

**Summary Report of the  
Rat Skin Transcutaneous Electrical Resistance (TER)  
*In Vitro* Assay  
for Assessing Dermal Corrosivity**

Prepared for

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## PURPOSE

This report focuses on the performance of the Rat Skin Transcutaneous Electrical Resistance (TER) assay to determine the usefulness and limitations of the assay for the identification of potential human corrosive chemicals. This report also discusses how Rat Skin TER compares to the *in vivo* rabbit skin corrosivity test and to other *in vitro* corrosivity tests (EPISKIN, EpiDerm [EPI-200], and Corrositex). The data and assessments reviewed included an inter-laboratory trial (Botham et al., 1992), a prevalidation study (Botham et al., 1995), and a validation study (Barratt et al., 1998; Fentem et al., 1998). Additionally, an independent analysis of the Rat Skin TER performance data, taking into account the totality of the database, was conducted.

## EVALUATION OF REGULATORY AND SCIENTIFIC RATIONALE

The Rat Skin TER assay has been in use for over five years (Botham et al., 1995). This assay is one of several *in vitro* corrosivity assays evaluated as alternatives to the *in vivo* rabbit corrosivity test by the European Centre for the Validation of Alternative Methods (ECVAM) in a formal validation study (Fentem et al., 1998).

The assay has been approved by the ECVAM Scientific Advisory Committee for use in corrosivity testing in Europe (Balls and Corcelle, 1998) and has also been evaluated and accepted for its intended use by the European Scientific Committee for Cosmetic Products and Non-food Products (SCCNFP) (SCCNFP, 1999). This method has been adopted for regulatory use within the European Union (EU) by the European Commission (EU, 2000).

## EVALUATION OF THE TEST METHOD

In the Rat Skin TER assay, test materials (liquids: 150  $\mu$ L; solids 100 mg plus 150  $\mu$ L of water) are applied for 2 and 24 hours to the epidermal surfaces of skin discs obtained from the skin of humanely killed young rats. Nine to 15 discs can be prepared from one rat pelt. Pelts must give a resistance value greater than 10 k to be acceptable for use in the test. To test each chemical, three skin discs are used per time period, in addition to a concurrent positive and negative control. Corrosive materials are identified by the ability of the chemical to produce a loss of normal stratum corneum integrity and barrier function, which is measured as a reduction of the inherent transcutaneous electrical resistance below a predetermined threshold level of 5 k. The validation protocol developed by ECVAM included a dye-binding assay, which is used to reduce the number of false positives encountered in the prevalidation study for surfactants and solvents. The scientific and mechanistic basis of the test and the rationale for using a 5 k criterion for identifying potential human corrosivity were not discussed by Botham et al. (1995) or Fentem et al. (1998).

## EVALUATION OF TEST METHOD DATA QUALITY

The Rat Skin TER assay was evaluated in three studies: an inter-laboratory trial (Botham et al., 1992), a prevalidation study (Botham et al., 1995), and an ECVAM validation study (Fentem et al., 1998). The inter-laboratory trial was based on an evaluation of 20 chemicals (6 corrosives/14 noncorrosives), while the prevalidation and ECVAM validation studies evaluated 50 chemicals (25C/25NC) and 60 chemicals (27C/33NC), respectively. The main

criterion for including chemicals in the study was that their corrosivity classification was based on unequivocal animal data (Barratt et al., 1998). The ECVAM validation chemical test set included organic acids (6C/5NC), organic bases (7C/3NC), neutral organics (9NC), phenols (2C/3NC), inorganic acids (6C/1 NC), inorganic bases (2C/2NC), inorganic salts (1C/2NC), electrophiles (3C/5NC), and soaps/surfactants (3NC). Despite the small numbers of chemicals in some categories, ECVAM concluded that the set of test chemicals used in the validation study represented the best possible group for evaluating the performance characteristics of the *in vitro* assays, given the limited availability of unequivocal animal data (Barratt et al., 1998).

In the validation study, each chemical was tested twice in each of three different laboratories. The tests were stated to have been conducted in the "spirit" of GLP (Fentem et al., 1998). 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 on at least three separate occasions.

#### 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 Rat Skin TER assay against the corresponding *in vivo* rabbit corrosivity data. The database used in the Rat Skin TER evaluation consisted of data from three published sources (Botham et al., 1992; Botham et al., 1995; Fentem et al., 1998).

For ease of comparison, chemicals evaluated in the Rat Skin TER 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.

The results of the overall performance analysis for the Rat Skin TER assay are presented in **Table 4.1**. Based on a database of 122 chemical and chemical mixtures, this assay had an accuracy of 81% (99/122 chemicals or chemical mixtures), a sensitivity of 94% (51/54 chemicals or chemical mixtures), a specificity of 71% (48/68 chemicals or chemical mixtures), a false positive rate of 29% (20/68), and a false negative rate of 6% (3/54). These performance characteristics were not different when the Botham et al. (1992 and 1995) studies were evaluated independently of the ECVAM validation study (Fentem et al., 1998) (**Tables 4.2** and **4.3**, respectively). The performance characteristics for the Rat Skin TER assay remained consistent when evaluated against various chemicals classes, including organic and inorganic acids and bases, organic and inorganic bases and base mixtures, organic and inorganic acids and acid mixtures. Based on the validation study results, which met pre-study acceptance criteria of no more than 20% false negatives and 20% false positives, the ECVAM concluded that the Rat Skin TER assay 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 types studied (Fentem et al., 1998; Balls and Corcelle, 1998). ECVAM concluded also that the Rat Skin TER assay

was not capable of classifying chemicals or chemical mixtures by packing group (i.e., it could not distinguish between known R35/I and R34/II & III chemicals). However, it was stated that taking into account the relative simplicity of the mechanism of action of corrosives, this method would be generally applicable across all chemical classes (Fentem et al. 1998).

#### **EVALUATION OF TEST METHOD RELIABILITY (REPEATABILITY/REPRODUCIBILITY)**

The Rat Skin TER assay has been evaluated for repeatability and/or reproducibility in three different studies. In the Botham et al. (1992) inter-laboratory trial, no statistically significant level of inter-laboratory variability was found for corrosives (6 compounds), noncorrosives (14 compounds), or for all test materials (20 compounds); variability among the three independent laboratories was assessed using ANOVA. An intra-laboratory analysis was not possible. In the prevalidation study (Botham et al., 1995), the agreement for the classifications obtained by both participating laboratories was 92% (23 of 25 C and 23 of 25 NC chemicals).

In the ECVAM validation study (Fentem et al., 1998), the 60 chemicals were each tested twice by each of three laboratories. Intra- and inter-laboratory reliability was evaluated 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 their analyses, ECVAM concluded that inter- and intra-laboratory variability was approximately equivalent, with no evidence

of systematic differences between experiments within a laboratory. Of the 60 chemicals tested, 37 gave the same corrosivity classification in both experiments in all three laboratories. For ten of the remaining 23 chemicals, only one experiment resulted in a classification differing from the other 5 predictions. Although there were differences for some chemicals in calls between experiments within and between laboratories, ECVAM concluded that the Rat Skin TER assay was reliable and reproducible. Due to the lack of quantitative data for individual chemicals in the published studies, no independent evaluation of repeatability or reproducibility for the Rat Skin TER assay could be conducted. However, 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.

**Table 4.1 Performance of the Rat Skin TER Assay in Predicting Corrosivity/Noncorrosivity Compared to *In Vivo* Findings (Overall)<sup>1</sup>**

Chemical or Product Class	Number of Chemicals	Accuracy		Sensitivity		Specificity	
		%	Number	%	Number	%	Number
<b>Overall</b>	122	81	(99/122)	94	(51/54)	71	(48/68)
<b>Organic and Inorganic Acids and Bases<sup>2</sup></b>	64	91	(58/64)	98	(44/45)	74	(14/19)
<b>Organic and Inorganic Bases and Base Mixtures<sup>3</sup></b>	27	93	(25/27)	100	(20/20)	71	(5/7)
<b>Organic and Inorganic Acids and Acid Mixtures</b>	31	94	(29/31)	100	(20/20)	82	(9/11)
<b>Amines</b>	21	95	(20/21)	100	(15/15)	83	(5/6)
<b>Inorganic Bases and Base Mixtures</b>	6	83	(5/6)	100	(5/5)	0	(0/1)
<b>Acid Derivatives</b>	6	67	(4/6)	80	(4/5)	0	(0/1)
<b>Surfactants</b>	21	62	(13/21)	100	(4/4)	53	(9/17)
<b>Industrial Chemicals</b>	26	73	(19/26)	50	(1/2)	75	(18/24)
<b>Cleaners and Detergents</b>	7	86	(6/7)	100	(2/2)	80	(4/5)

<sup>1</sup>This analysis contains data from Fentem et al. (1998), Botham et al. (1995), and Botham et al. (1992).

<sup>2</sup>This chemical class includes chemicals from the following chemical classes: organic and inorganic bases and base mixtures, organic and inorganic acids and acid mixture, and acid derivatives.

<sup>3</sup>This chemical class includes amines, inorganic bases, and base mixtures.

Table 4.2 Performance of the Rat Skin TER Assay in Predicting Corrosivity/Noncorrosivity Compared to *In Vivo* Findings (Fentem et al., 1998)

Chemical or Product Class	Number of Chemicals	Accuracy		Sensitivity		Specificity	
		%	Number	%	Number	%	Number
<b>Overall</b>	58	81	(47/58)	93	(25/27)	71	(22/31)
<b>Organic and Inorganic Acids and Bases<sup>1</sup></b>	39	85	(33/39)	96	(24/25)	64	(9/14)
<b>Organic and Inorganic Bases and Base Mixtures<sup>2</sup></b>	13	85	(11/13)	100	(9/9)	50	(2/4)
<b>Organic and Inorganic Acids and Acid Mixtures</b>	20	90	(18/20)	100	(11/11)	78	(7/9)
<b>Amines</b>	9	89	(8/9)	100	(6/6)	67	(2/3)
<b>Inorganic Bases and Base Mixtures</b>	4	75	(3/4)	100	(3/3)	0	(0/1)
<b>Acid Derivatives</b>	6	67	(4/6)	80	(4/5)	0	(0/1)
<b>Surfactants</b>	5	60	(3/5)	NA	(0/0)	60	(3/5)
<b>Industrial Chemicals</b>	10	80	(8/10)	100	(1/1)	78	(7/9)
<b>Cleaners and Detergents</b>	1	100	(1/1)	NA	(0/0)	100	(1/1)

NA = Not applicable

<sup>1</sup>This chemical class includes chemicals from the following chemical classes: organic and inorganic bases and base mixtures, organic and inorganic acids and acid mixture, and acid derivatives.

<sup>2</sup>This chemical class includes amines, inorganic bases, and base mixtures.

Table 4.3 Performance of the Rat Skin TER Assay in Predicting Corrosivity/Noncorrosivity Compared to *In Vivo* Findings (Botham et al., 1992; 1995)

Chemical or Product Class	Number of Chemicals	Accuracy		Sensitivity		Specificity	
		%	Number	%	Number	%	Number
<b>Overall</b>	65	82	(53/65)	96	(27/28)	70	(26/37)
<b>Organic and Inorganic Acids and Bases<sup>1</sup></b>	26	100	(26/26)	100	(21/21)	100	(5/5)
<b>Organic and Inorganic Bases and Base Mixtures<sup>2</sup></b>	14	100	(14/14)	100	(11/11)	100	(3/3)
<b>Organic and Inorganic Acids and Acid Mixtures</b>	12	100	(12/12)	100	(10/10)	100	(2/2)
<b>Amines</b>	12	100	(12/12)	100	(9/9)	100	(3/3)
<b>Inorganic Bases and Base Mixtures</b>	2	100	(2/2)	100	(2/2)	NA	(0/0)
<b>Acid Derivatives</b>	0	NA	(0/0)	NA	(0/0)	NA	(0/0)
<b>Surfactants</b>	16	63	(10/16)	100	(4/4)	50	(6/12)
<b>Industrial Chemicals</b>	16	69	(11/16)	0	(0/1)	73	(11/15)
<b>Cleaners and Detergents</b>	6	83	(5/6)	100	(2/2)	75	(3/4)

NA = Not applicable

<sup>1</sup>This chemical class includes chemicals from the following chemical classes: organic and inorganic bases and base mixtures, organic and inorganic acids and acid mixture, and acid derivatives.

<sup>2</sup>This chemical class includes amines, inorganic bases, and base mixtures.

## OTHER SCIENTIFIC REVIEWS

In March 1999, a search of the open literature was conducted to locate additional Rat Skin TER studies. Six databases (Medline, Toxline, Embase, Biosis, Caba, and LifeSci) were searched using the key terms "Transcutaneous" within one word of "electrical" within one word of "resistance"; and "TER" and "rat" or "rats". The search found no additional relevant studies conducted with this assay. In May 2001, another search was performed to locate additional TER studies. Four databases (PubMed, Web of Science, Toxline, and Current Contents Connect) were searched using the same search strategy and no additional relevant studies were found.

## OTHER CONSIDERATIONS

The cost for conducting the Rat Skin TER assay is reported by Syngenta Corporation (e-mail communication from Phil Botham, Syngenta CTL) to be approximately \$500-800 per test. When compared to other *in vitro* methods (EPISKIN , EpiDerm (EPI-200), and Corrositex ), the cost and the time necessary to conduct the Rat Skin TER assay are greater (**Table 4.4**). Additionally, TER requires the use of animals, whereas EPISKIN and Corrositex do not.

## RELATED ISSUES

### Refinement, Reduction, and Replacement

The Rat Skin TER assay does not eliminate the use of animals. However, if used in an integrated approach, TER provides for the reduction and refinement of animal use.

## Comparison to Other *In Vitro* Assays

General comparative information on the Rat Skin TER, EPISKIN , EpiDerm (EPI-200), and Corrositex assays is provided in **Tables 4.4** through **4.7**.



**Table 4.4 General Comparison of the Rat Skin TER, EPISKIN™, EpiDerm™ (EPI-200), and Corrositex® Assays**

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

<sup>a</sup> 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.

<sup>b</sup> 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 4.5 General Comparison of the Rat Skin TER Assay, EPISKIN™, EpiDerm™ (EPI-200), and Corrositex® Assays Based on a Weight-of-Evidence Approach<sup>a</sup> 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®
<b>Number of Chemicals</b>	122	60	24	163
<b>Overall Sensitivity<sup>b</sup></b>	94% (51/54)	82% (23/28)	92% (11/12)	85% (76/89)
<b>Overall Specificity<sup>b</sup></b>	71% (48/68)	84% (27/32)	83% (10/12)	70% (52/74)
<b>Overall Accuracy<sup>b</sup></b>	81% (99/122)	83% (50/60)	92% (22/24)	79% (128/163)
<b>False Positive Rate</b>	29% (20/68)	16% (5/32)	17% (2/12)	30% (22/74)
<b>False Negative Rate</b>	6% (3/54)	18% (5/28)	8% (1/12)	15% (13/89)
<b>Test Chemical Inter-laboratory Coefficient of Variation</b>	34.7 <sup>c</sup>	11.3 <sup>c</sup>	12.3 <sup>c</sup>	30.3 <sup>c</sup>
	3.8-322 <sup>d</sup>	3.9-148.8 <sup>d</sup>	0.9-51.2 <sup>d</sup>	7.7-252.5 <sup>d</sup>
	120 <sup>e</sup>	20 <sup>e</sup>	144 <sup>e</sup>	180 <sup>e</sup>

<sup>a</sup> 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.

<sup>b</sup> 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.

<sup>c</sup> Median value

<sup>d</sup> Range of values

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

**Table 4.6 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)**

	Rat Skin TER Assay	EPISKIN™ (prediction model B)	EpiDerm™ (EPI-200) (prediction)
<b>Number of Chemicals Tested in ECVAM Validation Study</b>	60 (Fentem et al., 1998)	60/24 <sup>a</sup> (Fentem et al., 1998)	24 (Liebsch et al., 2000)
<b>Sensitivity<sup>b</sup></b>	88% (140/159)	83% (201/243) / 88% (87/99)	88% (63/72)
<b>Specificity<sup>b</sup></b>	72% (142/196)	80% (237/297) / 79% (92/117)	86% (62/72)
<b>Accuracy<sup>b</sup></b>	79% (282/355) <sup>c</sup>	81% (438/540) / 83% (179/216)	87% (125/144)
<b>False Positive Rate<sup>b</sup></b>	28% (54/196)	20% (60/297) / 21% (25/117)	14% (10/72)
<b>False Negative Rate<sup>b</sup></b>	12% (19/159)	17% (42/243) / 12% (12/99)	13% (9/72)
<b>Number of Trials<sup>d</sup></b>	355	540 / 216	144
<b>Test Chemical Inter-laboratory Coefficient of Variation</b>	34.7 <sup>d</sup> 10-322 <sup>e</sup> 360 <sup>f</sup>	30.2 <sup>d</sup> 7.7-252.5 <sup>e</sup> 540 <sup>f</sup>	12.3 <sup>d</sup> 0.9-51. <sup>e</sup> 144 <sup>f</sup>

<sup>a</sup> 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).

<sup>b</sup> 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.

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

<sup>d</sup> Median value

<sup>e</sup> Range of values

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

**Table 4.7 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. <sup>a</sup>	Chemical	Type	<i>In Vivo</i>	Rat Skin TER	EPISKIN™ <sup>b</sup>	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 )	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
49	Eugenol	PHEN	NC	NC	NC	NC
4	Boron trifluoride dihydrate	INORGAC	R35/I	R35	R35/C	C

Table 4.7 (continued)

No. <sup>a</sup>	Chemical	Type	In Vivo	Rat Skin TER	EPISKIN™ <sup>b</sup>	EpiDerm™ (EPI-200)
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

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.

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

<sup>a</sup> Number assigned each chemical by the ECVAM Management Team.

<sup>b</sup> 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 the Rat Skin TER assay was an *in vitro* replacement assay for *in vivo* corrosivity testing (Fentem et al., 1998). NICEATM concurs with the ECVAM conclusion that the Rat Skin TER assay is both reliable and reproducible. For some chemical or product classes (e.g., cleaners and detergents), the small number of chemicals and/or the unbalanced distribution of corrosive and noncorrosive chemicals does not allow accurate conclusions to be made on the performance of this assay for these chemical classes.

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 Rat Skin TER assay 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, the Rat Skin TER assay sufficiently considers and

incorporates the 3Rs. The assay offers animal welfare advantages, including animal use refinement and reduction; this method reduces the number of animals used as skin from one humanely killed rat may be used to test up to five chemicals. Similarly, the use of the Rat Skin TER assay as part of an integrated approach reduces and refines the use of animals by providing a basis for decisions on further testing. Follow-up testing using *in vivo* methods, when deemed necessary, could employ fewer animals and test agent dilution schemes to minimize possible pain in any individual animal.