

ORIGINAL ARTICLE

Validation of human skin models for skin corrosivity tests in Japan

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Abstract

As shown in OECD test guidelines 430 and 431, the human skin epidermal assay and Transcutaneous Electrical Resistance Test (TER) were validated and peer reviewed as an alternative method to corrosivity testing; however, these methods have not been used widely in Japan. The problems related to techniques and evaluation are not clear. Therefore, we performed a validation study of EPI-200 (EpiDerm™), a 3-dimensional cultured epidermal model and Vitrolife-Skin™, a 3-dimensional cultured skin model made in Japan as a catch-up validation trial of alternatives for skin corrosivity testing using 13 chemicals including a positive control: 10% potassium hydroxide solution in Japan. From the obtained data, we identified the potential of utilizing these models to evaluate the corrosivity of a chemical.

Key words: Skin corrosivity, cultured epidermal model, cultured skin model, validation

Introduction

Over the last decade, the European Centre for the Validation of Alternative Methods (ECVAM) has supported formal validation studies using *in vitro* tests as a replacement for the *in vivo* rabbit test for predicting skin corrosivity (Botham, et al., 1995, Barratt, et al., 1998, Fentem et al., 1998, Liebsch et al., 2000). As a result, two new test methods for skin corrosion, which incorporates a rat skin

transcutaneous electrical resistance assay (TER) and two human skin epidermal assays, were included in Annex V of Directive 67/548/EEC in mid-2000, thereby making the use of *in vitro* alternatives for skin corrosivity testing of chemicals mandatory in the European Union (EC, 2000). As human epidermal model assays, two methods based on commercial human epidermal models, EPISKIN™ (EPISKIN, Chaponost, France) and

EpiDerm™ (MatTek, Ashford, MA, USA), were also endorsed.

Meanwhile, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) in the USA prepared final recommendations on these methods for their consideration and acceptance where appropriate (NIH Publication No.02-4502; ICCVAM, 2002). As a result, these assays were published as an alternative method to corrosivity testing as shown in the OECD test guidelines 430(OECD 430; 2004), and 431(OECD 431; 2004).

In Japan, these methods have not been widely used. The problems related to techniques and evaluation are not clear. In the present study, therefore we performed a catch-up validation trial to evaluate skin corrosivity using the human epidermal and skin models, that is, evaluations were made based on the ECVAM experimental protocol.

We performed a validation study of EPI-200 (EpiDerm™), a 3-dimensional cultured epidermal model and Vitrolife-Skin™, a 3-dimensional cultured skin model as validation trials of alternative for skin corrosivity testing in Japan. From the obtained data, we investigated the possibility of utilizing these models to evaluate the corrosivity of a chemical. We may suggest using these models to the ad hoc. committee of toxicology at MHLW in Japan.

Materials and Methods

Study management and organization

The study was performed according to the Japanese

Society for the Alternative to Animal Testing Experiments (JSAAE) validation scheme as shown in Fig.1. The chairman was Dr. I Yoshimura at the Fac. Eng. Tokyo Univ. Science, who is head of the validation committee in JSAAE. Dr. Ohno at the National Institute of Health Sciences (NIHS) prepared the protocol and supported this validation with a grant from MHLW. Six Laboratories joined the study as shown in Table 1, and a blind trial with 13 chemicals including a positive control (10% potassium hydroxide solution) was performed using the protocol. In addition, Dr. Y. Ohno, the chemical distributor, coded and distributed the test chemicals to be used in the blind trial. After submission of all coded data to biostatisticians, an independent biostatistical analysis of the blind trial was performed at the Fac. Med. Kyoto Univ. and Fac. Eng. Tokyo Univ. Science. The study director at each laboratory, a chemical distributor, biostatisticians and kit suppliers were organized into study management teams in this validation assay as shown in Fig. 1. Finally, the chairman reported the outcome of this validation and forwarded this report to JSAAE.

Technical transfer and preliminary tests

The management team performed the technical transfer by kit suppliers at NIHS, Tokyo on January 28, 2004. After that, technicians performed the preliminary test using 10% potassium hydroxide solution and benzalkonium chloride 10 % solution. A qualified technician from each laboratory participated in the technical transfer and the preliminary

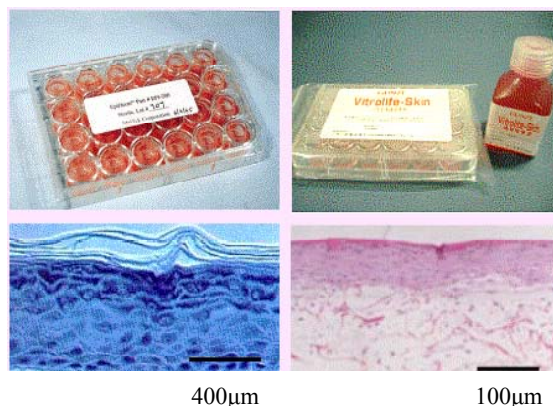
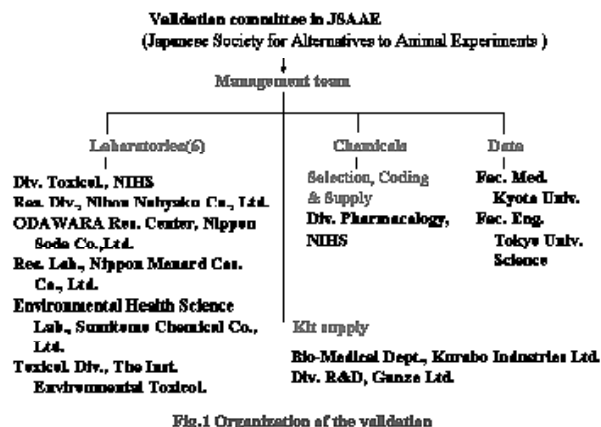


Fig.2 EpiDerm™

Fig.3 Vitrolife-Skin™

test. All technicians obtained good results in this test.

Cultured epidermal and skin models

EpiDerm™ (EPI-200) models were purchased from KURABO Corporation (Osaka, Japan) as kits containing 24 models as shown in Fig.2, with sufficient amounts of Dulbecco's modified Eagle's medium (DMEM)-based assay medium, and phosphate-buffered saline (PBS) solution. These kits are made by MatTek Corporation (Ashland, MA, USA). The human epidermal model consisting of an epidermis with cornified layers was prepared as previously described (Liebsch et al., 2000).

Vitrolife-Skin™ models were supplied from Gunze Corporation Ltd. (Kyoto, Japan) as kits containing 24 models, collagen sponges without cells and sufficient amounts of DMEM-based assay medium, as shown in Fig.3. The human skin model consisting of a dermis and epidermis with cornified layers was prepared as previously described (Morikawa et al., 2002; Morota et al., 1998; Morota et al., 1999).

Materials

A total of 13 test chemicals including a positive control (10 % potassium hydroxide solution) were selected from the chemicals tested in the ECVAM skin corrosive validation study (Fentem et al., 1998, Liebsch et al., 2000). The chemical distributors selected test chemicals considering a balanced representation of the chemical classes, rate of corrosion or non-corrosion, solubility etc. from the total 60 chemicals tested in the ECVAM validation study. Test chemicals included six of which are known to be corrosive *in vivo*, six which are non-corrosive, six liquids, four solids and two powders, excluding the positive control. Each laboratory was sent the rotated 11 chemicals, including the positive control, in 13 test chemicals as shown in Table 2. Therefore, five data items from each laboratory for each chemical were obtained. All blinded test chemicals were treated as powerful drugs or poisons in each laboratory. The management team considered the minimum appropriate number of chemicals for catch up validation.

All test chemicals used were from the same batch and were purchased from Sigma Aldrich (Milwaukee, USA) and Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and were supplied to each laboratory by the chemical distributors. Phosphate-buffered saline (PBS) and isopropanol were obtained from Wako Pure Chemical Industries,

Table 1 List of members in skin corrosivity validation assay

Japanese Society for Alternative to Animal Experiments Validation Executive Committee

	Organization	Name
Chairman	Tokyo University of Science, Faculty of Engineering, Dept. Management Science	Isao Yoshimura
	National Institute of Health Science, Biological Safety Research Center, Division of Pharmacology	Yasuo Ohno

Study Director

	Organization	Name
	National Institute of Health Sciences, Biological Safety Research Center, Division of Toxicology	Tomoko Ando
	Nihon Nohyaku Co., Ltd., Research Division, Toxicological & Pharmaceutical Research Center	Katsuhiro Inagaki
	Nippon Soda Co., Ltd., Odawara Research Center, Toxicological Research Department	Mami Kuboki
	Nippon Menard Cosmetic Co., Ltd., Research Laboratories	Hajime Kojima
	Sumitomo Chemical Co., Ltd., Environmental Health Science Laboratory, Biochemistry Group	Yosuke Nakamura
	The Institute of Environmental Toxicology, Toxicology Division II, Laboratory of Immunotoxicology	Tadashi Kosaka

Kit supplier

	Organization	Name
	Kurabo Industries Ltd., Bio-medical Department	Hisashi Torishima
	Kurabo Industries Ltd., Biomedical Department	Michiru Genno
	Gunze Limited, Division of Research & Development	Noriyuki Morikawa

Coordinator

	Organization	Name
	Sumitomo Chemical Co., Ltd., Environmental Health Science Laboratory, Biochemistry Group	Naohiko Isobe
	Nippon Soda Co., Ltd., Agro Product Division, Regulatory Affairs Group	Yukihiro Kanaguchi
	National Institute of Health Sciences, Biological Safety Research Center, Division of Toxicology	Jun Kanno
	The Institute of Environmental Toxicology, Toxicology Division II	Takanori Harada
	Nihon Nohyaku Co., Ltd., Research Division, Toxicological & Pharmaceutical Research Center	Masaru Nogata
	Nippon Soda Co., Ltd., Agro Product Division, Regulatory Affairs Group	Mitsuo Hattori
	Nippon Soda Co., Ltd., Odawara Research Center, Toxicological Research Department	Yoshinobu Fujii
	The Institute of Environmental Toxicology, Toxicology Division II, Laboratory of Neurotoxicology	Sayaka Ishimine
	Sumitomo Chemical Co., Ltd., Environmental Health Science Laboratory, Biochemistry Group	Takashi Morimoto

Ltd. and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and MTT formazan were obtained from Sigma Aldrich. They were supplied by the management team.

Methods

Chemical application procedure according to the ECVAM validation study.

The experimental steps of the method were performed according to the protocol used in phase III of the EpiDerm™ skin corrosivity test (Liebsch et al., 2000) with slight modifications. The EpiDerm™ models were equilibrated at 37°C and 5% CO₂ within one hour after receiving a kit and placed in 1 mL of DMEM-based assay medium in 6-well plates before use. If kept for a few days, it was preserved in a refrigerator. The Vitrolife-Skin™ models were placed in 250 µL of DMEM-based assay medium in 24-well plates and equilibrated for several hours' incubation (37°C, 5% CO₂) within a few days after receiving a kit. One hour before dosing, the models were transferred in 1 mL of DMEM-based assay medium to 6-well plates. Test chemicals were applied directly to the stratum corneum of two replicate models per chemical. Liquids (50 µL) were applied using a positive displacement pipette. Solids were crushed to a powder, if necessary, and 25 mg was applied using a spatula with the addition of 25µL of dis-

tilled water to ensure good contact with the surface. Two models were dosed with 100 µL distilled water as a negative control. After exposure for three or 60 min. at room temperature (15-25°C), two replicate models for each exposure time were rinsed thoroughly with PBS to remove the test chemical from the surface.

Calculation of cell viability

The effects of the test chemicals on cell viability were determined using an MTT reduction assay. After blotting, the models were incubated in 0.3 mL (EpiDerm™) or 1 mL (Vitrolife-Skin™) of each DMEM-based assay medium containing 0.5 mg of MTT for an additional three hours at 37°C and 5% CO₂. Living cells were dyed dark-violet by the MTT reagents. After the models were washed with PBS, biopsies of Vitrolife-Skin™ models were taken using a biopsy punch (6 mm diameter), although this operation is not used in EpiDerm™ models. The biopsies were separated from the models using forceps, and placed into acidified isopropanol (2.0mL: EpiDerm™, 1.0 mL: Vitrolife-Skin™), after removing excess water by placing the samples on absorbent paper. Precipitated formazan was extracted overnight at room temperature with protection from light. The absorbance of the extracts was measured at 570 nm using a UV-VIS spectrophotometer. Adequate absorbance of spectrophotometers was checked using 0.1mg/mL solution of MTT formazan prior to the validation study. Cell viability of EpiDerm™ models determined by the MTT reduction assay method was expressed as follows:

$$\text{Cell viability} = \frac{A_t}{A_c} \times 100 (\%), \quad (1)$$

where A_t and A_c are the absorbancies of the extracts when test chemicals and a negative control, respectively, are applied to the cultured skin model.

In case of Vitrolife-Skin™, additional tests using collagen sponges without cells were performed, with the potential to interfere with the MTT assay, and thus cell viability was expressed as follows:

$$\text{Cell viability} = \left(\frac{A_t - A_{bt}}{A_c - A_{bc}} \right) \times 100 (\%), \quad (2)$$

where A_t and A_c are absorbancies of the extracts

Table 2 Test chemicals

No.	Name	C/NC	Comments
1	Potassium hydroxide(10%aq)	C	Positive control
2	Sulfuric acid(10% wt)	C	
3	Octanoic (Caprylic) acid	C	
4	Sodium hydroxide(4.88%)	C	
5	Phenol	C	
6	Chromium trioxide	C	
7	Phosphoric acid	C	
8	Sodium perborate	NC	
9	Tetrachloroethylene	NC	
10	Potassium hydroxide(5% aq)	NC	
11	4-Amino-1,2,4-triazole	NC	
12	L-Lactic acid	NC	
13	Isopropanol (2-propanol)	NC	

when test chemicals and a negative control, respectively, are applied to the viable Vitro-life-Skin™ model, and A_{bl} and A_{bc} are the values obtained for a blank test using a test chemical and the negative control, respectively, with a collagen sponge without cells.

Prediction models

Predictions of *in vitro* corrosiveness/non-corrosiveness were made according to the refined final prediction model (PM2) used in phase III of the EpiDerm™ skin corrosivity test (Liebsch et al., 2000). Hence, chemicals that reduced cell viability to less than 50% upon exposure to the Vitro-life-Skin™ model for three min. were predicted to

be ‘corrosive’ *in vivo*. If 3 min. exposure produced cell viability of $\geq 50\%$, the chemical was classified as ‘non-corrosive’ after a 3 min. exposure, but the same chemical was still be classified as ‘corrosive’ if viability after a 60 min. exposure was below 15%. The results obtained using the EpiDerm™ and Vitro-life-Skin™ models in this study were compared with the results of ECVAM validation studies using EpiDerm™ (Liebsch et al., 2000) and EPISKIN™ (Fentem et al., 1998) for skin corrosivity testing.

This test was repeated twice. If different results from the two tests were obtained, a third test was performed at each laboratory and used for final judgment.

Table 3 Data from each laboratory

Chemical Lab.	Potassium hydroxide (10%). Corrosive						Sulfuric acid (10%). Corrosive					Tetrachloroethylene. Non-Coro				
	NIHS	NN	NS	NM	SC	IET	NIHS	NN	NS	NM	SC	NIHS	NN	NS	NM	IET
Blind No.	1	2	3	4	5	6	13	14	15	16	17	18	19	20	21	22
EpiDerm -test 1-	C	C	C	C	C	C	NC	NC	NC	C	C	NC	NC	NC	NC	NC
EpiDerm -test 2-	C	C	C	C	C	C	NC	C	C	C	C	NC	NC	NC	NC	NC
EpiDerm re-trial	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
Judgement	C	C	C	C	C	C	NC	C	C	C	C	NC	NC	NC	NC	NC
Vitro-life-Skin -	C	C	C	C	C	C	C	C	C	C	C	NC	NC	NC	NC	NC
Vitro-life-Skin -	C	C	C	C	C	C	C	C	NC	C	C	NC	NC	NC	NC	NC
Vitro-life-Skin re-	C	C	C	C	C	C	C	C	C	C	C	NC	NC	NC	NC	NC
Judgement	C	C	C	C	C	C	C	C	C	C	C	NC	NC	NC	NC	NC

Chemical Lab.	Octanoic acid. Corrosive					Potassium hydroxide(5%). Non-					Sodium hydroxide(4.88%) Corrosive				
	NIHS	NN	NS	NM	IET	NIHS	NN	NS	SC	IET	NIHS	NN	NS	SC	IET
Blind No.	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
EpiDerm -test 1-	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
EpiDerm -test 2-	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
EpiDerm re-trial	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
Judgement	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
Vitro-life-Skin -	C	C	NC	C	C	C	C	C	C	C	C	NC	C	C	C
Vitro-life-Skin -	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
Vitro-life-Skin re-	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
Judgement	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C

Chemical Lab.	4-Amino-1,2,4-triazole. Non-Coro.					Phosphoric acid. Corrosive					L-Lactic acid. Non-Coro.				
	NIHS	NN	NM	SC	IET	NN	NS	NM	SC	IET	NIHS	NS	NM	SC	IET
Blind No.	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
EpiDerm -test 1-	NC	NC	NC	NC	NC	C	C	C	C	C	NC	C	C	NC	C
EpiDerm -test 2-	NC	NC	NC	NC	NC	C	C	C	C	C	C	C	C	C	C
EpiDerm re-trial	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
Judgement	NC	NC	NC	NC	NC	C	C	C	C	C	C	C	C	C	C
Vitro-life-Skin -	NC	NC	NC	NC	NC	C	C	C	C	C	C	C	C	C	C
Vitro-life-Skin -	NC	NC	NC	NC	NC	C	C	C	C	C	C	C	C	C	C
Vitro-life-Skin re-	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
Judgement	NC	NC	NC	NC	NC	C	C	C	C	C	C	C	C	C	C

Chemical Lab.	Isopropanol. Non-Coro.					Phenol. Corrosive					Sodium perborate. Non-Coro.					Chromium trioxide. Corrosive				
	NN	NS	NM	SC	IET	NIHS	NN	NM	SC	IET	NIHS	NN	NS	NM	SC	NIHS	NS	NM	SC	IET
Blind No.	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72
EpiDerm -test 1-	NC	NC	NC	NC	NC	C	C	C	C	C	NC	NC	NC	NC	NC	C	C	C	C	C
EpiDerm -test 2-	NC	NC	NC	NC	NC	C	C	C	C	C	NC	NC	NC	NC	NC	C	C	C	C	C
EpiDerm re-trial	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
Judgement	NC	NC	NC	NC	NC	C	C	C	C	C	NC	NC	NC	NC	NC	C	C	C	C	C
Vitro-life-Skin -	NC	NC	NC	NC	NC	C	C	C	C	C	NC	NC	NC	NC	NC	C	C	C	C	C
Vitro-life-Skin -	NC	NC	NC	NC	NC	C	C	C	C	C	NC	NC	NC	NC	NC	C	C	C	C	C
Vitro-life-Skin re-	C	C	C	C	C	C	C	C	C	C	NC	NC	NC	NC	NC	C	C	C	C	C
Judgement	NC	NC	NC	NC	NC	C	C	C	C	C	NC	NC	NC	NC	NC	C	C	C	C	C

Laboratory:

NIHS: Div. Toxicol., NIHS

NS: ODAWARA Res. Center, Nippon Soda Co., Ltd.

SC: Environmental Health Science Lab., Sumitomo Chemical Co., Ltd.

NN: Res. Div., Nihon Nohyaku Co., Ltd.

NM: Res. Lab., Nippon Menard Cosmetic Co., Ltd.

IET: Toxicol. Div., The Inst.Environmental Toxicol.

C: Corrosive, NC: Non-Corrosive

Results

This validation study was not performed under GLP. However, all data obtained in each laboratory followed GLP compliance and spirit. Their records (data and detailed documents) could be checked after the assays, and raw data was sent to Tokyo Univ. of Science for analysis by biostatisticians. All documents were checked by the chairperson, biostatisticians and chemical distributors, and are stored in the NIHS.

Predictivity

Using cell viability after exposure to test chemicals for three or 60 min., the chemical classifications

according to the EpiDerm™ prediction model are shown in Table 3. Data for positive controls in the two models were evaluated correctly at all laboratories. The EpiDerm™ data summarized in Table 4 excluded the positive control data. Of 30 classifications of six chemicals in the corrosive class, 29 classifications of EpiDerm™ were correctly predicted to be corrosive, and sensitivity was 96.7%. All six chemicals in the corrosive class were correctly predicted excluding one laboratory. Lab.1 gave a negative classification of sulfuric acid from two data sets, but this chemical is corrosive. Cell viability values after expose to sulfuric acid for 60 min. were 18.54% and 38.80%, and these values

Table 4 Contingency table for EpiDerm™ predictions

<i>Vitro</i> <i>Vivo</i>	Corrosive	Non-Corrosive
Corrosive	29	1
Non-Corrosive	10 (5% KOH, Lactic Acid)	20

Table 5 Contingency table for Vitrolife-Skin™ predictions

<i>Vitro</i> <i>Vivo</i>	Corrosive	Non-Corrosive
Corrosive	30	0
Non-Corrosive	10 (5% KOH, Lactic Acid)	20

Table 6 Key statistical parameters for the four tests

	EpiDerm™	Vitrolife-Skin™	EpiDerm™ (ECVAM)	EPISKIN™ (ECVAM)
No. of Chemicals	12	12	24	60
Sensitivity	100% (12/12)	100% (12/12)	92%	82%
Specificity	66.7% (4/6)	66.7% (4/6)	83%	84%
Accuracy	83.3% (10/12)	83.3% (10/12)	92%	83%
False positive rate	16.7% (2/12)	16.7% (2/12)	17%	19%
False negative rate	0% (0/12)	0% (0/12)	8%	14%

were slightly high compared to 15%, which is the border line. On the other hand, of 30 classification of six chemicals in the non-corrosive class, 20 classifications of EpiDermTM were correctly predicted to be non-corrosive, and specificity was 66.7%, but two were false positives. There were 5% potassium hydroxide and lactic acid. All the laboratories gave them positive classifications from two data sets, which is a non-corrosive chemical. Positive predictivity was 74.4% (29 true corrosive classifications / 39 corrosive classifications in this assay). Negative predictivity was 95.2% (20 true non-corrosive classifications / 21 non-corrosive classifications in this assay). The total consistency rate was 81.7% (49 true classifications / 60 classifications in this assay).

The Vitrolife-SkinTM data are summarized in Table 5, excluding the positive control data. Of 30 classifications of six chemicals in the corrosive class, 30 of Vitrolife-SkinTM were correctly predicted to be corrosive, and sensitivity was 100%. All six chemicals in the corrosive class were correctly predicted.

On the other hand, of 30 classification of six chemicals in the non-corrosive class, 20 of Vitrolife-SkinTM were correctly predicted to be non-corrosive, and specificity was 66.7%, but two were false positives. They were 5% potassium hydroxide and lactic acid, which all laboratories gave a positive classification from two data sets. This chemical is non-corrosive. Positive predictivity was 75% (30 true corrosive classifications / 40 corrosive classifications in this assay). Negative predictivity was 100% (20 true non-corrosive classifications / 20 non-corrosive classifications in this assay). The total consistency rate was 83.8% (50 true classifications / 60 classifications in this assay).

Predictability of these two models was similar to the results obtained by the ECVAM validation study.

Intralaboratory variation

Most chemicals did not show any great differences in scores on tests repeated at each laboratory. Different classifications of EpiDermTM accounted for 6.66% (4/60). These data are not shown in the Tables. Cell viabilities of sulfuric acid after exposure for 60 min. in Lab. 2 were 17.26%, 9.46% and 12.02%, and those in Lab.3 were 15.72%, 10.58% and 9.01%, respectively. On the other hand, cell viabilities of lactic acid after exposure for 60 min. in Lab. 1 were 16.55%, 13.39% and 7.19%, while

those in Lab.5 were 15.85%, 12.01% and 15.89%, respectively. These cell viabilities were around 15% after exposure for 60 min. (the success criteria).

Different classifications of Vitrolife-SkinTM accounted for 5.0% (3/60). Cell viabilities of sulfuric acid after exposure for 60 min. in Lab. 3 were 5.90%, 16.09% and 6.34%, while after exposure to octanoic acid for 60 min in Lab.3 were 21.37%, 11.77% and 10.71%. These cell viabilities were around 15% after exposure for 60 min (the success criteria). Meanwhile, cell viabilities of sodium hydroxide (4.88%) after exposure for 3 min. in Lab. 2 were also 55.12%, 15.41% and 17.51%. These cell viabilities were around 50% after exposure for 3 min. (the success criteria).

These cell viabilities were in an extremely narrow range despite the different classifications. Therefore, intralaboratory variation between the two models is presumed to be small.

Interlaboratory variation

In EpiDermTM, inter-laboratory variation was significant for only sulfuric acid. The classification of sulfuric acid in Lab. 1 was different from the data in the other four laboratories. In the data of Lab.1, not shown in the Tables, cell viabilities after exposure for 60 min. were 18.54% and 38.80%, and these values were almost the same as the positive classification. For Vitrolife-SkinTM, inter-laboratory variation was not significant. From these results, the feasibility of using EpiDermTM and Vitrolife-SkinTM was suggested by the experiment.

Discussion

From the obtained data, we confirmed the potential of using EpiDermTM and Vitrolife-SkinTM as methods to evaluate the corrosivity of a chemical. We consider the data from these models has high predictivity, and low intra- and inter-laboratory variation.

With Vitrolife-SkinTM, however, it is necessary to use limited blank data using collagen sponges without cells.

Modified points of Vitrolife-SkinTM from the ECVAM skin corrosivity validation study

Application volume

Although the surface of the Vitrolife-skinTM model (0.5 cm²) is similar to that of EpiDermTM (0.63cm²), 50 µL of Liquid chemical was often insufficient for the surface. In this study, therefore,

the application volume of liquids was increased from 50 μL , the volume used in the phase III protocol in the EpiDermTM skin corrosivity test, to 100 μL . For the same reason, 50 mg of solid chemical was applied and 50 μL of water was added to ensure good contact with the surface (in contrast to the Phase III protocol, in which 25mg of solid and an additional 25 μL of water were applied. Additional tests using collagen sponges without cells, the Vitrolife-SkinTM model uses a collagen sponge without cells to construct the dermal layer, and this allows test chemicals to be easily absorbed and bound, compared with epidermal models consisting of only an epidermal layer and supporting material. In a previous study, tests using collagen sponges without cells, instead of non-viable Vitrolife-SkinTM models, were performed for several test chemicals with the potential to interfere with the MTT assay (Mirokawa, 2006). For 3-methoxypropylamine and n-heptylamine, these experiments suggested about 50-60% and 80% "viability", respectively, due to a chemical reaction with the MTT medium. Hence, the 70-80% viability obtained for 3-methoxypropylamine with the Vitrolife-SkinTM model should be corrected to about 20%. In the same way, the 120% viability obtained for n-heptylamine should be decreased to about 40%. Therefore, these two chemicals, which were incorrectly classified as negatives by testing without using blank collagen sponges, should correctly be classified as corrosive by adding blank collagen sponges, in agreement with the results from the EpiDermTM model. The additional test for the other six chemicals gave results of around 15% "viability", such that the Vitrolife-SkinTM *in vitro* prediction of corrosivity was not changed.

Therefore, we obtained blank data using collagen sponges without cells in the validation of Vitrolife-SkinTM. In this validation study, we detected solubilization, swelling and color change after exposure to chemicals, and the need to use blank collagen sponges without cells.

Comparison of skin models

As shown in Table 6, there was no difference in sensitivity, specificity, accuracy, false positive rate or false negative rate between EpiDermTM and Vitrolife-SkinTM in this validation study. The result in this validation study may be due to no difference in structure between a two-layer skin model consisting of a dermis and epidermis (Vitrolife-SkinTM) and epidermal models (EpiDermTM). The barrier

function of cornified layers of the cultured epidermal and skin model is less effective compared with human skin tissue (Kojima *et al.*, 2000). In addition, as chemical exposure times become longer, stronger cytotoxicity occurs due to the accumulation of chemicals which permeate the cornified layer of the skin model. However, it is considered the barrier function of these model is similar.

The sensitivity was 92% in phase III of the EpiDermTM study and 82% in EPISKINTM study, and the present values (100%) were higher than data of these previous validation assays. The specificity, however, was 83% in phase III of the EpiDermTM study and 84% in the EPISKINTM study, and the present ones (66.7%) were lower than those. We consider these accuracy and false positive rates to be no different between the present validation and previous validation study. On the other hand, none of the false negative rates in present validation study were lower than data from previous validation studies. This issue must be handled carefully, because this assay is a catch-up validation trial, and the number of chemicals and classes is small.

Though peer review of these models is in progress, the ad hoc. committee of toxicology at MHLW in Japan has approved the utilization of these models to evaluate the corrosivity of a chemical.

Acknowledgment

This validation was funded by a grant from MHLW.

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(Received: October 24, 2007/
Accepted: January 25, 2008)

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