acids	ORGAC	R34/II&III	R35	R34	N/A
7	3,3'-Dithiodipropionic acid	ORGAC	NC	NC	NC	N/A
12	Dodecanoic acid (lauric acid)	ORGAC	NC	NC	NC	NC
26	Isotearic acid	ORGAC	NC	NC	NC	NC
34	70/30 Oleine/octanoic acid	ORGAC	NC	NC	NC	N/A
58	10-Undecenoic acid	ORGAC	NC	NC	R34	N/A
2	1,2-Diaminopropane	ORGBA	R35/I	R35	R34/C	C
15	Dimethyldipropylenetriamine	ORGBA	R35/I	R35	R34/C	C
38	Tallow amine	ORGBA	R35/II	2R34/2NC/2NQ	NC	N/A
55	1-(2-Aminoethyl)piperazine	ORGBA	R34/II	R35	NC	N/A
13	3-Methoxypropylamine	ORGBA	R34/II&III	R35	R34	N/A
17	Dimethylisopropylamine	ORGBA	R34/II&III	R35	R34/C	C
45	n-Heptylamine	ORGBA	R34/II&III	R35	NC	C
10	2,4-Xylidine (2,4-Dimethylaniline)	ORGBA	NC	R34	R34	N/A
35	Hydrogenated tallow amine	ORGBA	NC	NC	NC	NC
59	4-Amino-1,2,4-triazole	ORGBA	NC	NC	NC	NC
8	Isopropanol	NORG	NC	NC	NC	N/A
11	2-Phenylethanol	NORG	NC	NC	NC	N/A
16	Methyl trimethylacetate (referred to as Methyl 2,2-dimethylpropanoate in EpiDerm (EPI-200))	NORG	NC	NC	NC	C
19	Tetrachloroethylene	NORG	NC	NC	NC	NC
22	n-Butyl propionate	NORG	NC	NC	NC	N/A
27	Methyl palmitate	NORG	NC	NC	NC	N/A
44	Benzyl acetone	NORG	NC	NC	NC	NC
51	Methyl laurate	NORG	NC	NC	NC	N/A
56	1,9-Decadiene	NORG	NC	NC	NC	NC
3	Carvacrol	PHEN	R34/II&III	R34	R34	N/A
23	2-tert-Butylphenol	PHEN	R34/II&III	R35	R34/C	C
9	<i>o</i> -Methoxyphenol (Guaiacol)	PHEN	NC	NC	R34	N/A
30	4,4-Methylene-bis-(2,6-di-tert-butylphenol)	PHEN	NC	NC	NC	N/A

Table 3.6 (continued)

No. ^a	Chemical	Type	In Vivo	Rat Skin TER	EPISKIN TM b	EpiDerm TM (EPI-200)
49	Eugenol	PHEN	NC	NC	NC	NC
4	Boron trifluoride dihydrate	INORGAC	R35/I	R35	R35/C	C
28	Phosphorus tribromide	INORGAC	R35/I	R35	R35/C	C
32	Phosphorus pentachloride	INORGAC	R35/I	R35	R34	N/A
25	Sulfuric acid (10% wt.)	INORGAC	R34/II&III	R34	R34	N/A
57	Phosphoric acid	INORGAC	R34/II	R35	R34	N/A
43	Hydrochloric acid (14.4% wt)	INORGAC	R34/II&III	R35	R34	N/A
53	Sulfamic acid	INORGAC	NC	R34	R34/C	C
18	Potassium hydroxide (10% aq.)	INORGBA	R34/II&III	R35	R34/C	C
42	2-Mercaptoethanol, Na salt (45% aq.)	INORGBA	R34/II&III	R35	NC	N/A
21	Potassium hydroxide (5% aq.)	INORGBA	NC	R35	R34	N/A
24	Sodium carbonate (50% aq.)	INORGBA	NC	R34	NC	NC
20	Ferric [iron (III)] chloride	INORGSAL	R34/II	R35	R34	N/A
52	Sodium bicarbonate	INORGSAL	NC	R34	NC	N/A
54	Sodium bisulfite	INORGSAL	NC	3R34/3NC	NC	N/A
5	Methacrolein	ELECTRO	R34/II&III	NC	R34/C	NC
14	Allyl bromide	ELECTRO	R34/II&III	R35	R34	N/A
48	Glycol bromoacetate (85%)	ELECTRO	R34/II&III	NC	R34/C	C
6	Phenethyl bromide	ELECTRO	NC	NC	NC	N/A
31	2-Bromobutane	ELECTRO	NC	3R34/3R35	NC	N/A
33	4-(Methylthio)-benzaldehyde	ELECTRO	NC	NC	NC	N/A
39	2-Ethoxyethyl methacrylate	ELECTRO	NC	NC	NC	N/A
46	Cinnamaldehyde	ELECTRO	NC	NC	NC	N/A
37	Sodium undecylenate (33% aq.)	SOAP	NC	R35	R34	N/A
41	20/80 Coconut/palm soap	SOAP	NC	NC	NC	N/A
60	Sodium lauryl sulfate (20% aq.)	SOAP	NC	R35	NC	NC

Definitions are as follows: C = Corrosive; NC = Noncorrosive; R34 is equivalent to packing groups II and/or III; R35 is equivalent of packing group I, except for tallow amine (R35/II); NQ = Non-qualified; N/A = Not applicable because not tested; ORGAC = Organic acid; ORGBA = Organic base; NORG = Neutral organics; PHEN = phenol; INORGAC = Inorganic acid; INORGBA = Inorganic base; INORGSAL = Inorganic salt; ELECTRO = Electrophile; SOAP = Soap surfactant

Overall corrosivity classifications were determined by the majority of the reported results obtained from each assay. If results do not show a majority, a definitive classification could not be determined.

^a Number assigned each chemical by the ECVAM Management Team.

^b For EPISKIN, prediction model B was the more complex prediction model and was the only model considered in detail by the ECVAM Management Team (Fentem et al., 1998).

SUMMARY CONCLUSIONS AND RECOMMENDATIONS

ECVAM concluded that EpiDerm (EPI-200) was an *in vitro* replacement assay for *in vivo* corrosivity testing. Although there were differences for some chemicals in calls between experiments within and between laboratories, ECVAM concluded that EpiDerm (EPI-200) was both reliable and reproducible; NICEATM concurs with that conclusion.

The two major questions to be addressed for *in vitro* corrosivity assays are:

1. Has the assay been evaluated sufficiently and is its performance satisfactory to support the proposed use for assessing the corrosivity potential of chemicals and chemical mixtures?
2. Does the assay adequately consider and incorporate, where scientifically feasible, the 3Rs of animal use (refinement, reduction, and replacement alternatives)? Does the assay offer advantages with respect to animal welfare considerations?

In response to the first question, the performance characteristics of the EpiDerm (EPI-200) method indicates, in specific testing circumstances, that this test may be considered useful as part of an integrated testing strategy for assessing the dermal corrosion potential of chemicals.

In response to the second question, EpiDerm (EPI-200) sufficiently considers and incorporates the 3Rs. Specifically, the use of EpiDerm (EPI-200) offers advantages with respect to animal welfare considerations, including animal use refinement, reduction, and replacement. Similarly, the use of the EpiDerm (EPI-

200) assay as part of an integrated approach reduces and refines the use of animals by providing a basis for decisions on further testing. When these methods are used as part of an integrated testing strategy for corrosivity, there is a reduction in the number of animals required because positive results typically eliminate the need for animal testing, and when further testing in animals is determined to be necessary, only one animal is required to confirm a corrosive chemical. Follow-up testing using *in vivo* methods, when deemed necessary, could also employ test agent dilution schemes to minimize possible pain in any individual animal.

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