5.2 SKIN CORROSION STUDIES

5.2.1 RESULTS OBTAINED WITH THE SKINETHIC MODEL

As described in the Introduction (section 3.2.2), the SkinEthic skin corrosion study had two main objectives:

1. to screen performance of the SkinEthic model in the *in vitro* skin corrosion test using the validated EpiDerm skin corrosion test protocol (Phase I).
2. to evaluate the performance of the SkinEthic skin corrosion test between laboratories with a set of reference chemicals applying the requirements and criteria of the OECD TG 431 (Phase II).

5.2.1.1 Phase I

For the evaluation of the predictive ability of the SkinEthic model in the *in vitro* skin corrosion test, twenty non-coded chemicals, covering diverse chemical groups were evaluated. In the first experiments, problems with spreading of hydrophilic substances on the tissue surface of the SkinEthic model occurred, which caused frequent underprediction. Test substances collected at the edge of insert in form of drop and thus only minor part of the tissue was exposed to the test chemical. Subsequently, in the MTT viability assay, no relevant result could be obtained. A modification of the dosing procedure using nylon mesh as a spreading tool helped to solve this problem (for details see 4.2.2.1*)*.

The experimental outcome of Phase I is summarised in Table 23. The results obtained with SkinEthic reconstructed human skin model were in concordance with the *in vivo* classification, and with results obtained previously in the ECVAM skin corrosion validation studies with the EpiDerm and EPISKIN models (Fentem *et al*., 1998; Liebsch *et al*., 2000). Only one chemical (10 % sulphuric acid) was not classified in concordance with classification given in the OECD TG 431. However, the classification given in Annex I of the Directive 67/548/EEC (EU COM, 1967; ECB, 2005) differs, and is corresponding to the result obtained with SkinEthic model. The result, which would be in agreement with classification of the OECD TG 431, was achievable only after a 4-h exposure (see Table 23).

In summary, the performance of the model in phase I was good, and the assay was prepared for evaluation in an inter-laboratory study. To assure high formality, phase II was performed as a blind trial. Before entering phase II, detailed standard operation procedure (SOP) and spreadsheets were distributed, and participating laboratories - BASF (Ludwigshafen, Germany) and SAFEPHARM (Derby, UK) - were trained for all procedures.

Table 23. Results obtained in Phase I.

No	Chemical name	*In vivo* class (C/NC)	No of tests	Mean 3 min	Mean 1 hour	Mean 4 h	SkinEthic prediction
1	SLS (20 % aq)	NC	1	99.3	92.6	-	NC
2	Boron trifluoride dihydrate	C	1	11.4	6.0	-	C
3	Benzylacetone	NC	1	102.3	77.6	-	NC
4	H_2SO_4 (10 % wt.)	C	3	92.5	62.9	-	NC
				103.3	86.7	5.7	C
				94.7	61.4	3.7	C
5	HCl (14.4 % wt.)	C	4	99	99		NC
				86.4	4.4	-	C
				88.3	5.4	-	C
				92.5	5.3	5.4	C
6	n-Heptylamine	C	1	66.1	102.3	-	NC
	n-Heptylamine (results after correction with "killed controls)	C	1	36.0	1.0	-	C
7	2,4-Xylidine	NC	2	78.3	14.4	-	C
				82.1	10.2	-	C
8	Tetrachloroethylene	NC	3	89.7	16.3	-	NC
				92.7	14.7	-	C
				101.4	25.6	-	NC
9	Octanoic acid (caprylic acid)	C	1	24.8	6.5	-	C
10	55/45 Octanoic/decanoic acid	C	1	38.4	10.1	-	C
11	4-Amino-1,2,4-triazole	NC	2	104.1	106.8	-	NC
				94.9	103.2	-	NC
12	2-tert-Buthylphenol	C	1	26.4	21.3	-	C
13	1,2- Diaminopropane	C	1	16.0	15.3	-	C
14	Phenethyl bromide	NC	1	122.7	100.8	-	NC
15	4-(Methylthio)-benzaldehyde	NC	1	108.2	112.4	-	NC
16	Potassium hydroxide 10% aq.	C	1	79.8	8.9	-	C
17	Isostearic acid	NC	1	100.0	94.4	-	NC
18	Acrylic acid	C	1	3.7	3.6	-	C
19	1,6 - Dibromhexane	NC	1	121.7	121.7	-	NC
20	8 N KOH	C	4	78.3	3.3	-	C
				86.3	4.9	-	C
				12.6	4.3	-	C
				75.0	5.7	-	C

C - corrosive; NC - non corrosive; S - solid; L - liquid

5.2.1.2 Phase II

Assay prediction

In phase II, three independent test runs of 12 samples (using three tissues per time-point and chemical) were performed in each of three laboratories. 10 % sulphuric acid was excluded from coding for reasons mentioned in paragraph 4.1.2.1.

According to the prediction model and classification given in OECD TG 431, in the group of 6 corrosive chemicals, misclassification for chemical #6 (10 % sulphuric acid) was obtained in all three laboratories. In the group of 6 non-corrosive substances, an over-prediction was observed in 3 out of 9 tests for chemical # 10 (tetrachloroethylene). For this chemical, viability values close to the classification cut-off were obtained. All results from phase II are summarised in Tables 24 and 25.

Table 24. Results obtained in Phase II.

No.	Chemical name	In vivo class	Run	BASF					SAFEPHARM					ZEBET				
				3 min		1 h		Classification	3 min		1 h		Classification	3 min		1 h		Classific
				Mean %	SD	Mean %	SD	C/NC	Mean %	SD	Mean %	SD	C/NC	Mean %	SD	Mean %	SD	C/NC
1	1,2-Diaminopropane	C	1	10.8	3.3	3.3	0.4	C	10.0	0.2	3.5	0.3	C	11.5	0.4	15.4	1.0	C
			2	18.8	4.2	9.3	1.0	C	9.3	0.4	6.4	0.8	C	−1.0	0.1	10.9	1.5	C
			3	20.4	6.2	6.7	0.5	C	8.5	0.6	5.3	0.3	C	21.4	0.7	14.2	1.0	C
2	Acrylic acid	C	1	3.3	0.4	1.8	0.2	C	3.1	0.2	3.0	0.1	C	2.6	0.6	2.4	0.3	C
			2	2.4	0.9	1.9	0.2	C	2.9	0.2	2.8	0.3	C	−1.1	0.1	2.8	0.3	C
			3	2.0	0.5	1.2	0.2	C	3.1	0.3	2.6	0.1	C	4.4	0.9	2.9	0.5	C
3	2-tert-Butylphenol	C	1	17.5	0.5	10.1	0.7	C	17.0	0.7	12.7	0.9	C	24.1	2.3	20.2	0.4	C
			2	14.8	1.7	12.3	0.5	C	13.9	1.1	10.8	0.8	C	27.6	0.8	14.2	1.8	C
			3	20.2	2.1	10.9	0.8	C	11.6	1.2	9.2	0.8	C	17.8	0.3	15.8	0.9	C
4	Potassium hydroxide (10% aq)	C	1	112.6	13.7	4.8	0.3	C	73.9	16.5	4.6	0.6	C	80.9	8.0	6.0	2.1	C
			2	96.3	2.8	3.0	0.1	C	93.3	7.3	3.4	0.2	C	38.7	8.9	4.6	0.5	C
			3	91.7	5.5	5.4	2.7	C	86.5	7.8	4.9	0.8	C	80.2	9.7	6.9	0.1	C
5	Octanoic acid	C	1	16.9	4.6	3.3	1.7	C	53.9	4.5	2.0	0.3	C	41.3	13.0	1.8	0.6	C
			2	46.3	3.8	2.6	0.3	C	73.9	14.7	2.5	1.0	C	35.4	7.1	1.6	0.3	C
			3	20.5	3.4	2.2	0.2	C	63.1	6.4	2.7	0.7	C	24.2	3.9	3.4	0.8	C
7	4-Amino-1,2,4-triazole	NC	1	112.7	3.7	108.7	9.2	NC	85.0	17.1	102.6	18.9	NC	96.5	3.6	103.5	2.8	NC
			2	103.7	1.1	101.5	6.9	NC	106.9	7.6	73.6	10.0	NC	102.1	2.4	95.9	5.2	NC
			3	93.7	8.5	95.1	3.9	NC	92.8	8.7	104.8	13.7	NC	104.6	2.1	98.7	1.5	NC
8	Eugenol	NC	1	113.8	17.4	32.6	6.9	NC	107.2	12.1	32.4	3.7	NC	64.8	1.2	36.2	3.2	NC
			2	83.8	2.8	30.5	2.0	NC	107.2	24.2	28.4	4.7	NC	60.1	0.8	43.8	3.1	NC
			3	79.8	4.5	24.2	1.7	NC	107.5	7.7	24.1	2.3	NC	88.2	7.6	37.4	6.9	NC
9	Phenethyl bromide	NC	1	139.4	6.4	76.0	5.5	NC	111.4	4.2	77.8	4.3	NC	95.7	6.3	93.8	8.4	NC
			2	113.9	14.4	89.6	6.7	NC	117.5	2.7	86.4	3.6	NC	110.7	1.2	90.3	4.3	NC
			3	119.1	4.8	87.0	3.6	NC	103.8	1.0	92.4	3.9	NC	98.6	3.1	98.3	2.1	NC
10	Tetrachloroethylene	NC	1	104.0	11.2	15.1	1.8	NC	94.5	9.7	5.7	1.1	C	105.5	3.4	21.2	3.0	NC
			2	93.7	12.0	11.5	6.1	C	102.3	8.5	11.4	1.9	C	103.8	2.3	30.8	6.3	NC
			3	89.6	2.8	17.3	2.7	NC	108.4	5.6	30.6	2.9	NC	91.0	4.3	48.6	8.4	NC
11	Isostearic acid	NC	1	112.4	1.6	105.6	2.6	NC	99.7	4.0	105.2	3.0	NC	103.9	6.6	104.9	2.5	NC
			2	103.4	4.0	88.4	8.0	NC	96.2	2.2	94.9	1.3	NC	102.8	4.1	98.4	0.3	NC
			3	98.0	2.3	101.7	5.7	NC	107.7	4.3	96.1	3.0	NC	86.8	2.3	100.8	4.3	NC
12	4-(Methylthio)-benzaldehyde	NC	1	122.1	10.7	75.2	5.5	NC	100.2	8.7	82.7	8.4	NC	90.7	8.2	107.7	3.6	NC
			2	100.0	2.2	85.6	5.4	NC	97.5	4.5	88.4	1.2	NC	127.0	0.9	113.8	4.0	NC
			3	97.5	4.1	68.1	4.6	NC	109.5	1.2	93.3	0.7	NC	96.7	2.1	95.5	4.5	NC

C — corrosive; NC — non-corrosive.

Table 25. Results obtained in Phase II for sulphuric acid.

Laboratory	**Run**	**sulphuric acid (10 % wt.)**							
		3 min		**1 hour**		**Class.**	**4 hours**		**Class.**
		mean %	SD	mean %	SD	C/NC	mean %	SD	C/NC
BASF	1	118.2	25.7	35.8	3.9	NC	2.3	0.3	C
	2	103.9	8.4	73.7	4.1	NC	4.9	4.5	C
	3	118.6	12.3	74.5	6.3	NC	11.3	15.4	C
SAFEPHARM	1	107.3	4.0	56.6	47.5	NC	3.7	2.1	C
	2	109.3	21.2	12.6	11.6	C	2.0	0.4	C
	3	102.9	2.8	92.0	6.1	NC	5.5	2.1	C
ZEBET	1	106.0	4.4	114.8	5.6	NC	47.4	6.6	NC
	2	121.7	4.5	101.6	6.1	NC	1.7	0.6	C
	3	102.9	2.8	92.0	6.1	NC	5.5	2.1	C

C-corrosive, NC-non corrosive

2x2 contingency table statistics were used to determine sensitivity, specificity, positive and negative predictive value and accuracy (see Table 26). When compared to *in vivo* classification given in OECD TG 431, high sensitivity (85.2%) as well as high specificity (94.4%) was obtained. The overall accuracy of the method was 93.2%, thus it can be concluded that the SkinEthic model provided excellent predictivity of the corrosive potential for the12 chemicals tested in the skin corrosion assay.

Table 26. 2x2 contingency table statistic for Phase II.

		***In vivo* classification**	
		Corrosive	Non-corrosive
***In vitro* prediction**	Corrosive	46	3
	Non-corrosive	8	51
Statistics for shadowed area of 2x2 table			
Sensitivity:	85.2		
Specificity:	94.4		
Positive predictive value:	93.9		
Negative predictive value:	86.4		
Accuracy:	93.2		
Test set prevalence	1		

Assay variability

The multivariate statistical analysis was performed simultaneously for the endpoint determinations at 3 min and 1 h. The analysis took into account 3 independent factors: “chemicals”, “laboratories”, and “run”, which contribute to the final variability. The results obtained in the GLM multivariate procedure are summarised in Table 27.

Table 27. Results of the analysis of variance for the two endpoint values “3 minutes” and “1 hour” by the factors ‘chemicals’, ‘laboratories’ and ‘run’.

Source	Dependent Variable	Sum of Squares	df[1]	Mean Square[2]	Relative Mean Square (%)	F[3]	Significance
Chemicals	3 minutes	179575.5	13	13813.5	95.2	92.6	0.000000
	1 hour	211638.2	13	16279.9	92.3	155.4	0.000000
Laboratories (inter-laboratory variability)	3 minutes	891.4	2	445.7	3.1	3.0	0.054515
	1 hour	2306.9	2	1153.4	6.5	11.0	0.000044
Run (intra-laboratory variability)	3 minutes	202.3	2	101.2	0.7	0.68	0.509537
	1 hour	203.0	2	101.5	0.6	0.97	0.382687
Error (other variability)	3 minutes	16104.4	108	149.1	1.0		
	1 hour	11312.4	108	104.7	0.6		
Total	3 minutes	891673.6	126	14509.5			
	1 hour	485951.6	126	17639.5			

[1] df= degrees of freedom. [2] The mean square of each effect is calculated by dividing the sum of squares by its degrees of freedom.

[3] F - statistic is calculated by dividing the mean square by the mean square error (12 chemicals plus positive control).

The factor ”chemicals” (for both time-points) and factor “laboratories” (for time-point 1 h), resulted in significance values of less than 0.001. This means that both groups contribute to the variability of the assay. A more straightforward way to recognise the contribution of both factors to the assay variability is to determine the relative mean square values.

The relative mean square value for the factor “laboratories” for both endpoints is small (at 3 min—3.1% and at 1 h—6.5%). This means, that although the results are regarded as “significantly” variable, the contribution of laboratories to the final variability is not high. Intra-laboratory variability represented by the factor “run” is very small (for 3 min—0.7% and for 1 h—0.6%), showing good reproducibility of results within a laboratory.

The relative mean square value for the factor “chemicals” is very large (95.2% for the time-point 3min, 92.3% for time-point 1 h). This was expected, since the results obtained with a few test chemicals range from corrosives to non-corrosives. Also variance of the biological responses of different skin model batches may be expected.

In summary, no significant differences between runs in single laboratories were found, indicating very good intralaboratory performance of the assay (see Figures 28 and 29). The diferences between laboratories were low, and not significant for the endpoint value “3 min” and slightly significant for the endpoint value “1 h”. Therefore, it can be concluded that the assay proved to be sufficiently reliable.

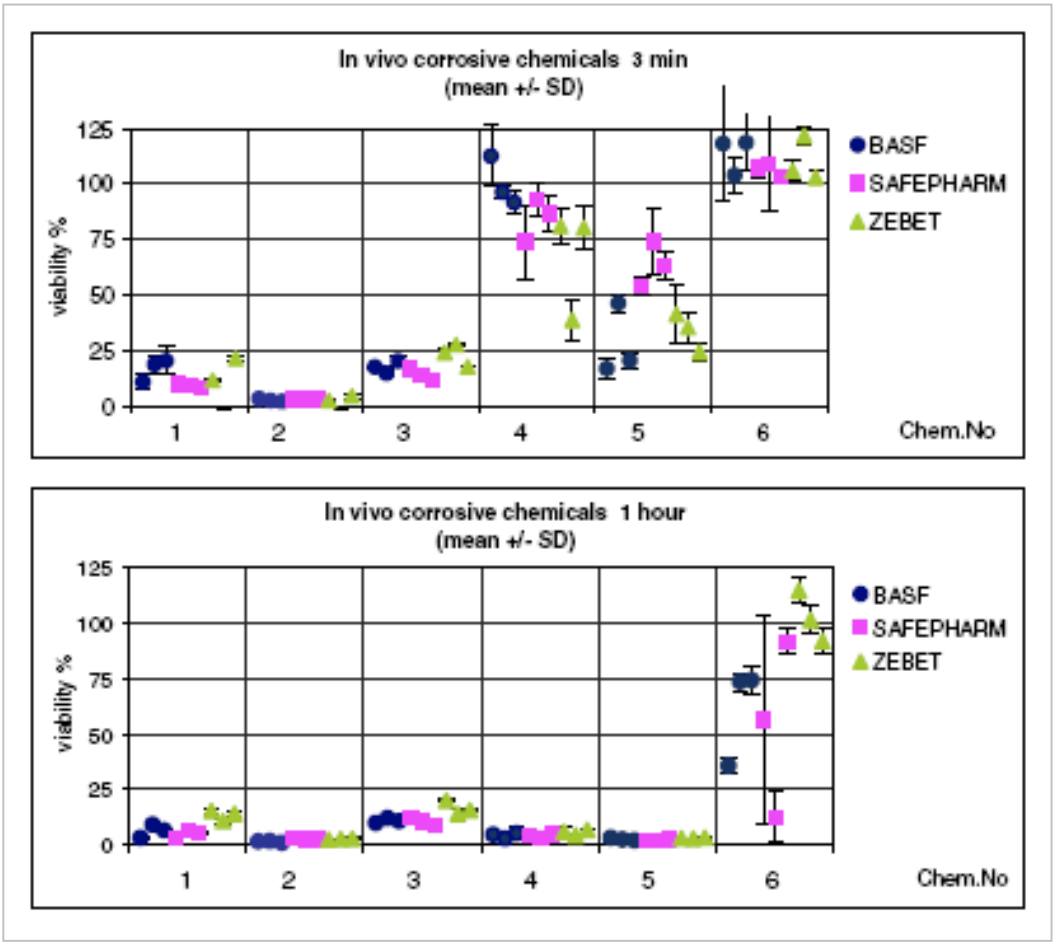

Figure 28. *In vivo* corrosive chemicals: six *in vivo* corrosive chemicals were tested three times in three laboratories. The results are displayed as means of each independent run and standard deviation.

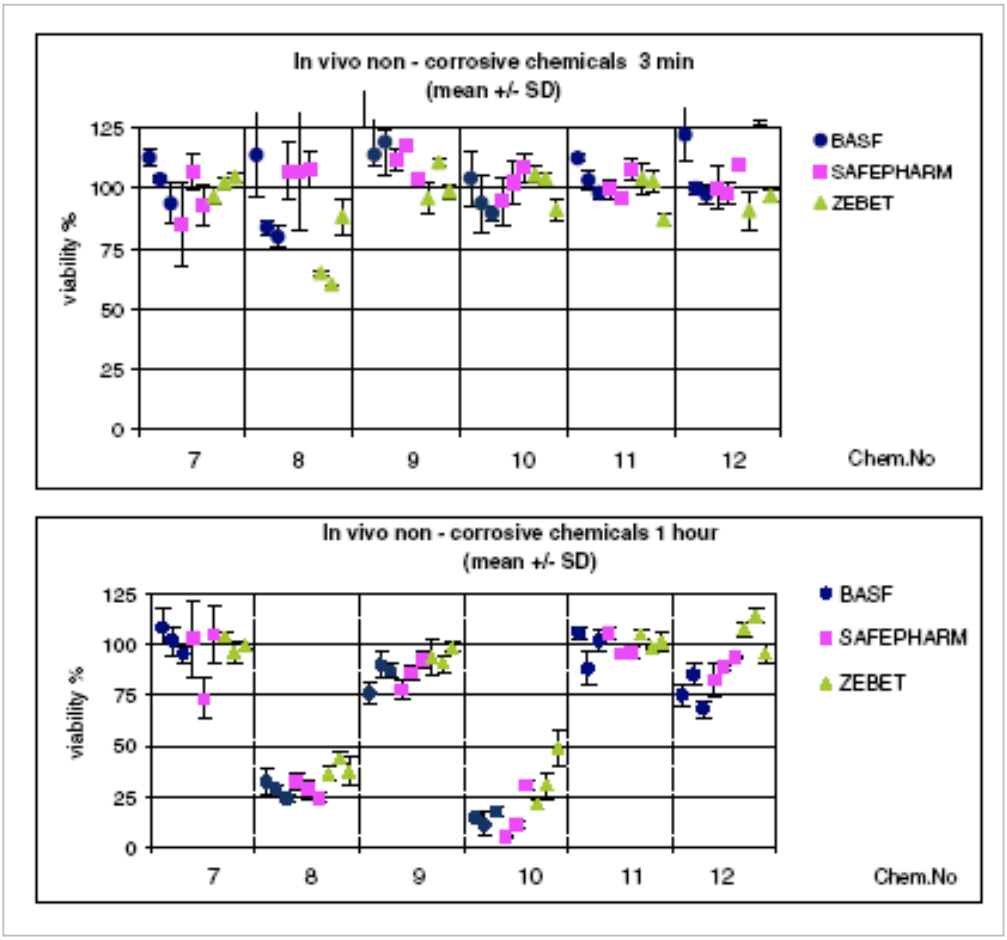

Figure 29. *In vivo* non-corrosive chemicals: six *in vivo* corrosive chemicals were tested three times in three laboratories. The results are displayed as means of each independent run and standard deviation.

5.2.2 DISCUSSION

Method performance in phase I

In phase I, the transferability of the EpiDerm skin corrosion protocol and prediction model to SkinEthic model was proven. Several disagreements in classification were observed at the beginning of the study, these being due to the strong hydrophobic nature of the stratum corneum of the SkinEthic model. This technical problem was overcome by using nylon mesh for spreading all liquid test chemicals.

Among the substances tested in phase I, some had been over-predicted or caused technical problems in previous validation studies (Fentem *et al*., 1998; Liebsch *et al*., 2000), e.g. 2,4-xylidine. This chemical has been consistently overpredicted by all reconstructed skin models in all skin corrosion and irritation studies (Fentem *et al*., 1998; Fentem *et al*., 2001; Liebsch *et al*., 2000; Portes *et al*., 2002; Heylings *et al*., 2003; Kandárová *et al*., 2005; Cotovio *et al*., 2005). The same result was obtained with 2,4-xylidine in the SkinEthic skin corrosion test. This seems to be due to the high toxicity of the substance. In addition, 2,4-xylidine shows

good solubility in lipids and may therefore easily penetrate the stratum corneum and damage the viable layers of reconstructed epidermis.

Tetrachlorethylene has also provided unbalanced results in previous studies. Like 2,4-xylidine, it is highly soluble in lipids. In addition, tetrachloroethylene reacts with plastic inserts and can not easily be removed from the tissue surface during the washing procedure. An over-prediction has been observed with this test chemical in one of three tests in phase I. Similar results have been obtained with the EPISKIN model in the ECVAM skin corrosion validation study (Fentem *et al*., 1998). In one of three testing laboratories, this chemical has been consistently over-predicted.

Minor problems were observed when testing solutions of inorganic acids at low concentrations. At the beginning of the study, several under-predictions for sulphuric acid 10 wt % and one under-prediction for hydrochloric acid 14.4 wt % were obtained. As described earlier, spreading of this substance on the tissue surface caused problems. In addition, due to interaction of these acids with nylon, the mesh could not be used as spreading tool. However, use of a more resistant material than nylon would probably not improve prediction in case of 10 % sulphuric acid.

It is important to note, that disagreement exists in the classification of several solutions of inorganic acids. For instance, hydrochloric acid 14.4 % and sulphuric acid 10 % were classified as "*in vivo* corrosive" in the ECVAM skin corrosion validation study (Fentem *et al*., 1998). However, according to Annex I of the Directive 67/548/ EEC (EU COM, 1967; ECB, 2005) solutions of hydrochloric acid should be classified as "irritating" in the range of 10–25%. Similarly, 5 -15% solutions of sulphuric acid are classified in Annex I only as "R38" (= irritating to the skin) (EU COM, 1967; ECB, 2005).

Method performance in phase II

Predictions of classification

In phase II, clear and correct classifications were generally obtained. Only a few misclassifications or borderline results were observed (for details see Tables 24, 25 and Figures 28 and 29).

To evaluate the observation for 10 % sulphuric acid in phase I, it was decided that the corrosive properties of this chemical should be evaluated by applying the general PM (3min/1 h) with an additional exposure of 4 h (as for the *in vivo* skin corrosion test according to OECD TG 404). For that reason, the chemical was excluded from coding. All data obtained for this chemical are summarised in Table 25. According to the PM (3min/1 h), the chemical would have been classified "non-corrosive" in 8 of 9 cases. This classification is in agreement with

Directive 67/548/EEC and its amendments (EU COM, 1967). However, according to the classification given in OECD TG 431, this chemical should be predicted “corrosive”. This was, however, achievable only after 4-h exposure.

According to the OECD SIDS database *“10 % sulphuric acid appears to be non-irritating nor corrosive to the skin of rabbit, guinea pig and human”* (OECD SIDS, 2001). This statement is based mainly on two studies performed by Nixon *et al*. (1975) and Nixon *et al*. (1990), where effects of 10 % sulphuric acid on intact and abraded skin of rabbits, guinea pigs and humans were evaluated. The exposure time in these experiments was 4 h and effects were scored after 4, 24 and 48 h. Negligible or slight irritation responses were obtained. Similar results were obtained with low concentrations of some other acids (10 % hydrochloric acid and 10 % acetic acid) when tested in humans in the studies of Robinson *et al*. (2001) and Basketter *et al*. (1999, 2004). Based on this knowledge, the OECD TG 431 should be amended with regard to this chemical and its classification.

Sources of variability

The statistical analysis shows that the intra-laboratory and inter-laboratory variability were acceptably low for most cases. Considerable variability was observed for 10% sulphuric acid at the endpoint 1-h which was caused mainly by spreading problems. Nylon mesh could not be used here as spreading support due to the chemical specific interaction with nylon. Due to the high hydrophobicity of SkinEthic’s epidermal surface, the chemical had tendency to collect at the edge of the insert, therefore multiple mechanical respreading of the chemical on the surface (using a bull-headed glass stick) was necessary. This procedure could consequently lead to a different degree of tissue damage, rejected by variable results in the viability assay.

Another factor that could contribute to the inter-laboratory variability was the different order of testing the chemicals. The sample coding procedure assured randomisation of the test order in the three testing laboratories. In addition, laboratories intentionally changed the testing order of the coded samples in the three independent runs.

It was known that there is a critical point in the test protocol which may contribute to variability: tissues are washed with PBS after exposure, and kept on the “holding plate", until washing of all inserts in the experimental set is completed. Thereafter, all inserts together are transferred into the MTT medium for 3 h for determination of the tissue viability. Thus, tissues may stay on the holding plate for quite a long period of time (e.g. if in one testing set 6 substances, negative and positive controls were tested, each on 3 tissues, the first tissue must stay in the holding plate for 24 min, the second for 23 min, etc. until the last tissue has been washed). If a test chemical has not properly been removed from the tissue surface during the washing procedure, the remaining traces may cause undesirable post-exposure.

After decoding of samples and analysis of data provided by each laboratory, it can be concluded that most probably differences in time on holding plate were responsible for over-predictions of tetrachloroethylene and for the variability observed after 3 min for potassium hydroxide 10 % and octanoic acid. These three chemicals have induced slight, but significant differences between runs and also differences between testing laboratories. Nevertheless, we conclude that the robustness of the assay and its predictive capacity were not affected.

Limitations of the assay

No special limitations of the SkinEthic skin corrosion assay were identified in this study, except for the well established interference of some test chemicals with the MTT assay. In the set of 12 OECD reference chemicals, 6 chemicals interacted directly with MTT. This fact did not affect the results for 1,2-diaminopropane, 2-tertbuthylphenol and potassium hydroxide, since these chemicals were clearly predicted as corrosive. When testing phenethyl bromide and 4-(methylthio)-benzaldehyde, only slight MTT reduction was observed, which did not affect a correct prediction. However, problems were observed with eugenol, which is a strong MTT reducer, and, in addition, has the capacity to spontaneously polymerise.

Therefore, in addition to the blind trial, the level of chemical conversion of MTT by eugenol was evaluated. For this purpose, a procedure described by Liebsch *et al*. (2000) was used, applying eugenol onto non-viable (freeze-killed) control tissues. The binding of the chemical to the tissue can be assessed, and thus also the amount of MTT converted to formazan in the corrosivity test by residual test material.

It is important to notice, that this procedure is not yet properly evaluated and needs optimisation. Particularly, the preparation of freeze-killed controls seems to be a crucial step. It was observed that the binding of test material to frozen tissue can be higher than the binding to viable tissues. This result may be explained by formation of ice crystals during the freezing procedure, which may damage structure of the skin model and thus increase its permeability. Consequently, penetration of the test material into the damaged tissues will be higher and thus also the amount of residuals bound to the tissue. From experiments performed in all of the laboratories, it can be concluded that eugenol is able to significantly affect the viability of tissues (see Table 24). Because of the high conversion of MTT to formazan by eugenol itself, a final estimation of the corrosive properties of this chemical based on the above-described technique may sometimes lead to borderline prediction.

5.2.3 RESULTS OBTAINED WITH EST-1000 MODEL

For the evaluation of performance of the EST-1000 model in the *in vitro* skin corrosion test the twelve reference chemicals endorsed by the OECD TG 431 were tested in one single experiment. The test was performed according to the standard operation procedure developed previously for SkinEthic model. Duplicate tissues were exposed for 3 min and one hour to the test chemicals. The classification was based on the same prediction model as used in the study with SkinEthic model.

Outcome of this "ministudy" is summarised in Table 28 and Figure 30. The results obtained with the EST-model were in most of cases in concordance with classification given in the OECD TG 431. Similarly as is some experiments with SkinEthic model, misclassification for tetrachloroethylene was obtained. In comparison to SkinEthic model, the EST-1000 model was more sensitive to potassium hydroxide (10 %) and positive control (8N KOH), where severe loss of cell viability occurred already after 3 min. After one hour, both tissues exposed to positive control were completely digested and major parts lost during the washing procedure. This effect might be linked to the different barrier properties of the EST-1000 model.

Table 28. Classification of 12 OECD reference chemicals in the EST-1000 skin corrosion test.

Chemical name	***In vivo***	**3 minutes**			**1 hour**			**Classification**
	class	OD mean	tissue viability [%]	tissue difference [%]	OD mean	Tissue viability [%]	tissue difference [%]	(C/NC)
Acrylic acid	severe corrosive	0.037	2.3	0.16	0.034	2.0	0.05	C*
1,2 Diaminopropane	severe corrosive	0.240	14.9	0.41	0.106	6.2	0.22	C*
2-Tertbuthyl phenol	corrosive	0.361	22.4	0.80	0.217	12.7	0.70	C*
Octanoic acid	corrosive	1.010	62.7	3.70	0.019	1.1	0.08	C
Sulphuric acid (10 %)	corrosive (§)	1.556	96.6	1.07	0.036	2.1	0.34	C
Potassium hydroxide (10 %)	corrosive	0.132	8.2	1.16	0.066	3.9	0.14	C*
Tetrachloroethylene	non corrosive	1.161	72.1	6.60	0.247	14.4	1.92	C
4-Amino-1,2,4-triazole	non corrosive	1.531	95.1	2.44	1.610	94.1	0.95	NC
Phenethyl bromide	non corrosive	1.317	81.8	7.34	1.223	71.5	2.67	NC
Isostearic acid	non corrosive	1.589	98.6	2.39	1.429	83.5	1.61	NC
4-Methylthio benzaldehyd	non corrosive	1.375	85.4	2.73	1.157	67.7	2.21	NC
Eugenol	non corrosive	0.996	61.9	1.53	0.581	33.9	1.92	NC
Negative control	non corrosive	1.611	100.0	0.39	1.711	100.0	0.95	NC
Positive control (8N KOH)	corrosive	0.002	0.1	0.01	0.010	0.6	0.06	C

C - corrosive after 3 min, C - corrosive after 1 hour, NC - non corrosive*

(§) - classified as non corrosive according to Annex I of the Directive 67/548/ EEC (ECB, 2005).

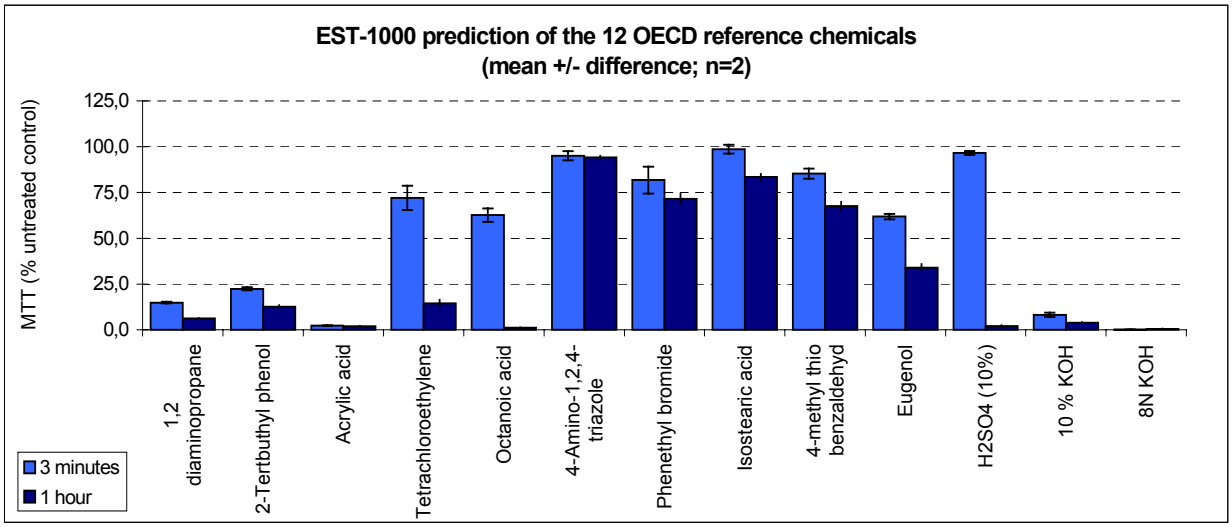

Figure 30. Prediction of corrosive potential of 12 OECD reference chemicals by EST-1000 model.

5.2.4 DISCUSSION

Based on previously performed experiments with the EST-1000 model (Hoffmann *et al*, 2005) and outcome given above, the EST model proved to be an useful tool for the screening of skin corrosion potential of chemicals.

Similarly as for the other models produced in plastic inserts (e.g. EpiDerm, SkinEthic), interaction between some chemicals and the wall of the insert may occur (e.g. in case of tetrachloroethylene). The reaction leads to formation of adverse products, that can be more or less toxic than the original chemical was. Another problem present chemicals strongly reducing MTT (e.g. eugenol). As described previously, due to their ability to directly reduce MTT, the prediction of the corrosive effect may be complicated.

Based on recommendations of the OECD TG 431, for the regulatory acceptance of the EST-1000 skin corrosion test, additional experiments with EST model (preferably between laboratories) should be performed, to confirm the assay reproducibility over time. In addition, more data related to the lipid profile of the model and tissue morphology should be produced to support results of study published by Hoffmann *et al*. in 2005.