September 11, 2009

Informational materials for peer review of the SIRC cytotoxicity test and the three dimensional dermal model (MATREX^{TM}) test

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Table 1 Information obtained from the Draize eye test and the alternative methods

Information from the Draize test	CAM	Erythrocyte	Skin model	SIRC	Human cultured cells	Animal cultured cells	EYTEX
<1> Corneal opacity							
a Degeneration of membrane parenchyma (collagen)	▲	×	○	×	×	×	○
b Swelling of collagen (depending on the intensity of epithelial/endothelial disorders)	▲	×	○	×	×	×	○
c Degeneration/exfoliation of epithelial cells (due to cytotoxicity)	▲	▲	○	○	○	○	×
<2> Iris							
a Transcorneal absorption and damage to the iris	×	×	×	×	×	×	×
b Light reflex	×	×	×	×	×	×	×
<3> Conjunctiva							
a Redness (inflammatory vascular dilation)	○	×	▲	▲	▲	▲	×
b Edema (inflammatory edema)	▲	×	▲	▲	▲	▲	×
c Secretion (excessive lacrimation/inflammatory infiltrating reaction)	×	×	×	×	×	×	×
<4> Information from follow-up							
a Repair	▲	×	▲	▲	▲	▲	×
b Presence of delayed onset	▲	▲	▲	▲	▲	▲	×
<5> Information about observation items excluded from the Draize test							
a Corneal ulcer (damage to/lack of corneal epithelium)	×	×	×	×	×	×	×
b Irregularity of cornea (dryness/concave formation)	×	×	×	×	×	×	×
c Improvement in disorders following eye irrigation	○	×	○	▲	▲	▲	×
d Evaluation of pain (observation of behavior/No. of nictitations/closed eye)	×	×	×	×	×	×	×
e Detection of disorders due to physical stimulation (insoluble substance)	×	×	×	×	×	×	×

Note: Evaluations based on the literature before starting validation: ○: possible introduction, ▲: investigation needed to establish introduction, ×: impossible to introduce

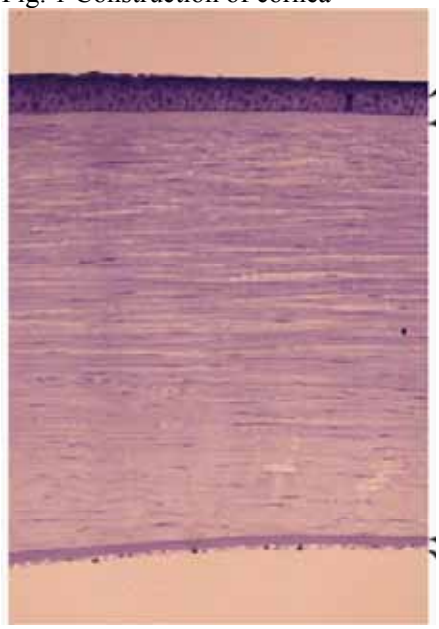
The table that reported by Kaneko et al. (1996) is translated into English.

Table 2 Scale for scoring ocular lesions in the Draize eye test

(1) Cornea	
(A) Opacity-degree of density (area most dense taken for reading)	
No Opacity	0
Scattered or diffuse area, details of iris clearly visible	1
Easily discernible translucent areas, details of iris slightly obscured	2
Opalescent areas, no details of iris visible, size of pupil barely discernible	3
Opaque, iris invisible	4
(B) Area of cornea involved	
One quarter (or less) but not zero	1
Greater than one quarter, but less than half	2
Greater than half, but less than three quarters	3
Greater than three quarters, up to whole area	4
A × B × 5	Total maximum = 80
(2) Iris	
(A) Values	
Normal	0
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive)	1
No reaction to light, hemorrhage, gross destruction (any or all of these)	2
A × 5	Total maximum = 10
(3) Conjunctivae	
(A) Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)	
Vessels normal	0
Vessels definitely injected above normal	1
More diffuse, deeper crimson red, individual vessels not easily discernible	2
Diffuse beefy red	3
(B) Chemosis	
No swelling	0
Any swelling above normal (includes nictitating membrane)	1
Obvious swelling with partial eversion of lids	2
Swelling with lids about half closed	3
Swelling with lids about half closed to completely closed	4
(C) Discharge	
No discharge	0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hairs just adjacent to lids	2
Discharge with moistening of the lids and hairs, and considerable area around the eye	3
Score (A + B + C) × 2	Total maximum = 20

The table is the same as that reported by Draize et al. (1959)

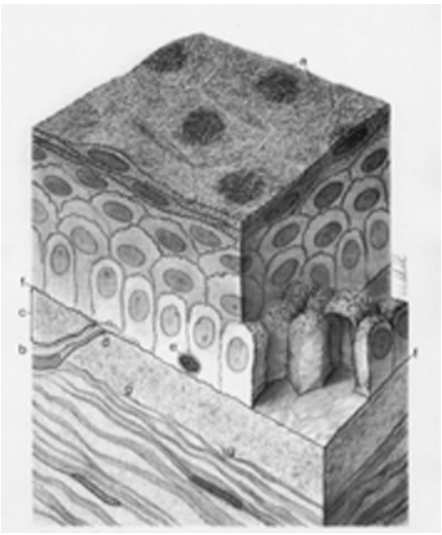
Fig. 1 Construction of cornea



Epithelial layer
Bowman's layer

Stroma

Descemet's membrane
Endothelial layer



The figure from Hirano (2008) is translated into English.

Table 3 List of the methods evaluated and the participation of each organization

Methods	Organizations participating in the validation study																					Number of participants*		
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	3rd	2nd	1st
Chorioallantoic membrane																									
HET–CAM	●	●			●		●					●											5	5	5
CAM–TB	●	●			●		●					●											5	5	5
Red blood cells																									
RBC		●	●		●						●	●								●			6	9	7
Haemoglobin																									
HD#	●		●									●	●	●						●			6	8	8
Artificial skin models																									
SKIN^{2TM} (ZK1100)	●		●	●							●					●	●			●		●	8	6	8
MATREX™					●	●			●													●	4	7	3
Normal cells from rabbit cornea																									
CornePack*#										●			●				●		●			●	5	6	7
Cell lines from rabbit cornea																									
SIRC–CVS	●	●					●									●					●		5	6	6
SIRC–NRU	●	●		●			●									●					●		6	7	7
Cell lines from the other mammals																									
HeLa–MTT						●				●	●	●	●			●			●				6	8	8
CHL–CVS							●		●			●											4	7	7
EYTEX™			●		●			●							●			●		●		●	7	5	5
Sum	6	4	4	3	4	3	5	1	2	2	3	6	3	1	1	4	2	1	2	4	2	4	67	79	76

V: suppliers of test kits. *: number of participants to each validation.
HET–CAM: hen's egg–chorioallantoic membrane method; CAM–TB: chorioallantoic membrane-trypan blue staining method; RBC: haemolysis method; HD: haemoglobin denaturation method; SIRC–CVS: cytotoxicity tests on SIRC cells using crystal violet staining; HeLa–MTT: cytotoxicity tests on HeLa cells using MTT reduction; CHL–CVS: cytotoxicity test on CHL cells using crystal violet staining; #: major changes in protocols were made before the second validation.

The table is the same as that reported by Ohno et al.(1999).

Table 4
List of the co-operating organizations for the Japanese validaton

Administrative organizations
Ministry of Health and Welfare
National Institute of Health Sciences
(Div. Pharmacol. Div. Toxicol.
and Div. Genetics Mutagen.)

Universities
Yokohama-City University
Showa University

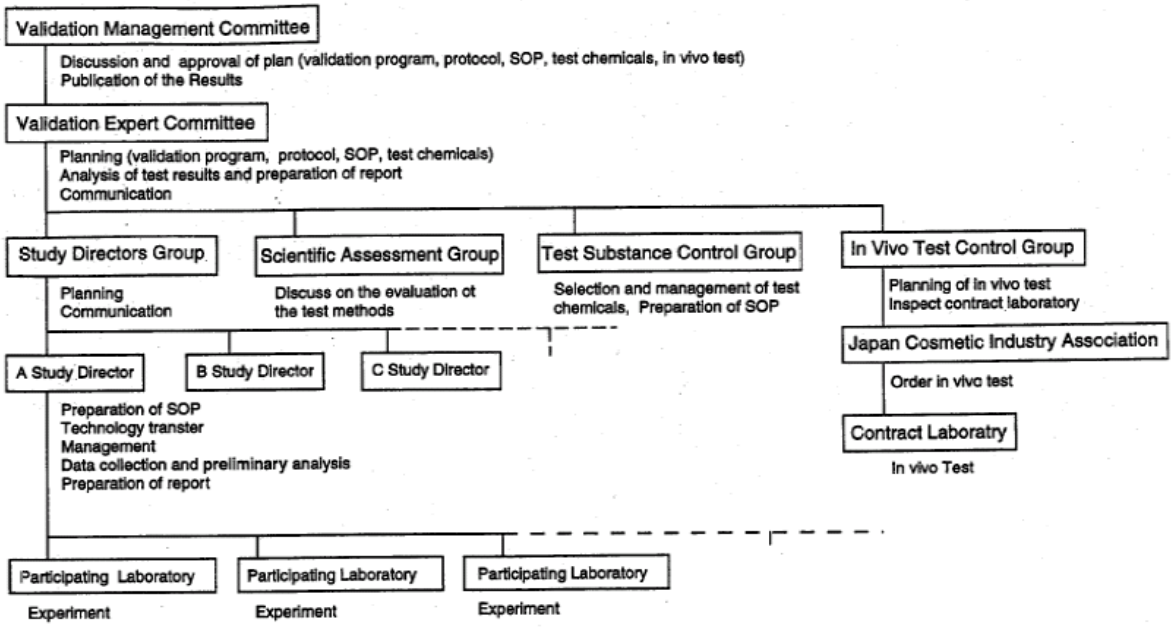
Kit suppliers
Oriental Yeast Co. Ltd
Kurabo Industries, Ltd
Invitro International Japan, Ltd
Toyobo Co., Ltd

Others
RIKEN Gene Bank
Japan Seigiken Research Centre Co. Ltd

Japan Cosmetic Industry Corporation
Shiseido Safety & Analytical Res. Center
POLA Corp.
Kanebo Ltd
KOSE Corp.
Lion Corp.
KAO Corp.
SUNSTAR Inc.
OPPEN Cosmetic Co. Ltd
NOEVIR Co. Ltd
Kaminomoto Co. Ltd
Procter & Gamble Far East, Inc.
Nippon Menard Cosmetic Co. Ltd
Yakult Central Institute for Microbiological Res.
Ajinomoto Co. Inc.
Cow Brand Soap Kyoshinsha Co., Ltd
Hoyu Co. Ltd
CLUB COSMETICS Co. Ltd
Nippon Shikizai Inc.

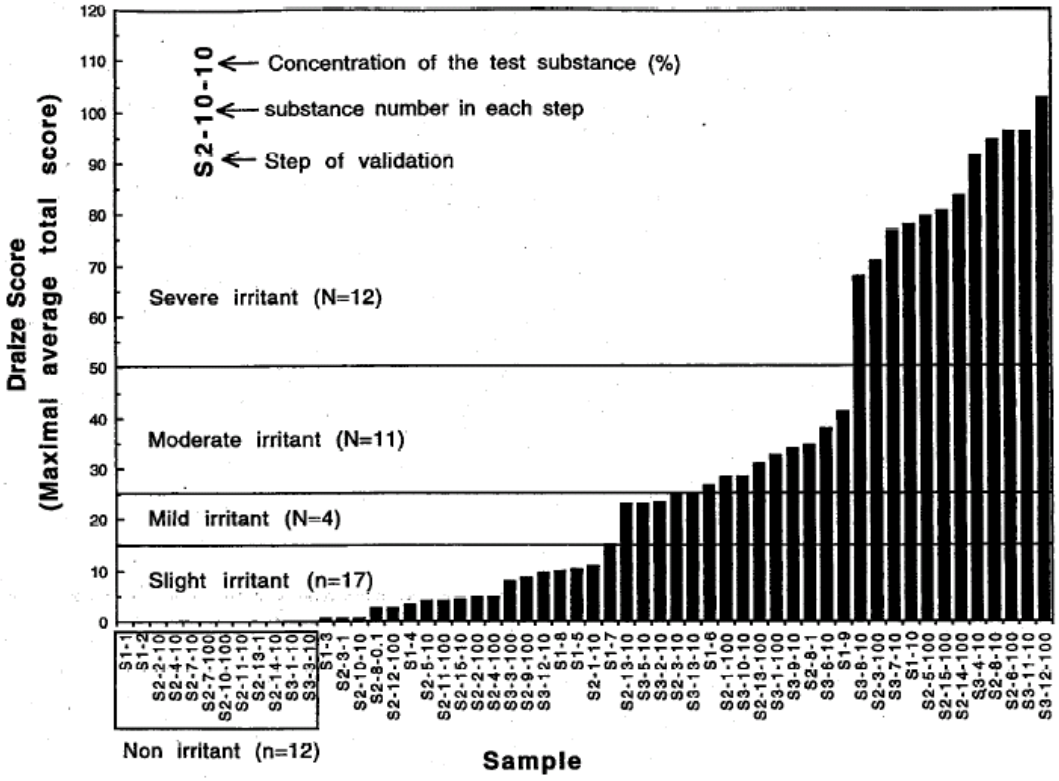
The table is the same as that reported by Ohno et al.(1999).

Fig. 2 Organization of the second and third validations



The figure is the same as that reported by Ohno et al.(1999).

Fig. # Eye irritation potential of test samples used in the Japanese validaton study



Abscissa indicates the sample number and ordinate indicates the maximal average scores (MAS). The first two characters of the sample number indicate the stage of the validation, the next two numerals indicate the identification number of the test substance in each validation, and the last numerals indicate the concentration of the test substance which was applied to the eyes of the rabbits. Chemical names corresponding to each number of the test substances are indicated separately.

Sample no.	and chemical name
S1-1	Isotonic sodium chloride solution
S1-2	Polyoxyethylene hydrogenated caster oil (60 E.O.)
S1-3	Polyoxyethylene sorbitan monolaurate (20 E.O.)
S1-4	Polyethyleneglycol monolaurate (10 E.O.)
S1-5	Sodium N-lauryl sarcosinate (30% solution)
S1-6	Sodium hydrogenated tallow L-glutamate
S1-7	Sodium lauryl sulfate
S1-8	Sodium polyoxyethylene laurylether sulfate (2 E.O.) (27% solution)
S1-9	Polyoxyethylene octylphenylether (10 E.O.)
S1-10	Benzalkonium chloride
S2-1	Sucrose fatty scid ester
S2-2	Glycerin
S2-3	Acid red 92
S2-4	Polyoxyethylene sorbitan monooleate (20E.O.)
S2-5	Calcium thioglycolate
S2-6	Distearyldimethylammonium chloride
S2-7	2-Ethylhexyl p-dimethylamino benzonate
S2-8	Cetylpyridinium chloride
S2-9	Methyl p-hydroxybenzoate
S2-10	Isopropyl myristate
S2-11	Polyethylene glycol 400
S2-12	Silicic anhydride
S2-13	Benzyl alcohol
S2-14	Sodium salicylate
S2-15	m-Phenylenediamine
S3-1	Ethanol
S3-2	Monoethanolamine
S3-3	Triethanolamine
S3-4	Stearyltrimethylammonium chloride
S3-5	Diisopropanolamine
S3-6	Potassium laurate
S3-7	Cetyltrimethylammonium bromide
S3-8	Acetic acid
S3-9	Butanol
S3-10	Chlorhexidine gluconate (20% solution)
S3-11	Domiphen bromide
S3-12	Lactic acid
S3-13	Glycolic acid
S3-14	Di (2-ethylhexyl) sodium sulfosuccinate

Table 5 List of the test substances and their characteristics

Substance no.	Name	Test substances		10% Aqueous solutions	
		Class	Nature	Nature	pH
S1-1	Isotonic sodium chloride solution	–	Solution	Solution	5.71
S1-2	Polyoxyethylene hydrogenated castor oil (60 E.O.)	Surfactants (nonionic)	White wax	Solution	4.17
S1-3	Polyoxyethylene sorbitan monolaurate (20 E.O.) (Tween 80)	Surfactants (nonionic)	Yellow liquid	Solution	6.79
S1-4	Polyethyleneglycol monolaurate (10 E.O.)	Surfactants (nonionic)	Liquid	Solution	3.86
S1-5	Sodium *N*-lauroyl sarcosinate (30% solution)	Surfactants (anionic)	Liquid	Solution	7.57
S1-6	Sodium *N*-hydrogenated tallow L-glutamate	Surfactants (anionic)	White powder	Suspension	6.85
S1-7	Sodium lauryl sulfate	Surfactants (anionic)	White flake	Solution	5.98
S1-8	Sodium polyoxyethylene laurylether sulfate (2E.O.) (27% solution)	Surfactants (anionic)	Liquid	Solution	6.65
S1-9	Polyoxyethylene octylphenylether (10 E.O.) (Triton X-100)	Surfactants (nonionic)	Liquid	Solution	6.35
S1-10	Benzalkonium chloride	Surfactants (cationic)	White powder	Suspension	4.97
S2-1	Sucrose fatty acid ester	Surfactants (nonionic)	White powder	Suspension	6.86
S2-2	Glycerin	Polyols	Liquid	Solution	5.96
S2-3	Acid Red 92	Colour additives	Red powder	Red solution	8.27
S2-4	Polyoxyethylene sorbitan monooleate (20 E.O.)	Surfactants (nonionic)	Liquid	Solution	6.23
S2-5	Calcium thioglycolate	Organic salts	White powder	turbid sol.	**11.57**
S2-6	Distearyldimethylammonium chloride	Surfactants (cationic)	White flake	turbid sol.	5.51
S2-7	2-Ethylhexyl *p*-dimethylamino benzoate	PABA derivatives	Liquid	Suspension	4.74
S2-8	Cetylpyridinium chloride	Surfactants (cationic)	White powder	Solution	4.41
S2-9	Methyl *p*-hydroxybenzoate	Esters	White powder	Suspension	4.99
S2-10	Isopropyl myristate	Esters	Liquid	Suspension	6.72
S2-11	Polyethylene glycol 400	Polyols	Liquid	Solution	5.05
S2-12	Silicic acid	Inorganics	White powder	Suspension	5.74
S2-13	Benzyl alcohol	Alcohols	Liquid	Suspension	6.44
S2-14	Sodium salicylate	Organic salts	Particle	Solution	6.50
S2-15	*m*-Phenylene diamine	Amines	Black pellet	Solution	8.56
S3-1	Ethanol	Alcohols	Volatile liquid	Solution	5.90
S3-2	Monoethanolamine	Alkanolamines	Liquid	Solution	**12.58**
S3-3	Triethanolamine	Alkanolamines	Liquid	Solution	**11.26**
S3-4	Stearyltrimethylammonium chloride	Surfactants (cationic)	Solid, Liquid	Solution	4.24
S3-5	Diisopropanolamine	Alkanolamines	White powder	Solution	**11.89**
S3-6	Potassium laurate	Surfactants (anionic)	White powder	Solution	10.49
S3-7	Cetyltrimethylammonium bromide	Surfactants (cationic)	Wax	Solution	5.89
S3-8	Acetic acid	Carboxilic acids	Liquid	Solution	**2.40**
S3-9	Butanol	Alcohols	Volatile liquid	Suspension	7.31
S3-10	Chlorhexidine gluconate solution (20% solution)	Organic salts	Liquid	Solution	6.56
S3-11	Domiphen bromide	Surfactants (cationic)	White powder	Solution	6.22
S3-12	Lactic acid	Carboxilic acids	Liquid	Solution	**1.94**
S3-13	Glycolic acid	Carboxilic acids	White powder	Solution	**1.76**
S3-14	Di(2-ethylhexyl) sodium sulfosuccinate	Surfactants (anionic)	White powder	Suspension	6.54

Table 6 GHS classification of serious eye damage / eye irritation

Caterory of GHS	Decision by in vivo test (Draize test) result	Decision by existing classification
1	・At least in one animal, effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of 21 days after installation of the test material ・At least in 2 of 3 tested animals, the average values of the scores following grading at 24, 48 and 72 hours after installation of the test material are 3 or more in corneal opacity, and more than 1.5 in iritis	・The substance which is classified as Severe or Corrosive (very strong irritation or corrosiveness corresponding to AOI 80 or more) is classified as Category 1 (however, when irreversible lesion is not observed, the substance is determined as irritating to the eye (Category 2A)).
2A	・In the Draize test conducted using 3 animals, the average values of the scores following grading at 24, 48 and 72 hours after installation of the test material in two or more animals are 1 or more in corneal opacity, 1 or more in iritis, 2 or more in conjunctival redness and 2 or more in conjunctival edema. ・The effects are fully reversed within an observation period of 21 days.	・The substance which is classified as Moderate (strong irritation corresponding to AOI 30–80) is classified as Category 2A.
2B	・In the Draize test conducted using 3 animals, the average values of the scores following grading at 24, 48 and 72 hours after installation of the test material in two or more animals are 1 or more in corneal opacity, 1 or more in iritis, 2 or more in conjunctival redness and 2 or more in conjunctival edema. ・The substance is classified as mildly irritating to the eye (Category 2B) when the above description applies to the substance and the effect reverses within 7 days.	・The substance which is classified as Mild is classified as Category 2B.

Decision by physico-chemical properties: In the case of pH ≤ 2, ≥ 11.5, the substance is classified as Category 1 (determined with buffer capacity taken into consideration (Booman et al. (1989) proposed 0.2 meq HCL/g in eye irritation).

The table is the same as technical guidance document on the GHS classification.

Table 7 Grading of eye irritation by Kay and Calandra method

Scoring Index (Maximal average score)	Grading
0.0 - 5.0	None irritant
5.1 - 15.0	Minimal irritant
15.1 - 30.0	Mild irritant
30.1 - 60.0	Moderate irritant
60.1 - 80.0	Severe irritant
80.1 - 110.0	Extreme irritant

The grading was reported by Kay and Calandra (1962).

Table 8 Grading of eye irritation reported by Ohno et al.(1999)

Scoring Index (Maximal average score)	Grading
0.0 - 15.0	Slight
15.1 - 25.0	Mild irritant
25.1 - 50.0	Moderate irritant
50.1 - 110.0	Severe irritant

The grading was reported by Ohno et al. (1999).

Table 9 Grading of eye irritation reported by Ohno et al.(2004).

Scoring Index (Maximal average score)	Grading
0.0 - 5.0	Slight
5.1 - 25.0	Mild irritant
25.1 - 50.0	Moderate irritant
50.1 - 110.0	Severe irritant

The grading was reported by Ohno (2004).

Table 10 Draize eye test results in the Japanese validation study （Concentration ：10%）

Substance （Concentration ：10%）	MAS	GHS
Ethanol	0.0	NI
2-Ethylhexyl p-dimethylamino benzonate	0.0	NI
Glycerin	0.0	NI
Polyethylene glycol 400	0.0	NI
Polyoxyethylene hydrogenated caster oil (60 E.O.)	0.0	NI
Polyoxyethylene sorbitan monooleate (20E.O.)	0.0	NI
Sodium salicylate	0.0	NI
Triethanolamine	0.0	NI
Isopropyl myristate	0.7	NI
Polyoxyethylene sorbitan monolaurate (20 E.O.)	0.7	NI
Polyethyleneglycol monolaurate (10 E.O.)	3.3	NI
Calcium thioglycolate	4.0	NI
m-Phenylenediamine <lack of stability>	4.3	NI
Lactic acid	9.7	NI
Sodium polyoxyethylene laurylether sulfate (2 E.O.) (27% solution)	10.0	NI
Sodium N-lauryl sarcosinate (30% solution)	10.3	NI
Sucrose fatty acid ester	11.0	NI
Diisopropanolamine	23.0	NI
Sodium lauryl sulfate	15.0[$]	1or2A
Benzyl alcohol	23.0	1or2A
Monoethanolamine	23.3	2B
Acid red 92	25.0	1or2A
Glycolic acid	25.0	2B
Sodium hydrogenated tallow L-glutamate	26.7	1or2A
Chlorhexidine gluconate (20% solution)	28.3	2A
Butanol	34.0	1or2A
Potassium laurate	38.0	1or2A
Polyoxyethylene octylphenylether (10 E.O.)	41.3	1or2A
Di (2-ethylhexyl) sodium sulfosuccinate	57.0	1or2A
Acetic acid	68.0	1or2A
Cetyltrimethylammonium bromide	76.7	1or2A
Benzalkonium chloride	78.0	1or2A
Stearyltrimethylammonium chloride	91.3	1or2A
Cetylpyridinium chloride	94.7	1
Domiphen bromide	96.3	1

MAS: Maximal average score of the Draize eye test.
GHS category 1: Severe or corrosive irritant, 2A: Irritant, 2B: Irritant, NI: Non irritant.
1or2A: The Draize eye test results couldn't discriminate between 1 and 2A for no observation data on day 21. The observation was performed to day 14.
$: Sodium laury sulfate was evaluated as positive because 2 of 3 individuals had the corneal damage of 15 and 10 (for the maximal corneal score), respectively.

Table 11 Draize eye test results in the Japanese validation study （as is）

Substance （as is）	Physical state	MAS	GHS
2-Ethylhexyl p-dimethylamino benzonate	Liquid	0.0	NI
Isopropyl myristate	Liquid	0.0	NI
Isotonic sodium chloride solution	Liquid	0.0	NI
Silicic anhydride	Powder	2.7	NI
Polyethylene glycol 400	Liquid	4.0	NI
Glycerin	Liquid	4.7	NI
Polyoxyethylene sorbitan monooleate (20 E.O.)	Liquid	4.7	NI
Triethanolamine	Liquid	8.0	NI
Methyl p-hydroxybenzoate	Powder	8.7	NI
Sucrose fatty acid ester	Powder	28.3	1or2A
Benzyl alcohol	Liquid	31.0	1or2A
Ethanol	Liquid	32.7	1or2A
Acid red 92	Powder	71.0	1or2A
Calcium thioglycolate	Powder	79.7	1
m-Phenylenediamine	Powder	80.7	1or2A
Sodium salicylate	Powder	83.7	1or2A
Distearyldimethylammonium chloride	Powder	96.3	1
Lactic acid	Liquid	102.7	1

Table 12 Draize eye test results in the Japanese validation study （Concentration ：1%）

Substace (Concentration:1%)	MAS	GHS
Benzyl alcohol	0	NI
Acid Red 92	0.7	NI
Cetylpyridinium chloride	34.7	1or2A

Table 13 Draize eye test results in the Japanese validation study （Concentration ：0.1%）

Substance (Concentration:0.1%)	MAS	GHS
Cetylpyridinium chloride	2.7	NI

Table 14 Draize eye test results in the Japanese validation study

Substance （Concentration ：10%）	Concentration	MAS	GHS
2-Ethylhexyl p-dimethylamino benzonate	as is	0.0	NI
Isopropyl myristate	as is	0.0	NI
Isotonic sodium chloride solution	as is	0.0	NI
Silicic anhydride	as is	2.7	NI
Polyethylene glycol 400	as is	4.0	NI
Glycerin	as is	4.7	NI
Polyoxyethylene sorbitan monooleate (20 E.O.)	as is	4.7	NI
Triethanolamine	as is	8.0	NI
Methyl p-hydroxybenzoate	as is	8.7	NI
Sucrose fatty acid ester	as is	28.3	1or2A
Benzyl alcohol	as is	31.0	1or2A
Ethanol	as is	32.7	1or2A
Acid red 92	as is	71.0	1or2A
Calcium thioglycolate	as is	79.7	1
Sodium salicylate	as is	83.7	1or2A
Distearyldimethylammonium chloride	as is	96.3	1
Lactic acid	as is	102.7	1
Ethanol	10	0.0	NI
2-Ethylhexyl p-dimethylamino benzonate	10	0.0	NI
Glycerin	10	0.0	NI
Polyethylene glycol 400	10	0.0	NI
Polyoxyethylene hydrogenated caster oil (60 E.O.)	10	0.0	NI
Polyoxyethylene sorbitan monooleate (20E.O.)	10	0.0	NI
Sodium salicylate	10	0.0	NI
Triethanolamine	10	0.0	NI
Isopropyl myristate	10	0.7	NI
Polyoxyethylene sorbitan monolaurate (20 E.O.)	10	0.7	NI
Polyethyleneglycol monolaurate (10 E.O.)	10	3.3	NI
Calcium thioglycolate	10	4.0	NI
Lactic acid	10	9.7	NI
Sodium polyoxyethylene laurylether sulfate (2 E.O.) (27% solution)	10	10.0	NI
Sodium N-lauryl sarcosinate (30% solution)	10	10.3	NI
Sucrose fatty acid ester	10	11.0	NI
Diisopropanolamine	10	23.0	NI
Sodium lauryl sulfate	10	15.0$	1or2A
Benzyl alcohol	10	23.0	1or2A
Monoethanolamine	10	23.3	2B
Acid red 92	10	25.0	1or2A
Glycolic acid	10	25.0	2B
Sodium hydrogenated tallow L-glutamate	10	26.7	1or2A
Chlorhexidine gluconate (20% solution)	10	28.3	2A
Butanol	10	34.0	1or2A
Potassium laurate	10	38.0	1or2A
Polyoxyethylene octylphenylether (10 E.O.)	10	41.3	1or2A
Di (2-ethylhexyl) sodium sulfosuccinate	10	57.0	1or2A
Acetic acid	10	68.0	1or2A
Cetyltrimethylammonium bromide	10	76.7	1or2A
Benzalkonium chloride	10	78.0	1or2A
Stearyltrimethylammonium chloride	10	91.3	1or2A
Cetylpyridinium chloride	10	94.7	1
Domiphen bromide	10	96.3	1
Benzyl alcohol	1	0	NI
Acid Red 92	1	0.7	NI
Cetylpyridinium chloride	1	34.7	1or2A
Cetylpyridinium chloride	0.1	2.7	NI

MAS: Maximal average score of the Draize eye test.

GHS category 1: Severe or corrosive irritant, 2A: Irritant, 2B: Irritant, NI: Non irritant.

1or2A: The Draize eye test results couldn't discriminate between 1 and 2A for no observation data on day 21. The observation was performed to day 14.

$: Sodium laury sulfate was evaluated as positive because 2 of 3 individuals had the corneal damage of 15 and 10 (at the maximal corneal score), respectively.

Table 15 Draize eye test results in the Japanese validation study
-GHS classification by considering pH-

Substance (Concentration :10%)	pH	MAS	GHS
Ethanol	5.90	0.0	NI
2-Ethylhexyl p-dimethylamino benzonate	4.74	0.0	NI
Glycerin	5.96	0.0	NI
Polyethylene glycol 400	5.05	0.0	NI
Polyoxyethylene hydrogenated caster oil (60 E.O.)	4.17	0.0	NI
Polyoxyethylene sorbitan monooleate (20E.O.)	6.23	0.0	NI
Sodium salicylate	6.50	0.0	NI
Triethanolamine	11.26	0.0	NI
Isopropyl myristate	6.72	0.7	NI
Polyoxyethylene sorbitan monolaurate (20 E.O.)	6.79	0.7	NI
Polyethyleneglycol monolaurate (10 E.O.)	3.86	3.3	NI
Sodium polyoxyethylene laurylether sulfate (2 E.O.) (27% solution)	6.65	10.0	NI
Sodium N-lauryl sarcosinate (30% solution)	7.57	10.3	NI
Sucrose fatty acid ester	6.86	11.0	NI
Calcium thioglycolate	11.57	4.0	1*
Lactic acid	1.94	9.7	1*
Sodium lauryl sulfate	5.98	15.0[$]	2Aor1
Benzyl alcohol	6.44	23.0	2Aor1
Diisopropanolamine	11.89	23.0	1*
Monoethanolamine	12.58	23.3	1*
Acid red 92	8.27	25.0	2Aor1
Glycolic acid	1.76	25.0	1*
Sodium hydrogenated tallow L-glutamate	6.85	26.7	2Aor1
Chlorhexidine gluconate (20% solution)	6.56	28.3	2A
Butanol	7.31	34.0	2Aor1
Potassium laurate	10.49	38.0	2Aor1
Polyoxyethylene octylphenylether (10 E.O.)	6.35	41.3	2Aor1
Di (2-ethylhexyl) sodium sulfosuccinate	6.54	57.0	2Aor1
Acetic acid	2.40	68.0	2Aor1
Cetyltrimethylammonium bromide	5.89	76.7	2Aor1
Benzalkonium chloride	4.97	78.0	2Aor1
Stearyltrimethylammonium chloride	4.24	91.3	2Aor1
Cetylpyridinium chloride	4.41	94.7	1
Domiphen bromide	6.22	96.3	1

MAS: Maximal average score of the Draize eye test.
GHS category 1: Severe or corrosive irritant, 2A: Irritant, 2B: Irritant, NI: Non irritant.
1or2A: The Draize eye test results couldn't discriminate between 1 and 2A for no observation data on day 21. The observation was performed to day 14.
$: Sodium laury sulfate was evaluated as positive because 2 of 3 individuals had the corneal damage of 15 and 10 (at the maximal corneal score), respectively.
: Category 1 means the classification on the basis of pH (pH≤2 or pH≥11.5: severe or corrosive irritant).

Table 16 Recovery time in the Draize eye test of the Japanese validation study
-GHS classification by considering pH-

Substance (Concentration :10%)	pH	MAS	GHS	Recovery time (hr)
Ethanol	5.90	0.0	NI	0
2-Ethylhexyl p-dimethylamino benzonate	4.74	0.0	NI	0
Glycerin	5.96	0.0	NI	0
Polyethylene glycol 400	5.05	0.0	NI	0
Polyoxyethylene hydrogenated caster oil (60 E.O.)	4.17	0.0	NI	0
Polyoxyethylene sorbitan monooleate (20E.O.)	6.23	0.0	NI	0
Sodium salicylate	6.50	0.0	NI	0
Triethanolamine	11.26	0.0	NI	0
Isopropyl myristate	6.72	0.7	NI	4
Polyoxyethylene sorbitan monolaurate (20 E.O.)	6.79	0.7	NI	4
Polyethyleneglycol monolaurate (10 E.O.)	3.86	3.3	NI	24
Sodium polyoxyethylene laurylether sulfate (2 E.O.) (27% solution)	6.65	10.0	NI	96
Sodium N-lauryl sarcosinate (30% solution)	7.57	10.3	NI	120
Sucrose fatty acid ester	6.86	11.0	NI	72
Calcium thioglycolate	11.57	4.0	1*	72
Lactic acid	1.94	9.7	1*	168<
Sodium lauryl sulfate	5.98	15.0$	1or2A	168<
Benzyl alcohol	6.44	23.0	1or2A	168<
Diisopropanolamine	11.89	23.0	1*	72
Monoethanolamine	12.58	23.3	1*	144
Acid red 92	8.27	25.0	1or2A	168<
Glycolic acid	1.76	25.0	1*	144
Sodium hydrogenated tallow L-glutamate	6.85	26.7	1or2A	168<
Chlorhexidine gluconate (20% solution)	6.56	28.3	2A	168<
Butanol	7.31	34.0	1or2A	168<
Potassium laurate	10.49	38.0	1or2A	168<
Polyoxyethylene octylphenylether (10 E.O.)	6.35	41.3	1or2A	168<
Di (2-ethylhexyl) sodium sulfosuccinate	6.54	57.0	1or2A	168<
Acetic acid	2.40	68.0	1or2A	168<
Cetyltrimethylammonium bromide	5.89	76.7	1or2A	168<
Benzalkonium chloride	4.97	78.0	1or2A	168<
Stearyltrimethylammonium chloride	4.24	91.3	1or2A	168<
Cetylpyridinium chloride	4.41	94.7	1	168<
Domiphen bromide	6.22	96.3	1	168<

MAS: Maximal average score of the Draize eye test.
GHS category 1: Severe or corrosive irritant, 2A: Irritant, 2B: Irritant, NI: Non irritant.
1or2A: The Draize eye test results couldn't discriminate between 1 and 2A for no observation data on day 21. The observation was performed to day 14.
$: Sodium laury sulfate was evaluated as positive because 2 of 3 individuals had the corneal damage of 15 and 10 (at the maximal corneal score), respectively.
: Category 1 means the classification on the basis of pH (pH≤2 or pH≥11.5: severe or corrosive irritant).

Table 17 Recovery time in the Draize eye test of the Japanese validation study （as is）

Substance （as is）	Physical state	MAS	GHS	Recovery time (hr)
2-Ethylhexyl p-dimethylamino benzonate	Liquid	0.0	NI	0
Isopropyl myristate	Liquid	0.0	NI	0
Isotonic sodium chloride solution	Liquid	0.0	NI	0
Silicic anhydride	Powder	2.7	NI	24
Polyethylene glycol 400	Liquid	4.0	NI	24
Glycerin	Liquid	4.7	NI	24
Polyoxyethylene sorbitan monooleate (20 E.O.)	Liquid	4.7	NI	48
Triethanolamine	Liquid	8.0	NI	72
Methyl p-hydroxybenzoate	Powder	8.7	NI	168
Sucrose fatty acid ester	Powder	28.3	1or2A	168<
Benzyl alcohol	Liquid	31.0	1or2A	168<
Ethanol	Liquid	32.7	1or2A	168<
Acid red 92	Powder	71.0	1or2A	168<
Calcium thioglycolate	Powder	79.7	1	168<
m-Phenylenediamine	Powder	80.7	1or2A	168<
Sodium salicylate	Powder	83.7	1or2A	168<
Distearyldimethylammonium chloride	Powder	96.3	1	168<
Lactic acid	Liquid	102.7	1	168<

Table 18 Recovery time in the Draize eye test of the Japanese validation study
（Concentration ：1%）

Substace (Concentration:1%)	MAS	GHS	Recovery time (hr)
Benzyl alcohol	0	NI	0
Acid Red 92	0.7	NI	24
Cetylpyridinium chloride	34.7	1or2A	168<

Table 19 Recovery time in the Draize eye test of the Japanese validation study
（Concentration ：0.1%）

Substance (Concentration:0.1%)	MAS	GHS	Recovery time (hr)
Cetylpyridinium chloride	2.7	NI	72

Table 20 Regional difference in the Draize eye test results (0<MAS<50) of the Japanese validation study

Substance	Concn (%)	MAS	GHS	Cornea	Iris	Conjunctivae	Recovery time (hr)
Acid Red 92	1	0.7	NI	0	0	0.7	24
Isopropyl myristate	10	0.7	NI	0	0	0.7	4
Polyoxyethylene sorbitan monolaurate (20 E.O.)	10	0.7	NI	0	0	0.7	4
Cetylpyridinium chloride	0.1	2.7	NI	0	0	2.7	72
Silicic anhydride	100	2.7	NI	0	0	2.7	24
Polyethyleneglycol monolaurate (10 E.O.)	10	3.3	NI	0	0	3.3	24
Polyethylene glycol 400	100	4.0	NI	0	0	4.0	24
Glycerin	100	4.7	NI	0	0	4.7	24
Polyoxyethylene sorbitan monooleate (20 E.O.)	100	4.7	NI	0	0	4.7	48
Sodium polyoxyethylene laurylether sulfate (2 E.O.) (27% solution)	10	10.0	NI	3.3	0	10.0	96
Sodium N-lauryl sarcosinate (30% solution)	10	10.3	NI	8.3	0	8.0	120
Sucrose fatty scid ester	10	11.0	NI	1.7	1.7	9.3	72
Calcium thioglycolate	10	4.0	I*	0	0	4.0	72
Lactic acid	10	9.7	I*	5.0	0	8.0	168<
Sodium lauryl sulfate	10	15.0$	I	8.3	0	10.0	168<
Benzyl alcohol	10	23.0	1or2A	15.0	1.7	10.0	168<
Diisopropanolamine	10	23.0	1*	16.7	1.7	4.7	72
Monoethanolamine	10	23.3	1*	13.3	1.7	10.0	144
Acid red 92	10	25.0	1or2A	20.0	1.7	10.0	168<
Glycolic acid	10	25.0	1*	15.0	0.0	14.0	144
Sodium hydrogenated tallow L-glutamate	10	26.7	1or2A	16.7	1.7	12.0	168<
Chlorhexidine gluconate (20% solution)	10	28.3	2A	18.3	1.7	12.7	168<
Sucrose fatty scid ester	100	28.3	1or2A	23.3	1.7	8.0	168<
Benzyl alcohol	100	31.0	1or2A	25.0	1.7	8.7	168<
Ethanol	100	32.7	1or2A	26.7	0.0	8.7	168<
Butanol	10	34.0	1or2A	30.0	1.7	10.0	168<
Potassium laurate	10	38.0	1or2A	30.0	1.7	10.0	168<
Cetylpyridinium chloride	1	34.7	1or2A	21.7	1.7	12.7	168<
Polyoxyethylene octylphenylether (10 E.O.)	10	41.3	1or2A	30.0	5.0	10.0	168<

MAS: Maximal average score of the Draize eye test.

GHS category 1: Severe or corrosive irritant, 2A: Irritant, 2B: Irritant, NI: Non irritant.

1or2A: The Draize eye test results couldn't discriminate between 1 and 2A for no observation data on day 21. The observation was performed to day 14.

$: Sodium laury sulfate was evaluated as positive because 2 of 3 individuals had the corneal damage of 15 and 10 (at the maximal corneal score), respectively.

: Category 1 means the classification on the basis of pH (pH≤2 or pH≥11.5: severe or corrosive irritant).

Table 21 Relationship between MMAS and GHS in the Draize eye test

	Test chemical	CAS No.	Supplier	Purity (%)	In vivo Draize		
					MMAS[a]	EU[b]	GHS[c]
1	3,3-Dimethylpentane	562-49-2	Aldrich	99	0.0	NI	NI
2	3-Methoxy-1,2-propanediol	623-39-2	Acros	98	0.0	NI	NI
3	Polyethylene glycol 400	25322-68-3	Aldrich	–	0.0	NI	NI
4	Glycerol	56-81-5	Sigma	99.5	1.7	NI	NI
5	Methyl cyclopentane	96-37-7	Fluka	>95	3.7	NI	NI
6	Tween 20	9005-64-5	Sigma	–	4.0	NI	NI
7	Methyl isobutyl ketone	108-10-1	Fluka	>99	4.8	NI	NI
8	Toluene	108-88-3	Acros	⩽99.5	9.0	NI	NI
9	Methyl amyl ketone	110-43-0	Aldrich	99	10.5/16.3	NI	NI
10	2-Methyl-1-pentanol	105-30-6	Acros	98.5	13.0	NI	2
11	Ethanol	64-17-5	Merck	⩽99.8	24.0	NI	2
12	Sodium hydroxide (1%)[d]	1310-73-2	Merck	⩽99	25.8	R36	2
13	Triton X-100 (5%)[d]	9002-93-1	Acros	SG	32.3	R36	2
14	1-Octanol	111-87-5	Aldrich	99	41.0	R36	2
15	2-Ethyl-1-hexanol	104-76-7	Aldrich	99	51.3	R36	2
16	*n*-Hexanol	111-27-3	Acros	98	64.8	R36	2
17	Acetone	67-64-1	Fluka	–	65.8	R36	2
18	Cyclohexanol	108-93-0	Aldrich	99	79.8	R41	1
19	Cetylpyridinium bromide (6%)[d]	140-72-7	Sigma	>99	85.8	R41	1
20	Benzalkonium chloride (10%)[d]	8001-54-5	Sigma	Ultra	108.0	R41	1

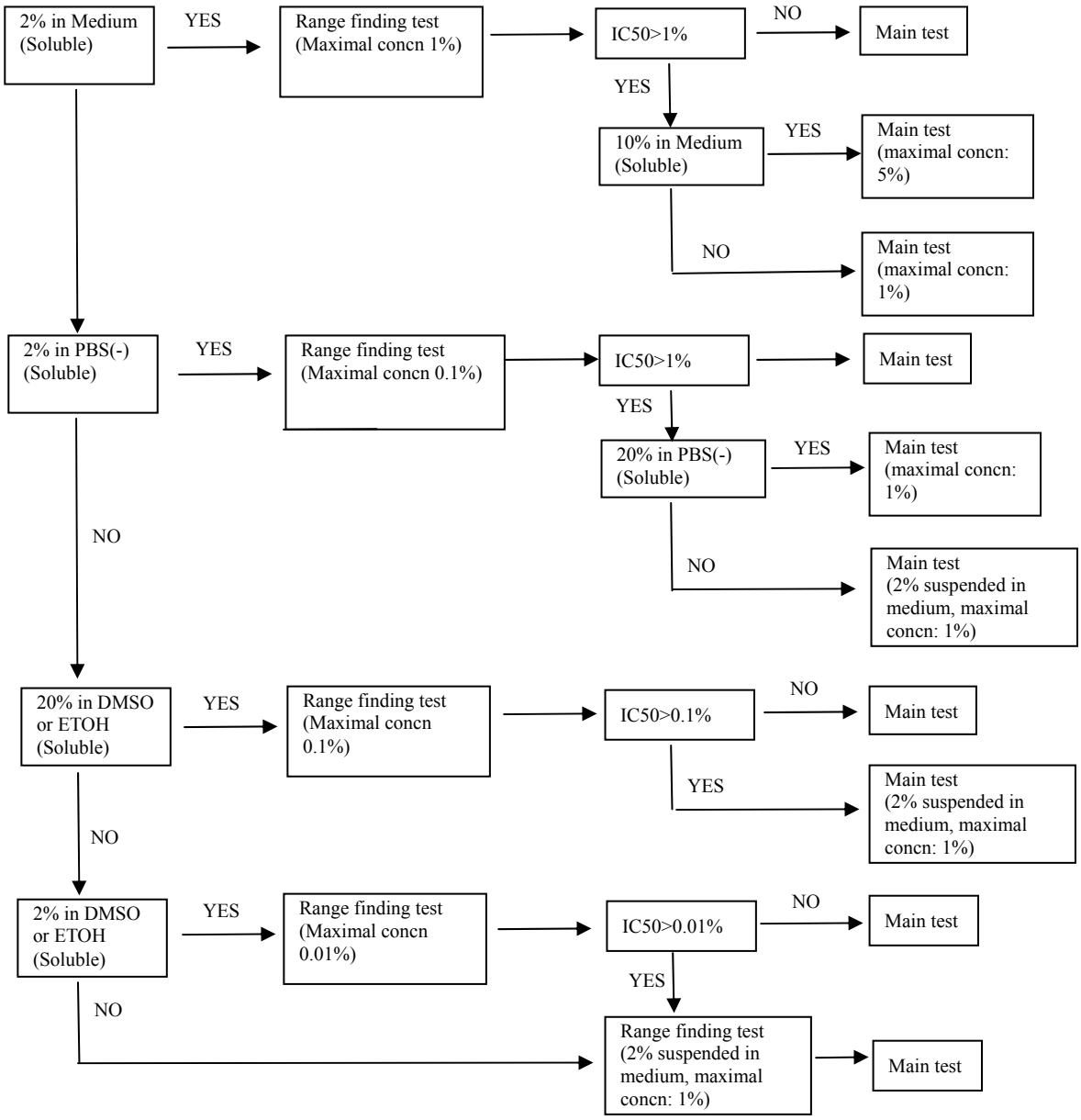
The data are the same as that reported by Goethem et al. (2006)

Table 22 Methods of the SIRC-NRU assay and the SIRC-CVS assay

Cell and culture conditions	SIRC cells derived from rabbit cornea were obtained from the American Type Culture Collection. These were cultured and distributed by the Japanese Cancer Research Resources Bank (JCRB) to each laboratory. The passage numbers of the cells used in each laboratory ranged from 417 to 425. The absence of contamination by mycoplasma was confirmed before each experiment by the JCRB and afterwards by several laboratories. SIRC cells were cultured in Eagle's MEM supplemented with 10% calf serum in a CO_2 incubator (5% in air) at 37°C. The mean (± SD) doubling time of the cells, determined in every laboratory was 20 hr (± 3.8 hr, n = 17).	
Cytotoxicity assay	*SIRC–NRU method* Various concentrations of each test substance were made up by dissolving the substance in appropriate solvent or suspending in culture medium. The solutions (100 μl) were then poured into each well of a 96-well microplate. SIRC cells were harvested from preculture bottles by trypsinization, washed once, and then resuspended (2×10^5 cells/ml) in the culture medium. A 100-μl aliquot of the cell suspension was introduced gradually into each well. The plates were maintained at room temperature for 20 min to allow the cells to settle on the bottom of the well. The cells were then cultured for 3 days. The culture medium was replaced with 250 μl fresh medium that contained neutral red (NR) (50 μg/ml) and incubated for another 3 hr. The medium was then removed and the cells were rapidly washed with an aqueous solution of 1% formaldehyde and 1% $CaCl_2$. NR that was incorporated into viable cells was extracted with 100 μl 1% acetic acid in 50% ethanol. After 15 min at room temperature, the microplates were gently agitated by a microplate shaker and the absorbance at 540 nm was measured by an automatic microplate reader. The mean absorbance of 10 wells that contained no test substance was regarded as the control value. The absorbance of the other wells was calculated as a percentage of the control absorbance. Five wells were used for each concentration of test substance. The concentration of each substance which inhibits cell viability to 50% of control (EC_{50}) was obtained from the dose–response curve.	*SIRC–CVS method* SIRC–CVS in the first phase of the validation study was performed according to the procedure for SIRC–NRU. After incubation for 3 days, dead cells were washed off with Ca^{++}-, Mg^{++}-free phosphate buffered saline (PBS (–)). The cells attached to the bottom of the plate were fixed and stained with 0.4% crystal violet solution in methanol for 30 min. The plate was washed with water, and the absorbance at 590 nm was measured by an automatic microplate reader. The SIRC–CVS method was modified for the second and third phases of validation for application to the plates used for SIRC–NRU investigation. The plates used for SIRC–NRU were washed off twice with PBS (–) and stained again with 0.4% crystal violet solution in methanol for 30 min. The other procedures were the same as these listed previously.
Minor Modifications of experimental procedure	At the time of the second and the third phases of the validation, the participating laboratories were asked to select the solvent for dissolving the test substances according to the detailed scheme described in the SOP. In addition, the SIRC–CVS was modified as described above.	

The contents are the same as those reported by Tani et al. (1999).

Fig. 3 Flow chart for preparation of test sample in cytotoxicity test



The figure that reported by Kojima (1999) is translated into English.

Table 23 Results of interlaboratory reproducibility on the SIRC-CVS assay
(Concentration: 10%, Negative reference: Tween 20)

Substance	MAS	GHS	IC50 of the SIRC-CVS assay (μg/mL)						
(Draize eye test was performed at 10% concentration)	at 10%	at 10%	Lab. A	Lab. B	Lab. C	Lab. D	Lab. E	Lab. F	Average±SD
Ethanol	0.0	NI	10000<	10000<	10000<	10000<	10000<	NT	10000<
2-Ethylhexyl p-dimethylamino benzonate	0.0	NI	381	1193	570	97.5	484	120	474±400
Glycerin	0.0	NI	12746	5347.5	5350	6750	12500	27000	11600±8260
Polyethylene glycol 400	0.0	NI	6854.5	50000<	47500	32750	34500	40000	35300<
Polyoxyethylene hydrogenated caster oil (60 E.O.)	0.0	NI	2945	2792	3487	2375	3687	3350	3110±490
Polyoxyethylene sorbitan monooleate (20E.O.)	0.0	NI	745	762	1075	1075	710	1400	963±272
Sodium salicylate	0.0	NI	840	559	1195	950	635	1525	952±364
Triethanolamine	0.0	NI	1440	1430	1750	1993	3850	NT	2090±1010
Isopropyl myristate	0.7	NI	10000<	10000<	10000<	6000	10000<	10000<	9330<
Polyoxyethylene sorbitan monolaurate (20 E.O.) =Tween 20	0.7	NI	541	794	737	675	1228	625	767±243
Polyethyleneglycol monolaurate (10 E.O.)	3.3	NI	330	406	245	305	574	123	348±128
Calcium thioglycolate	4.0	NI	300	660	287.5	420	292.5	600< (Retest)	392±159 (Data from 5 labs)
m-Phenylenediamine (Lack of stability)	4.3	NI	167	73	290	255	167	355	218±102
Lactic acid	9.7	NI	994	982	1315	1285	1575	NT	1230±248
Sodium polyoxyethylene laurylether sulfate (2 E.O.) (27% solution)	10.0	NI	686	662	865	765	773	735	747±72.3
Sodium N-lauryl sarcosinate (30% solution)	10.3	NI	454	490	338	425	495	430	439±57.5
Sucrose fatty acid ester	11.0	NI	250	304	292.5	315	294.5	257.5	286±26
Diisopropanolamine	23.0	NI	455	901	720	170	1250	NT	699±414
Sodium lauryl sulfate	15.0$	1or2A	182	172	117	190	198	149	168±30.1
Benzyl alcohol	23.0	1or2A	1148	888.5	1485	1100	830	1675	1190±335
Monoethanolamine	23.3	2B	4.46	9.8	5.9	10.5	17.5	NT	9.62±5.08
Acid red 92	25.0	1or2A	230	231	340	332.5	268.5	380	297±62.7
Glycolic acid	25.0	2B	914	682	890	778	1075	NT	868±148
Sodium hydrogenated tallow L-glutamate	26.7	1or2A	143	118	113	90.8	235	1115	140±56.1
Chlorhexidine gluconate (20% solution)	28.3	2A	67.2	44.8	67.5	45.8	112.5	NT	67.6±27.4
Butanol	34.0	1or2A	10000<	4395	10000<	10000<	10000<	NT	8880<
Potassium laurate	38.0	1or2A	103	117	73 #	110	150	NT	120±20.9 (Data from 4 labs)
Polyoxyethylene octylphenylether (10 E.O.)	41.3	1or2A	26.7	38.0	23.3	32.3	51.0	59.5	38.4±14.2
Di (2-ethylhexyl) sodium sulfosuccinate	57.0	1or2A	210	182	181	156	175	NT	181±19.5
Acetic acid	68.0	1or2A	681	691	690	795 #	820	NT	721±66.5 (Data from 4labs)
Cetyltrimethylammonium bromide	76.7	1or2A	2.95	3.21	1.72	2.3> #	2.50	NT	2.59±0.654 (Data from 4labs)
Benzalkonium chloride	78.0	1or2A	16.2	25.2	13.2	15.5	29.0	15.0	19.0±6.50
Stearyltrimethylammonium chloride	91.3	1or2A	1.07	1.47	1.31	1.17	2.90	NT	1.58±0.752
Cetylpyridinium chloride	94.7	1	0.53	0.96	2.55	0.88	2.25	2.85	1.67±0.99
Domiphen bromide	96.3	1	13.4	11.4	7.55	13.4	14.8	NT	12.1±2.81

The data were taken from Tani et al. (1999). The classification of positive or negative using MAS was based on 15 as a cut-off point As reported by Tani et al.(1999), m-phenylenediamine was excluded from the subsequent analysis due to instability.
MAS: Maximal average score of the Draize eye test.
GHS category 1: Severe or corrosive irritant, 2A: Irritant, 2B: Irritant, NI: Non irritant.
1or2A: The Draize eye test results couldn't discriminate between 1 and 2A for no observation data on day 21. The observation was performed to day 14.
$: Sodium laury sulfate was evaluated as positive because 2 of 3 individuals had the corneal damage of 15 and 10 (for the maximal corneal score), respectively.
#: Derail from SOP
SD: Standard deviation
NT: Not tested

Table 24 Results of interlaboratory reproducibility on the SIRC-CVS assay
(Concentration: 10%, Negative reference: Sucrose fatty acid ester)

Substance	MAS	GHS	IC50 of the SIRC-CVS assay (μg/mL)						
(Draize eye test was performed at 10% concentration)	at 10%	at 10%	Lab. A	Lab. B	Lab. C	Lab. D	Lab. E	Lab. F	Average±SD
Ethanol	0.0	NI	10000<	10000<	10000<	10000<	10000<	NT	10000<
2-Ethylhexyl p-dimethylamino benzonate	0.0	NI	381	1193	570	97.5	484	120	474±400
Glycerin	0.0	NI	12746	5347.5	5350	6750	12500	27000	11600±8260
Polyethylene glycol 400	0.0	NI	6854.5	50000<	47500	32750	34500	40000	35300<
Polyoxyethylene hydrogenated caster oil (60 E.O.)	0.0	NI	2945	2792	3487	2375	3687	3350	3110±490
Polyoxyethylene sorbitan monooleate (20E.O.)	0.0	NI	745	762	1075	1075	710	1400	963±272
Sodium salicylate	0.0	NI	840	559	1195	950	635	1525	952±364
Triethanolamine	0.0	NI	1440	1430	1750	1993	3850	NT	2090±1010
Isopropyl myristate	0.7	NI	10000<	10000<	10000<	6000	10000<	10000<	9330<
Polyoxyethylene sorbitan monolaurate (20 E.O.) =Tween 20	0.7	NI	541	794	737	675	1228	625	767±243
Polyethyleneglycol monolaurate (10 E.O.)	3.3	NI	330	406	245	305	574	123	348±128
Calcium thioglycolate	4.0	NI	300	660	287.5	420	292.5	600< (Retest)	392±159 (Data from 5 labs)
m-Phenylenediamine (Lack of stability)	4.3	NI	167	73	290	255	167	355	218±102
Lactic acid	9.7	NI	994	982	1315	1285	1575	NT	1230±248
Sodium polyoxyethylene laurylether sulfate (2 E.O.) (27% solution)	10.0	NI	686	662	865	765	773	735	747±72.3
Sodium N-lauryl sarcosinate (30% solution)	10.3	NI	454	490	338	425	495	430	439±57.5
Sucrose fatty acid ester	11.0	NI	250	304	292.5	315	294.5	257.5	286±26
Diisopropanolamine	23.0	NI	455	901	720	170	1250	NT	699±414
Sodium lauryl sulfate	15.0$	1or2A	182	172	117	190	198	149	168±30.1
Benzyl alcohol	23.0	1or2A	1148	888.5	1485	1100	830	1675	1190±335
Monoethanolamine	23.3	2B	4.46	9.8	5.9	10.5	17.5	NT	9.62±5.08
Acid red 92	25.0	1or2A	230	231	340	332.5	268.5	380	297±62.7
Glycolic acid	25.0	2B	914	682	890	778	1075	NT	868±148
Sodium hydrogenated tallow L-glutamate	26.7	1or2A	143	118	113	90.8	235	1115	140±56.1
Chlorhexidine gluconate (20% solution)	28.3	2A	67.2	44.8	67.5	45.8	112.5	NT	67.6±27.4
Butanol	34.0	1or2A	10000<	4395	10000<	10000<	10000<	NT	8880<
Potassium laurate	38.0	1or2A	103	117	73 #	110	150	NT	120±20.9 (Data from 4 labs)
Polyoxyethylene octylphenylether (10 E.O.)	41.3	1or2A	26.7	38.0	23.3	32.3	51.0	59.5	38.4±14.2
Di (2-ethylhexyl) sodium sulfosuccinate	57.0	1or2A	210	182	181	156	175	NT	181±19.5
Acetic acid	68.0	1or2A	681	691	690	795 #	820	NT	721±66.5 (Data from 4labs)
Cetyltrimethylammonium bromide	76.7	1or2A	2.95	3.21	1.72	2.3> #	2.50	NT	2.59±0.654 (Data from 4labs)
Benzalkonium chloride	78.0	1or2A	16.2	25.2	13.2	15.5	29.0	15.0	19.0±6.50
Stearyltrimethylammonium chloride	91.3	1or2A	1.07	1.47	1.31	1.17	2.90	NT	1.58±0.752
Cetylpyridinium chloride	94.7	1	0.53	0.96	2.55	0.88	2.25	2.85	1.67±0.99
Domiphen bromide	96.3	1	13.4	11.4	7.55	13.4	14.8	NT	12.1±2.81

The data were taken from Tani et al. (1999). The classification of positive or negative using MAS was based on 15 as a cut-off point As reported by Tani et al.(1999), m-phenylenediamine was excluded from the subsequent analysis due to instability.
MAS: Maximal average score of the Draize eye test.
GHS category 1: Severe or corrosive irritant, 2A: Irritant, 2B: Irritant, NI: Non irritant.
1or2A: The Draize eye test results couldn't discriminate between 1 and 2A for no observation data on day 21. The observation was performed to day 14.
$: Sodium laury sulfate was evaluated as positive because 2 of 3 individuals had the corneal damage of 15 and 10 (for the maximal corneal score), respectively.
#: Derail from SOP
SD: Standard deviation
NT: Not tested

Table 25 Results of interlaboratory reproducibility on the SIRC-CVS assay
(Remaining substances)

Substance (Draize eye test was not performed at 10% concentration)	MAS as is	GHS as is	IC50 of the SIRC-CVS assay (μg/mL)						
			Lab. A	Lab. B	Lab. C	Lab. D	Lab. E	Lab. F	Average±SD
Isotonic sodium chloride solution	0.0	NI	10000<	10000<	10000<	10000<	10000<	10000<	10000<
Silicic anhydride	2.7	NI	10000<	10000<	10000<	38750	10000<	10000<	14800<
Methyl p-hydroxybenzoate	8.7	NI	103	214	257	195	215.5	255	207±56.4
Distearyldimethylammonium chloride	96.3	1	18.5	43.8	57	35.5	32.1	39.7	37.8±12.8

Table 26 Results of interlaboratory reproducibility on the SIRC-CVS assay (as is)

Substance (Application was as is in the Draize eye test.)	MAS as is	GHS as is	IC50 of the SIRC-CVS assay (μg/mL)						
			Lab. A	Lab. B	Lab. C	Lab. D	Lab. E	Lab. F	Average±SD
2-Ethylhexyl p-dimethylamino benzonate	0.0	NI	381	1193	570	97.5	484	120	474±400
Isopropyl myristate	0.0	NI	10000<	10000<	10000<	6000	10000<	10000<	9330<
Isotonic sodium chloride solution	0.0	NI	10000<	10000<	10000<	10000<	10000<	10000<	10000<
Silicic anhydride	2.7	NI	10000<	10000<	10000<	38750	10000<	10000<	14800<
Polyethylene glycol 400	4.0	NI	6854.5	50000<	47500	32750	34500	40000	35300<
Glycerin	4.7	NI	12746	5347.5	5350	6750	12500	27000	11600±8260
Polyoxyethylene sorbitan monooleate (20E.O.)	4.7	NI	745	762	1075	1075	710	1400	963±272
Triethanolamine	8.0	NI	1440	1430	1750	1993	3850	NT	2090±1010
Methyl p-hydroxybenzoate	8.7	NI	103	214	257	195	215.5	255	207±56.4
Sucrose fatty acid ester	28.3	1or2A	250	304	292.5	315	294.5	257.5	286±26
Benzyl alcohol	31.0	1or2A	1148	888.5	1485	1100	830	1675	1190±335
Ethanol	32.7	1or2A	10000<	10000<	10000<	10000<	10000<	NT	10000<
Acid red 92	71.0	1or2A	230	231	340	332.5	268.5	380	297±62.7
Calcium thioglycolate	79.7	1	300	660	287.5	420	292.5	600< (Retest)	392±159 (Data from 5 labs)
m-Phenylenediamine (Lack of stability)	80.7	1or2A	167	73	290	255	167	355	218±102
Sodium salicylate	83.7	1or2A	840	559	1195	950	635	1525	952±364
Distearyldimethylammonium chloride	96.3	1	18.5	43.8	57	35.5	32.1	39.7	37.8±12.8
Lactic acid	102.7	1	994	982	1315	1285	1575	NT	1230±248

NT：Not tested

Table 27 Results of interlaboratory reproducibility on the SIRC-CVS assay
(Concentration: 1%)

Substance (Draize eye test was performed at 1% concentration)	MAS at 1%	GHS at 1%	IC50 of the SIRC-CVS assay (μg/mL)						
			Lab. A	Lab. B	Lab. C	Lab. D	Lab. E	Lab. F	Average±SD
Benzyl alcohol	0	NI	1148	888.5	1485	1100	830	1675	1190±335
Acid red 92	0.7	NI	230	231	340	332.5	268.5	380	297±62.7
Cetylpyridinium chloride	34.7	1or2A	0.53	0.96	2.55	0.88	2.245	2.85	1.67±0.99

Table 28 Results of interlaboratory reproducibility on the SIRC-CVS cytotoxicity test
(Concentration: 0.1%)

Substance (Draize eye test was performed at 0.1% concentration)	MAS at 10%	GHS at 10%	IC50 of the SIRC-CVS assay (μg/mL)						
			Lab. A	Lab. B	Lab. C	Lab. D	Lab. E	Lab. F	Average±SD
Cetylpyridinium chloride	2.7	NI	0.53	0.96	2.55	0.88	2.245	2.85	1.67±0.99

Table 29 Results of interlaboratory reproducibility on the SIRC-CVS assay
(Concentration: 10%, Negative reference: Tween 20)
-GHS classification by considering pH-

Substance (Draize eye test was performed at 10% concentration)	MAS at 10%	GHS at 10%	IC50 of the SIRC-CVS assay (μg/mL)						
			Lab. A	Lab. B	Lab. C	Lab. D	Lab. E	Lab. F	Average±SD
Ethanol	0.0	NI	10000<	10000<	10000<	10000<	10000<	NT	10000<
2-Ethylhexyl p-dimethylamino benzonate	0.0	NI	381	1193	570	97.5	484	120	474±400
Glycerin	0.0	NI	12746	5347.5	5350	6750	12500	27000	11600±8260
Polyethylene glycol 400	0.0	NI	6854.5	50000<	47500	32750	34500	40000	35300<
Polyoxyethylene hydrogenated caster oil (60 E.O.)	0.0	NI	2945	2792	3487	2375	3687	3350	3110±490
Polyoxyethylene sorbitan monooleate (20E.O.)	0.0	NI	745	762	1075	1075	710	1400	963±272
Sodium salicylate	0.0	NI	840	559	1195	950	635	1525	952±364
Triethanolamine	0.0	NI	1440	1430	1750	1993	3850	NT	2090±1010
Isopropyl myristate	0.7	NI	10000<	10000<	10000<	6000	10000<	10000<	9330<
Polyoxyethylene sorbitan monolaurate (20 E.O.) =Tween 20	0.7	NI	541	794	737	675	1228	625	767±243
Polyethyleneglycol monolaurate (10 E.O.)	3.3	NI	330	406	245	305	574	123	348±128
m-Phenylenediamine （Lack of stability）	4.3	NI	167	73	290	255	167	355	218±102
Sodium polyoxyethylene laurylether sulfate (2 E.O.) (27% solution)	10.0	NI	686	662	865	765	773	735	747±72.3
Sodium N-lauryl sarcosinate (30% solution)	10.3	NI	454	490	338	425	495	430	439±57.5
Sucrose fatty acid ester	11.0	NI	250	304	292.5	315	294.5	257.5	286±26
Calcium thioglycolate	4.0	1*	300	660	287.5	420	292.5	600< (Retest)	392±159 (Data from 5 labs)
Lactic acid	9.7	1*	994	982	1315	1285	1575	NT	1230±248
Sodium lauryl sulfate	15.0[$]	1or2A	182	172	117	190	198	149	168±30.1
Benzyl alcohol	23.0	1or2A	1148	888.5	1485	1100	830	1675	1190±335
Diisopropanolamine	23.0	1*	455	901	720	170	1250	NT	699±414
Monoethanolamine	23.3	1*	4.46	9.8	5.9	10.5	17.5	NT	9.62±5.08
Acid red 92	25.0	1or2A	230	231	340	332.5	268.5	380	297±62.7
Glycolic acid	25.0	1*	914	682	890	778	1075	NT	868±148
Sodium hydrogenated tallow L-glutamate	26.7	1or2A	143	118	113	90.8	235	1115	140±56.1
Chlorhexidine gluconate (20% solution)	28.3	2A	67.2	44.8	67.5	45.8	112.5	NT	67.6±27.4
Butanol	34.0	1or2A	10000<	4395	10000<	10000<	10000<	NT	8880<
Potassium laurate	38.0	1or2A	103	117	73 #	110	150	NT	120±20.9 (Data from 4 labs)
Polyoxyethylene octylphenylether (10 E.O.)	41.3	1or2A	26.7	38.0	23.3	32.3	51.0	59.5	38.4±14.2
Di (2-ethylhexyl) sodium sulfosuccinate	57.0	1or2A	210	182	181	156	175	NT	181±19.5
Acetic acid	68.0	1or2A	681	691	690	795 #	820	NT	721±66.5 (Data from 4labs)
Cetyltrimethylammonium bromide	76.7	1or2A	2.95	3.21	1.72	2.3> #	2.50	NT	2.59±0.654 (Data from 4labs)
Benzalkonium chloride	78.0	1or2A	16.2	25.2	13.2	15.5	29.0	15.0	19.0±6.50
Stearyltrimethylammonium chloride	91.3	1or2A	1.07	1.47	1.31	1.17	2.90	NT	1.58±0.752
Cetylpyridinium chloride	94.7	1	0.53	0.96	2.55	0.88	2.245	2.85	1.67±0.99
Domiphen bromide	96.3	1	13.4	11.4	7.55	13.4	14.8	NT	12.1±2.81

NT：Not tested
#:Derail from SOP

Table 30 Results of interlaboratory reproducibility on the SIRC-CVS assay
(Concentration: 10%, Negative reference: Sucrose fatty acid ester)
-GHS classification by considering pH-

Substance (Draize eye test was performed at 10% concentration)	MAS at 10%	GHS at 10%	IC50 of the SIRC-CVS assay (μg/mL) Lab. A	Lab. B	Lab. C	Lab. D	Lab. E	Lab. F	Average±SD
Ethanol	0.0	NI	10000<	10000<	10000<	10000<	10000<	NT	10000<
2-Ethylhexyl p-dimethylamino benzonate	0.0	NI	381	1193	570	97.5	484	120	474±400
Glycerin	0.0	NI	12746	5347.5	5350	6750	12500	27000	11600±8260
Polyethylene glycol 400	0.0	NI	6854.5	50000<	47500	32750	34500	40000	35300<
Polyoxyethylene hydrogenated caster oil (60 E.O.)	0.0	NI	2945	2792	3487	2375	3687	3350	3110±490
Polyoxyethylene sorbitan monooleate (20E.O.)	0.0	NI	745	762	1075	1075	710	1400	963±272
Sodium salicylate	0.0	NI	840	559	1195	950	635	1525	952±364
Triethanolamine	0.0	NI	1440	1430	1750	1993	3850	NT	2090±1010
Isopropyl myristate	0.7	NI	10000<	10000<	10000<	6000	10000<	10000<	9330<
Polyoxyethylene sorbitan monolaurate (20 E.O.) =Tween 20	0.7	NI	541	794	737	675	1228	625	767±243
Polyethyleneglycol monolaurate (10 E.O.)	3.3	NI	330	406	245	305	574	123	348±128
m-Phenylenediamine （Lack of stability）	4.3	NI	167	73	290	255	167	355	218±102
Sodium polyoxyethylene laurylether sulfate (2 E.O.) (27% solution)	10.0	NI	686	662	865	765	773	735	747±72.3
Sodium N-lauryl sarcosinate (30% solution)	10.3	NI	454	490	338	425	495	430	439±57.5
Sucrose fatty acid ester	11.0	NI	250	304	292.5	315	294.5	257.5	286±26
Calcium thioglycolate	4.0	1*	300	660	287.5	420	292.5	600< (Retest)	392±159 (Data from 5 labs)
Lactic acid	9.7	1*	994	982	1315	1285	1575	NT	1230±248
Sodium lauryl sulfate	15.0[$]	1or2A	182	172	117	190	198	149	168±30.1
Benzyl alcohol	23.0	1or2A	1148	888.5	1485	1100	830	1675	1190±335
Diisopropanolamine	23.0	1*	455	901	720	170	1250	NT	699±414
Monoethanolamine	23.3	1*	4.46	9.8	5.9	10.5	17.5	NT	9.62±5.08
Acid red 92	25.0	1or2A	230	231	340	332.5	268.5	380	297±62.7
Glycolic acid	25.0	1*	914	682	890	778	1075	NT	868±148
Sodium hydrogenated tallow L-glutamate	26.7	1or2A	143	118	113	90.8	235	1115	140±56.1
Chlorhexidine gluconate (20% solution)	28.3	2A	67.2	44.8	67.5	45.8	112.5	NT	67.6±27.4
Butanol	34.0	1or2A	10000<	4395	10000<	10000<	10000<	NT	8880<
Potassium laurate	38.0	1or2A	103	117	73 #	110	150	NT	120±20.9 (Data from 4 labs)
Polyoxyethylene octylphenylether (10 E.O.)	41.3	1or2A	26.7	38.0	23.3	32.3	51.0	59.5	38.4±14.2
Di (2-ethylhexyl) sodium sulfosuccinate	57.0	1or2A	210	182	181	156	175	NT	181±19.5
Acetic acid	68.0	1or2A	681	691	690	795 #	820	NT	721±66.5 (Data from 4labs)
Cetyltrimethylammonium bromide	76.7	1or2A	2.95	3.21	1.72	2.3> #	2.50	NT	2.59±0.654 (Data from 4labs)
Benzalkonium chloride	78.0	1or2A	16.2	25.2	13.2	15.5	29.0	15.0	19.0±6.50
Stearyltrimethylammonium chloride	91.3	1or2A	1.07	1.47	1.31	1.17	2.90	NT	1.58±0.752
Cetylpyridinium chloride	94.7	1	0.53	0.96	2.55	0.88	2.245	2.85	1.67±0.99
Domiphen bromide	96.3	1	13.4	11.4	7.55	13.4	14.8	NT	12.1±2.81

NT：Not tested
#:Derail from SOP

Table 31 Results of the SIRC-NRU assay and the SIRC-CVS assay in the Japanese validation study

Substance no.	SIRC–NRU n*	Solvent†	EC_{50}‡ (μg/ml)	SD	CV	Rank	SIRC–CVS n	Solvent	EC_{50} (μg/ml)	SD	CV	Rank
S1-1	7	–	10000<	–	–	34	6	–	10000<	–	–	32
S1-2	7	–	2910	1600	55.1%	31	6	–	3110	490	15.8%	31
S1-3	7	–	946	230	24.3%	27	6	–	767	243	31.6%	24
S1-4	7	–	428	107	25.0%	19	6	–	348	128	36.8%	17
S1-5	7	–	444	157	35.4%	20	6	–	439	57.5	13.1%	19
S1-6	7	–	147	34.5	23.6%	11	6	–	140	56.1	40.2%	11
S1-7	7	–	171	25.2	14.8%	12	6	–	168	30.1	17.9%	12
S1-8	7	–	675	134	19.8%	23	6	–	747	72.3	9.7%	23
S1-9	7	–	41.8	16.8	40.2%	7	6	–	38.4	14.2	36.9%	8
S1-10	7	–	18.0	6.40	35.4%	6	6	–	19.0	6.50	34.0%	6
S2-1	1	M	320	–	–		1	M	315	–	–	
	2	MS	230	28.3	12.3%		1	MS	250	–	–	
	2	E	290	2.1	0.7%		2	E	298	7.99	2.7%	
	2	D	266	51.6	19.3%		2	D	276	26.2	9.5%	
	7	M + MS + E + D	271	41.0	15.1%	15	6	M + MS + E + D	286	26	9.1%	15
S2-2	7	M	9760	5060	51.8%	33	6	M	11600	8260	71.2%	32
S2-3	7	M	316	57.0	18.1%	17	6	M	297	62.7	21.1%	16
S2-4	7	M	1250	257	20.4%	28	6	M	963	272	28.2%	27
S2-5	6	MS	475	134	31.7%		4	MS	325	63.4	19.5%	
							1	MS	600<	–	–	
	1	D	589	–	–		1	D	660	–	–	
	7	MS + D	484	121	25.1%	21	5	MS + D	392	159	40.6%	18
S2-6	6	E	46.8	21	47.3%		5	E	36.6	13.9	38.0%	
	1	P	50.2	–	–		1	P	43.8	–	–	
	7	E + P	47.4	17.6	37.1%	8	6	E + P	37.8	12.8	33.9%	7
S2-7	2	MS	194	112	58.0%		3	MS	240	200	83.3%	
	5	D	412	220	53.4%		3	D	591	444	75.1%	
	7	MS + D	350	210	60.1%	18	6	MS + D	474	400	84.4%	20
S2-8	5	M	1.40	0.91	60.7%		4	M	2	1.2	60.0%	
	2	P	2.74	2.06	75.2%		2	P	1.56	0.97	62.2%	
	7	M + P	2.00	1.00	50.0%	2	6	M + P	1.67	0.99	59.3%	2
S2-9	7	D	173	37.0	21.5%	14	6	D	207	56.4	27.2%	14
S2-10	6	MS	10000<	–	–		5	MS	10000<	–	–	
	1	MS	8400	–	–		1	MS	6000	–	–	
	7	MS	9770<	–	–	34	6	MS	9330<	–	–	32
S2-11	6	M	30900	13900	45.0%		5	M	32300	15400	47.5%	
	1	M	50000<	–	–		1	M	50000<	–	–	
	7	M	33600<	–	–	34	6	M	35300<	–	–	32
S2-12	6	MS	10000<	–	–		5	MS	10000<	–	–	
	1	MS	43000	–	–		1	MS	38800	–	–	
	7	MS	20400<	–	–	34	6	MS	14800<	–	–	32
S2-13	7	M	1370	543	39.6%	29	6	M	1190	335	28.2%	28
S2-14	7	M	856	439	51.2%	25	6	M	952	364	38.2%	26
S2-15§	6	M	255	–	–		5	M	190	–	–	
	1	E	365	–	–		1	E	355	–	–	
	7	M + E	271	–	–	–	6	M + E	218	–	–	–
S3-1	6	M	10000<	–	–	34	5	M	10000<	–	–	32
S3-2	6	M	12.6	6.31	50.1%	5	5	M	9.62	5.08	52.9%	4
S3-3	6	M	3140	820	26.1%	32	5	M	2090	1010	48.3%	30
S3-4	2	M	1.93	0.534	27.6%		2	M	1.27	0.283	22.4%	
	2	P	2.22	0.375	16.9%		1	P	1.17	–	–	
	2	MS	1.74	0.933	53.6%		2	MS	2.11	1.12	53.4%	
	6	M + P + MS	1.96	0.552	28.1%	1	5	M + P + MS	1.58	0.752	47.6%	1
S3-5	6	M	1370	391	28.6%	30	5	M	699	414	59.2%	21
S3-6	6	P	126	16.4	13.1%	10	4	P	120	20.9	17.4%	10
S3-7	2	M	3.30	0.633	19.2%		1	M	3.21	–	–	
	2	P	3.50	0.361	10.3%		1	P	2.95	–	–	
	1	E	1.80	–	–		1	E	2.50	–	–	
	1	MS	1.15	–	–		1	MS	1.72	–	–	
	6	M + P + E + MS	2.76	1.07	38.8%	3	4	M + P + E + MS	2.59	0.654	25.2%	3
S3-8	6	M	620	131	21.1%	22	4	M	721	66.5	9.2%	22
S3-9	2	M	3620	244	6.7%		1	M	4400	–	–	
	4	M	10000<	–	–		4	M	10000<	–	–	
	6	M	7870<	–	–	34	6	M	8880<	–	–	32
S3-10	6	D	92.2	37.3	40.5%	9	5	D	67.6	27.4	40.6%	9
S3-11	6	P	10.8	3.42	31.6%	4	5	P	12.1	2.81	23.3%	5
S3-12	6	M	938	289	30.8%	26	5	M	1230	248	20.2%	29
S3-13	6	M	774	197	25.4%	24	5	M	868	148	17.1%	25
S3-14	5	D	175	17.3	9.9%		5	D	181	19.5	10.8%	13
	1	MS	160	–	–							
	6	D + MS	172	16.5	9.6%	13						
S1-SLS	7	M	170	25.3	14.9%	–	6	M	162	33.9	20.9%	–
S2-SLS	7	M	170	15.0	8.8%	–	7	M	176	13.4	7.6%	–
S3-SLS	6	M	165	17.8	10.8%	–	5	M	167	23.2	13.8%	–

* = no. of data.
† = solvents were selected under a common SOP. (second and third phases of validation).
M = culture medium, MS: suspension in culture medium, P = PBS(–), D = DMSO, E = ethanol.
‡ = mean value of EC_{50} which was average of two EC_{50} results obtained in each laboratory.
§ = S2-15 was excluded from analysis due to instability.

Substance no.	and substance name
S1-1	Isotonic sodium chloride solution
S1-2	Polyoxyethylene hydrogenated caster oil (60 E.O.)
S1-3	Polyoxyethylene sorbitan monolaurate (20 E.O.)
S1-4	Polyethyleneglycol monolaurate (10 E.O.)
S1-5	Sodium N-lauryl sarcosinate (30% solution)
S1-6	Sodium hydrogenated tallow L-glutamate
S1-7	Sodium lauryl sulfate
S1-8	Sodium polyoxyethylene laurylether sulfate (2 E.O.) (27% solution)
S1-9	Polyoxyethylene octylphenylether (10 E.O.)
S1-10	Benzalkonium chloride
S2-1	Sucrose fatty scid ester
S2-2	Glycerin
S2-3	Acid red 92
S2-4	Polyoxyethylene sorbitan monooleate (20E.O.)
S2-5	Calcium thioglycolate
S2-7	2-Ethylhexyl p-dimethylamino benzonate
S2-8	Cetylpyridinium chloride
S2-10	Isopropyl myristate
S2-11	Polyethylene glycol 400
S2-13	Benzyl alcohol
S2-14	Sodium salicylate
S2-15	m-Phenylenediamine
S3-1	Ethanol
S3-2	Monoethanolamine
S3-3	Triethanolamine
S3-4	Stearyltrimethylammonium chloride
S3-5	Diisopropanolamine
S3-6	Potassium laurate
S3-7	Cetyltrimethylammonium bromide
S3-8	Acetic acid
S3-9	Butanol
S3-10	Chlorhexidine gluconate (20% solution)
S3-11	Domiphen bromide
S3-12	Lactic acid
S3-13	Glycolic acid
S3-14	Di (2-ethylhexyl) sodium sulfosuccinate

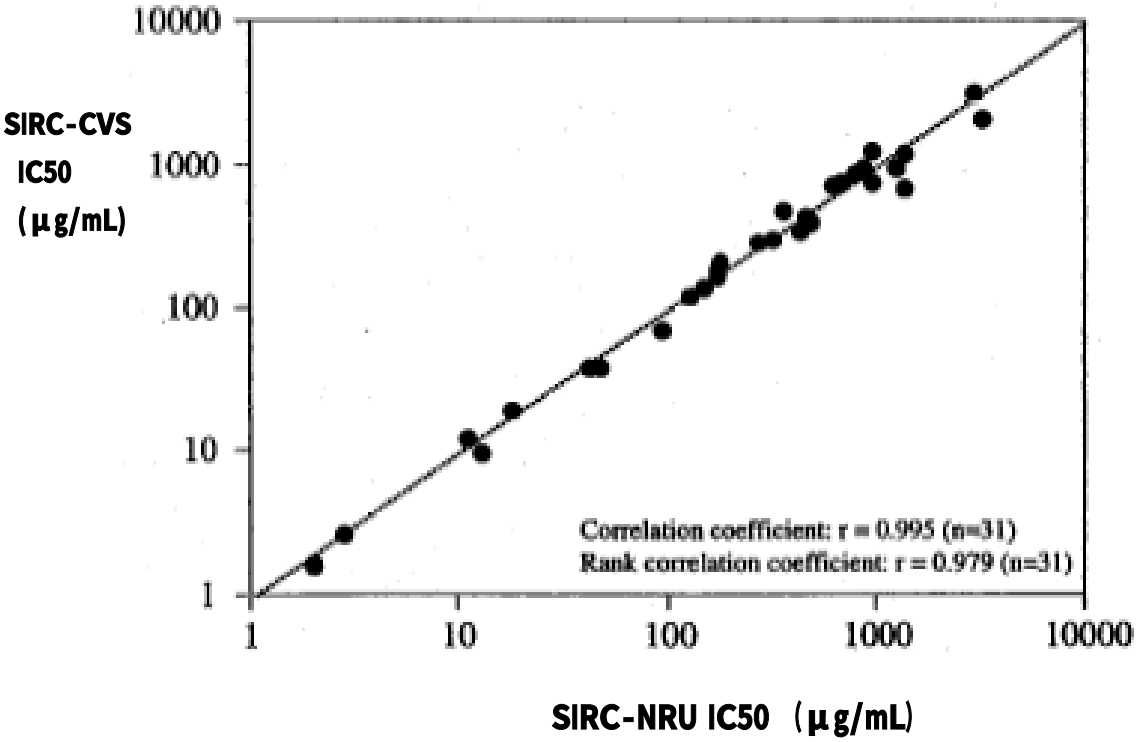
Data are the same as those reported by Tani et al.(1999).

Table 32 Correlation of the results between alternative methods in the Japanese validation study

	HET-CAM	CAM-TB	RBC	SKIN^{2TM}	MATREX^{TM}	CornePack*	SIRC-CVS	SIRC-NRU	HeLa-MTT	CHL-CVS	EYTEX^{TM}
HET-CAM	1.000										
CAM-TB	0.679	1.000									
RBC	−0.653	−0.743	1.000								
SKIN^{2TM}	−0.479	−0.811	**0.953**	1.000							
MATREX^{TM}	−0.628	−0.733	**0.936**	**0.931**	1.000						
CornePack*	−0.480	−0.690	0.892	0.846	**0.913**	1.000					
SIRC-CVS	−0.582	−0.820	0.809	0.838	0.813	0.773	1.000				
SIRC-NRU	−0.589	−0.823	0.814	0.821	0.814	0.768	**0.997**	1.000			
HeLa-MTT	−0.580	−0.831	0.838	0.872	0.840	0.812	**0.985**	**0.985**	1.000		
CHL-CVS	−0.545	−0.765	0.811	0.841	0.820	0.798	**0.972**	**0.968**	**0.969**	1.000	
EYTEX^{TM}	0.751	0.313	−0.542	−0.188	−0.389	−0.202	−0.397	−0.391	−0.370	−0.331	1.000

#: Non-irritants for which EC_{50} values are not determined are given maximum values.

Fig.4 Correlation between IC50 values obtained for SIRC-NRU assay and those of SIRC-CVS assay



Data are the same as those reported by Tani et al. (1999).

Table 33 Correlation of the results obtained by alternative methods and Draize eye test

Methods	Analysis using all data			Analysis excluding specific classes of chemicals			
		Correlation coefficients				Correlation coefficients	
	N	Pearson's linear	Spearman's rank	class###	N	Pearson's linear	Spearman's rank
Chorioallantoic membrane							
HET–CAM	52	0.688	0.802	1	46	0.702	0.831
				2	6	0.779	0.714
CAM–TB	55	0.718	0.838	1	48	0.801	0.863
				2	7	0.926	0.964
Red blood cells							
RBC	17	–0.631	0.643	3	16	–0.651	0.674
Haemoglobin							
RDC_{50}	8##	0.906	0.714				
1%RDR	23##	0.671	0.579				
1% λ max	31##	0.791	0.697				
Artificial skin models							
SKIN™ (ZK1100)#	30	–0.694	0.680	4	20	–0.842	
MATREX™#	30	–0.672	0.832	4	20	–0.754	
Normal cells from rabbit cornea							
CornePack*#	28	–0.538	0.588	4	21	–0.731	0.787
Cell lines from rabbit cornea							
SIRC–CVS#	29	–0.805	0.779	4	22	–0.924	0.945
SIRC–NRU#	30	–0.816	0.787	4	23	–0.916	0.931
Cell lines from the other mammals							
HeLa–MTT#	29	–0.799	0.745	4	22	–0.922	0.926
CHL–CVS#	29	–0.729	0.703	4	22	–0.864	0.880
EYTEX™	38	0.313					

#: log (EC_{50}) were correlated with Draize scores (maximal average total score). ##: include the data of substances of the first validation, for which the experiments were conducted afterwards, during the second and the third validations. ###: 1: liquid sample only, 2: powder sample only; 3: excluded strong alkali and acid samples; 4: excluded alcohol (lower mono-ol), strong acids and strong alkalis.

Data are the same as those of Ohno et al.(1999).

Table 34 Predictability of the alternative tests – Classification into positive and negative irritants

Methods	Analysis by using all data: No. of samples	Type of errors#	No. of errors	% of errors	Sample number falsely predicted	Analysis excluding specific chemical class##: No. of samples	Type of errors#	No. of errors	% of errors
Chorioallantoic membrane									
HET-CAM	52	FP	8	17.3	S1-4-10, S1-5-10, S1-8-10, S2-5-10, S2-9-100, S2-14-10, S3-3-100, S3-12-10	38	FP	7	18.4
		FN	1		S2-1-100		FN	0	
CAM-TB	55	FP	9	16.4	S1-4-10, S1-5-10, S1-8-10, S2-1-10, S2-3-1, S2-5-10, S2-8-0.1, S3-3-100, S3-12-10	41	FP	7	19.5
		FN	0				FN	1	
Red blood cells									
RBC	30	FP	4	30.0	S1-4, S1-5, S1-8, S2-1	24	FP	3	16.7
		FN	5		S2-13, S3-2, S3-5, S3-9, S3-10		FN	1	
Haemoglobin									
RDC_{50}	23###	FP	0####	26.1					
		FN	6####		S1-9, S2-13, S3-2, S3-5, S3-8, S3-9				
1% RDR	23###	FP	2####	34.7	S1-5, S1-8				
		FN	6####		S1-9, S2-13, S3-2, S3-5, S3-8, S3-9				
1% λmax	31###	FP	2####	29.0	S1-5, S1-8				
		FN	7####		S1-9, S2-13, S3-2, S3-5, S3-8, S3-9, S3-10				
Artificial dermal models									
SKIN^{2TM} (ZK1100)	33	FP	6	30.3	S1-3, S1-4, S1-5, S1-7, S1-8, S2-1	24	FP	5	25.0
		FN	4		S2-13, S3-5, S3-8, S3-9		FN	1	
MATREX™	34	FP	6	23.4	S1-3, S1-4, S1-5, S1-8, S2-1, S2-12	25	FP	5	20.0
		FN	2		S2-13, S3-9		FN	0	
Normal cells from rabbit cornea									
CornePack	35	FP	8	40.0	S1-2, S1-3, S1-4, S1-5, S1-8, S2-1, S2-4, S2-5	26	FP	4	15.4
		FN	6		S2-13, S3-2, S3-5, S3-8, S3-13, S3-9		FN	0	
Cell lines from rabbit cornea									
SIRC-CVS	34	FP	5	29.4	S1-4, S1-5, S2-1, S2-5, S2-7	25	FP	2	8.0
		FN	5		S2-13, S3-5, S3-8, S3-9, S3-13		FN	0	
SIRC-NRU	34	FP	6	29.4	S1-4, S1-5, S1-8, S2-1, S2-5, S2-7	25	FP	4	16.0
		FN	4		S2-13, S3-5,S3-9, S3-13		FN	0	
Cell lines from the other mammals									
HeLa-MTT	34	FP	5	29.4	S1-3, S1-4, S1-5, S1-8, S2-1	25	FP	3	16.0
		FN	5		S2-13, S3-5, S3-8, S3-9, S3-13		FN	1	
CHL-CVS	34	FP	6	29.4	S1-3, S1-4, S1-5, S2-1, S2-5, S2-7	25	FP	4	20.0
		FN	4		S2-13, S3-8, S3-9, S3-13		FN	1	
EYTEX™	54	FP	11	27.7	S1-7-10, S2-3-1, S2-4-100, S2-5-10, S2-9-100, S2-11-100 S2-13-10, S3-2-10, S3-3-10, S3-3-100, S3-4-10, S3-12-10	46	FP	15	37.0
		FN	5		S1-6-10, S1-9-10, S2-1-100, S2-6-100, S2-8-1, S3-9-10, S3-14-10		FN	2	

Eye irritation potentials were classified into two classes according to the Draize scores (0–15 and >15) and the number of false predictions was calculated from linear regression lines. #: FP: false positive, FN: false negative. ##: Regression lines were made excluding the data of powder sample in the case of HET-CAM and CAM-TB. The data for acids, alkalis, and alcohols (lower mono-ol) were excluded in the case of the other methods. ###: include the data of the test substances of the first validation, for which the experiments were conducted afterwards, during the second and the third validations. ####: estimated from the prediction according to their own potocols without using regression lines.

False positive: Polyethyleneglycol monolaurate (10 E.O.) , Sodium N-lauryl sarcosinate (30% solution), Sucrose fatty acid ester, Calcium thioglycolate, 2-Ethylhexyl p-dimethylamino benzonate
False negative: Benzyl alcohol, Diisopropanolamine, Acetic acid, Butanol, Glycolic acid

Substance no.	and substance name
S1-1	Isotonic sodium chloride solution
S1-2	Polyoxyethylene hydrogenated caster oil (60 E.O.)
S1-3	Polyoxyethylene sorbitan monolaurate (20 E.O.)
S1-4	Polyethyleneglycol monolaurate (10 E.O.)
S1-5	Sodium N-lauryl sarcosinate (30% solution)
S1-6	Sodium hydrogenated tallow L-glutamate
S1-7	Sodium lauryl sulfate
S1-8	Sodium polyoxyethylene laurylether sulfate (2 E.O.) (27% solution)
S1-9	Polyoxyethylene octylphenylether (10 E.O.)
S1-10	Benzalkonium chloride
S2-1	Sucrose fatty scid ester
S2-2	Glycerin
S2-3	Acid red 92
S2-4	Polyoxyethylene sorbitan monooleate (20E.O.)
S2-5	Calcium thioglycolate
S2-6	Distearyldimethylammonium chloride
S2-7	2-Ethylhexyl p-dimethylamino benzonate
S2-8	Cetylpyridinium chloride
S2-9	Methyl p-hydroxybenzoate
S2-10	Isopropyl myristate
S2-11	Polyethylene glycol 400
S2-12	Silicic anhydride
S2-13	Benzyl alcohol
S2-14	Sodium salicylate
S2-15	m-Phenylenediamine
S3-1	Ethanol
S3-2	Monoethanolamine
S3-3	Triethanolamine
S3-4	Stearyltrimethylammonium chloride
S3-5	Diisopropanolamine
S3-6	Potassium laurate
S3-7	Cetyltrimethylammonium bromide
S3-8	Acetic acid
S3-9	Butanol
S3-10	Chlorhexidine gluconate (20% solution)
S3-11	Domiphen bromide
S3-12	Lactic acid
S3-13	Glycolic acid
S3-14	Di (2-ethylhexyl) sodium sulfosuccinate

For example, "S2-3-1"means the application of substance "S2-3" at 1% concentration.

Fig. 5 Relationship between the SIRC-CVS assay and the Draize eye test

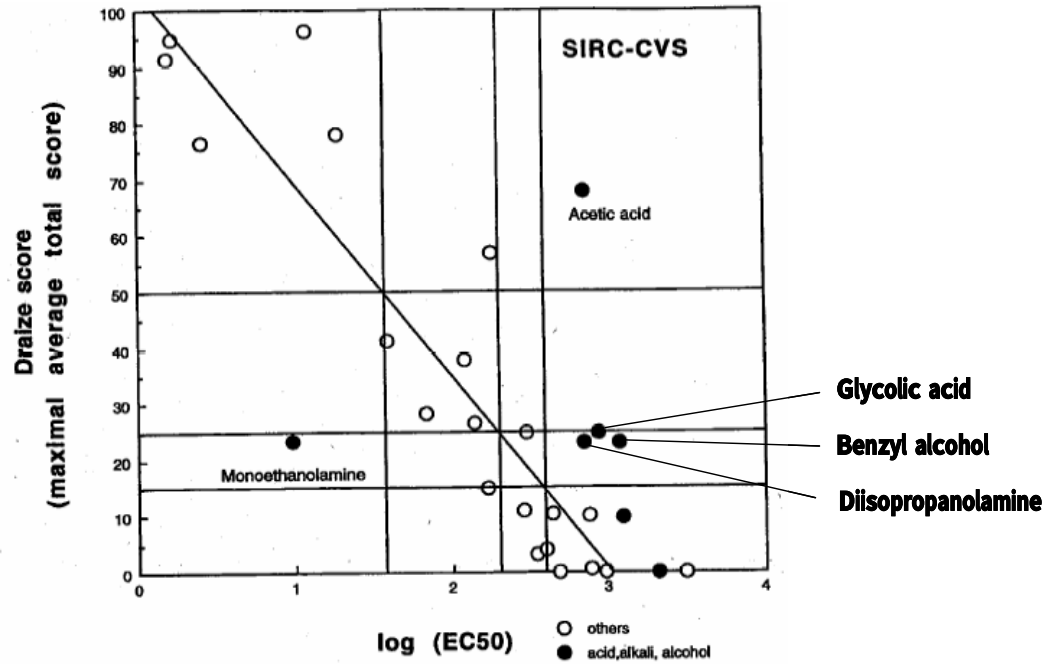


Fig. 6 Relationship between the SIRC-NRU assay and the Draize eye test

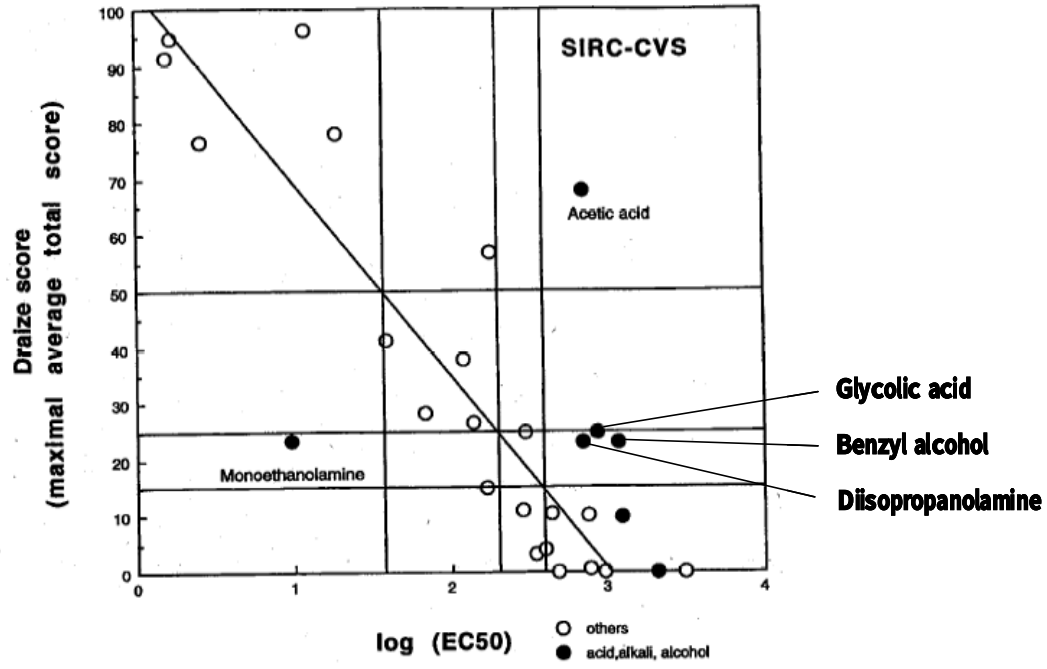


Fig. 7 Relationship between the HeLa-MTT assay and the Draize eye test

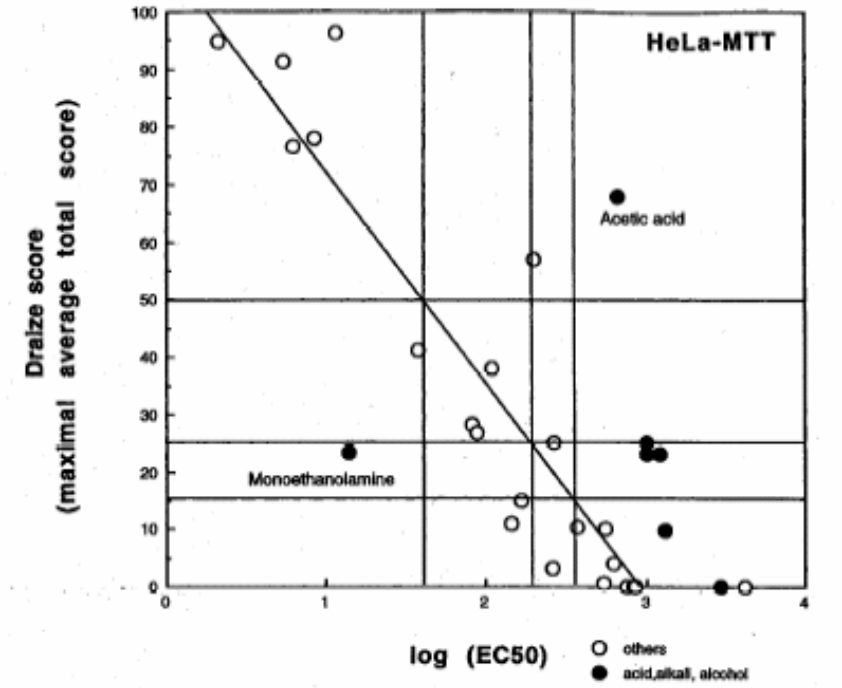


Fig. 8 Relationship between the CHL-CVS assay and the Draize eye test

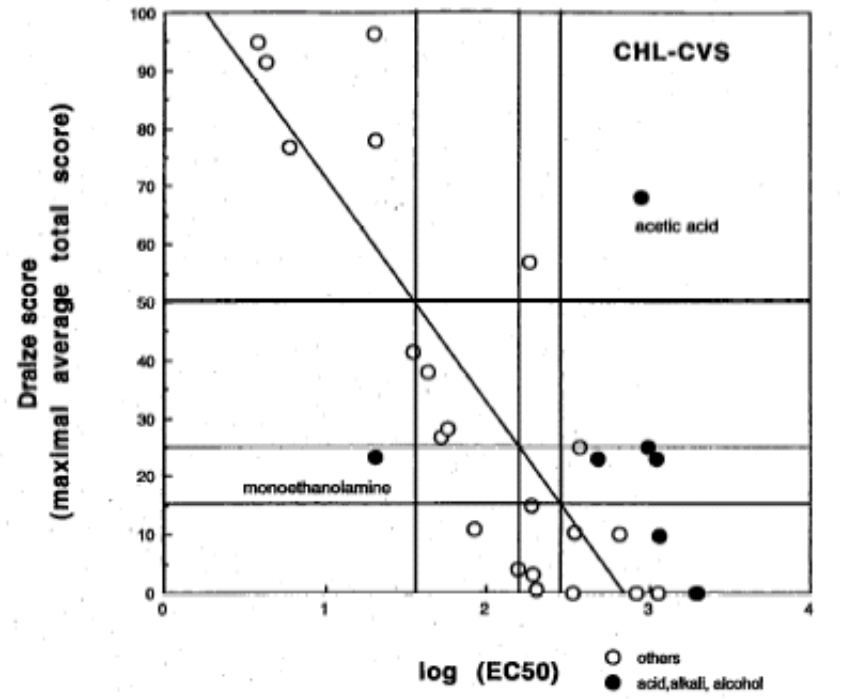


Table 35 Predicted irritancy of test samples based on the SIRC-CVS assay
(Concentration: 10%, Negative reference: Tween 20)

		In vitro (Classification by SIRC-CVS assay using Tween 20 as a reference substance for non-irritancy)	
		Positive	Negative
In vivo (Classification by GHS)	1, 2Aor 2B	Sodium lauryl sulfate Monoethanolamine Acid red 92 Sodium hydrogenated tallow L-glutamate Chlorhexidine gluconate (20% solution) Potassium laurate Polyoxyethylene octylphenylether (10 E.O.) Di (2-ethylhexyl) sodium sulfosuccinate Acetic acid Cetyltrimethylammonium bromide Benzalkonium chloride Stearyltrimethylammonium chloride Cetylpyridinium chloride Domiphen bromide 14	Benzyl alcohol Glycolic acid Butanol 3
	NI	2-Ethylhexyl p-dimethylamino benzonate Polyethyleneglycol monolaurate (10 E.O.) Calcium thioglycolate Sodium polyoxyethylene laurylether sulfate (2 E.O.) (27% solution) Sodium N-lauryl sarcosinate (30% solution) Sucrose fatty acid ester Diisopropanolamine 7	Ethanol Glycerin Polyethylene glycol 400 Polyoxyethylene hydrogenated caster oil (60 E.O.) Polyoxyethylene sorbitan monooleate (20E.O.) Sodium salicylate Triethanolamine Isopropyl myristate Polyoxyethylene sorbitan monolaurate (20 E.O.)=Tween 20 Lactic acid 10

Table 36 Predicted irritancy of test samples based on the SIRC-CVS assay
(Concentration: 10%, Negative reference: Sucrose fatty acid ester)

		In vitro (Classification by SIRC-CVS assay using Sucrose fatty acid ester as a reference substance for non-irritancy)	
		Positive	Negative
In vivo (Classification by GHS)	1, 2Aor 2B	Sodium lauryl sulfate Monoethanolamine Sodium hydrogenated tallow L-glutamate Chlorhexidine gluconate (20% solution) Potassium laurate Polyoxyethylene octylphenylether (10 E.O.) Di (2-ethylhexyl) sodium sulfosuccinate Cetyltrimethylammonium bromide Benzalkonium chloride Stearyltrimethylammonium chloride Cetylpyridinium chloride Domiphen bromide 12	Benzyl alcohol Acid red 92 Glycolic acid Butanol Acetic acid 5
	NI	0	Ethanol 2-Ethylhexyl p-dimethylamino benzonate Glycerin Polyethylene glycol 400 Polyoxyethylene hydrogenated caster oil (60 E.O.) Polyoxyethylene sorbitan monooleate (20E.O.) Sodium salicylate Triethanolamine Isopropyl myristate Polyoxyethylene sorbitan monolaurate (20 E.O.)=Tween 20 Polyethyleneglycol monolaurate (10 E.O.) Calcium thioglycolate Lactic acid Sodium polyoxyethylene laurylether sulfate (2 E.O.) (27% solution) Sodium N-lauryl sarcosinate (30% solution) Sucrose fatty acid ester Diisopropanolamine 17

Table 37 Predicted irritancy of test samples based on the SIRC-CVS assay
(Concentration: 10%, Negative reference: Tween 20)
-GHS classification by considering pH-

		In vitro (Classification by SIRC-CVS assay using Tween 20 as a reference substance for non-irritancy)	
		Positive	Negative
In vivo (Classification by GHS)	1, 2Aor 2B	Calcium thioglycolate Sodium lauryl sulfate Diisopropanolamine Monoethanolamine Acid red 92 Sodium hydrogenated tallow L-glutamate Chlorhexidine gluconate (20% solution) Potassium laurate Polyoxyethylene octylphenylether (10 E.O.) Di (2-ethylhexyl) sodium sulfosuccinate Acetic acid Cetyltrimethylammonium bromide Benzalkonium chloride Stearyltrimethylammonium chloride Cetylpyridinium chloride Domiphen bromide 16	Lactic acid Benzyl alcohol Glycolic acid Butanol 4
	NI	2-Ethylhexyl p-dimethylamino benzonate Polyethyleneglycol monolaurate (10 E.O.) Sodium polyoxyethylene laurylether sulfate (2 E.O.) (27% solution) Sodium N-lauryl sarcosinate (30% solution) Sucrose fatty acid ester 5	Ethanol Glycerin Polyethylene glycol 400 Polyoxyethylene hydrogenated caster oil (60 E.O.) Polyoxyethylene sorbitan monooleate (20E.O.) Sodium salicylate Triethanolamine Isopropyl myristate Polyoxyethylene sorbitan monolaurate (20 E.O.) =Tween 20 9

Table 38 Predicted irritancy of test samples based on the SIRC-CVS assay
(Concentration: 10%, Negative reference: Sucrose fatty acid ester)
-GHS classification by considering pH-

		In vitro (Classification by SIRC-CVS assay using Sucrose fatty acid ester as a reference substance for non-irritancy)	
		Positive	Negative
In vivo (Classification by GHS)	1, 2Aor 2B	Sodium lauryl sulfate Monoethanolamine Sodium hydrogenated tallow L-glutamate Chlorhexidine gluconate (20% solution) Potassium laurate Polyoxyethylene octylphenylether (10 E.O.) Di (2-ethylhexyl) sodium sulfosuccinate Cetyltrimethylammonium bromide Benzalkonium chloride Stearyltrimethylammonium chloride Cetylpyridinium chloride Domiphen bromide 12	Benzyl alcohol Acid red 92 Glycolic acid Butanol Acetic acid 5
	NI	0	Ethanol 2-Ethylhexyl p-dimethylamino benzonate Glycerin Polyethylene glycol 400 Polyoxyethylene hydrogenated caster oil (60 E.O.) Polyoxyethylene sorbitan monooleate (20E.O.) Sodium salicylate Triethanolamine Isopropyl myristate Polyoxyethylene sorbitan monolaurate (20 E.O.) =Tween 20 Polyethyleneglycol monolaurate (10 E.O.) Sodium polyoxyethylene laurylether sulfate (2 E.O.) (27% solution) Sodium N-lauryl sarcosinate (30% solution) Sucrose fatty acid ester 14

Table 39 Forty-eight substances

No	Substance	CAS	Supplier	*in vivo* data reported previously		GHS	Reference
			(*in vitro* test)	Classification at 10% concn	Classification at the applied concn	at 10% concn	
1	2-Bromo-2-nitropropane-1,3-diol	52-51-7	Fluorochem	Positive	Positive：100, 20, 10, 5% Negative：2, 0.5%	1, 2A or 2B	JACT 3(3):139-155,1984. JEPT 4(4):47-61, 1980.
2	Benzalkonium chloride	8001-54-5	Wako	Positive	Positive：2, 1, 0.5% Negative：0.1, 0.01%	1, 2A or 2B	JACT 8(4):589-625, 1989.
3	Cetrimonium chloride	112-02-7	Wako	Positive	Positive：2.5, 1.2, 0.5% Negative：0.1%	1, 2A or 2B	IJT 16(S3):195-220,1997.
4	Chlorhexidine digluconate	18472-51-0	Wako	Positive	Positive：20, 2% Negative：0.05%	1, 2A or 2B	JACT 12(3):201-23, 1993.
5	Chlorophene	120-32-1	Wako	Positive	Positive：100, 3% Negative：1, 0.3%	1, 2A or 2B	IJT 23(S1):1-27 2004.
6	Dioctyl sodium sulfosuccinate	577-11-7	Alfa Aesar	Positive	Positive：10% Negative：2, 0.5%	1, 2A or 2B	IJT 17(S4):1-20, 1998.
7	Lauramide DEA	120-40-1	Wako	Positive	Positive：20, 10%	1, 2A or 2B	JACT 5(5):415-54, 1986.
8	Phenethyl alcohol	60-12-8	Wako	Positive	Positive：100, 15, 5% Negative：0.3%	1, 2A or 2B	JACT 9(2):165-83, 1990.
9	Stearalkonium chloride	122-19-0	Wako	Positive	Positive：25, 4, 2.5% N egative：0.5%	1, 2A or 2B	JACT 1(2):57-69, 1982.
10	TEA-Lauryl sulfate	139-96-8	Wako	Positive	Positive：20, 10, 5, 2.5, 1.25%	1, 2A or 2B	JACT 1(4):143-67, 1982.
11	Acetyl tributyl citrate	77-90-7	Wako	Negative	Negative：100%	NI	IJT 21(S2):1-17, 2002.
12	Benzophenone-1	131-56-6	Wako	Negative	Positive：100% Negative：16, 8, 4%	NI	JACT 2(5):35-77, 1983.
13	Benzophenone-2	131-55-5	Wako	Negative	Positive：100% Negative：16, 8, 4%	NI	JACT 2(5):79-84, 1983.
14	Butylene glycol	107-88-0	Wako	Negative	Negative：100, 10%	NI	Hifu 26(5):1065-1074, 1984.
15	Carnauba wax	8015-86-9	Wako	Negative	Negative：50%	NI	JACT 3(3):1-41, 1984.
16	Cetyl alcohol	36653-82-4	Wako	Negative	Negative：100%	NI	JACT 7(3):359-413, 1988.
17	Cetyl palmitate	540-10-3	Wako	Negative	Negative：100%	NI	JACT 1(2):13-35, 1982.
18	Decyl oleate	3687-46-5	Wako	Negative	Negative：100%	NI	JACT 1(2):85-95, 1982.
19	Diazolidinyl urea	78491-02-8	MP Biomedicals	Negative	Negative：30%	NI	JACT 9(2):229-45, 1990.
20	Diethylhexyl adipate	103-23-1	Wako	Negative	Negative：100%	NI	JACT 3(3):101-30, 1984.
21	Diisopropyl adipate	6938-94-9	Wako	Negative	Negative：100%	NI	JACT 3(3):101-30, 1984.
22	Ethylhexyl palmitate	29806-73-3	Wako	Negative	Negative：100%	NI	JACT 1(2):13-35, 1982.
23	Ethylhexyl stearate	22047-49-0	Wako	Negative	Negative：100%	NI	JACT 4(5):107-46, 1985.
24	Glyceryl stearate	11099-07-3	Wako	Negative	Negative：100%	NI	JACT 1(4):169-192, 1982.
25	Hexylene glycol	107-41-5	Wako	Negative	Positive：100% Negative：25%	NI	JACT 4(5):223-48, 1985.
26	Isocetyl stearate	25339-09-7	Wako	Negative	Negative：100%	NI	JACT 4(5):107-46, 1985.
27	Isopropyl myristate	110-27-0	TCI	Negative	Negative：100%	NI	JACT 1(4):55-80, 1982.
28	Isopropyl palmitate	142-91-6	Wako	Negative	Negative：100%	NI	JACT 1(2):13-35, 1982.
29	Oleyl alcohol	143-28-2	Wako	Negative	Negative：100%	NI	JACT 4(5):1-29, 1985.
30	PEG-2 stearate	106-11-6	Wako	Negative	Negative：100%	NI	JACT 2(7):17-60, 1983.
31	PEG-40 stearate	9004-99-4	Wako	Negative	Negative：100%	NI	JACT 2(7):17-60, 1983.
32	Phytantriol	74563-64-7	Wako	Negative	Positive：100, 23% Negative：10, 3%	NI	IJT 26(Suppl. 1):107-117, 2007.
33	Propylene carbonate	108-32-7	Wako	Negative	Negative：100, 17.5, 10.5%	NI	JACT 6(1):23-51, 1987.
34	Castor seed oil	8001-79-4	Wako	Negative	Negative：100%	NI	JACT 7(6):721-739, 1988.
35	Safflower oil	8001-23-8	Wako	Negative	Negative：100%	NI	JACT 4(5):171-97, 1985.
36	Sesame (Sesamum indicum) oil	8008-74-0	Wako	Negative	Negative：100%	NI	JACT 12(3):261-77, 1993.
37	Sodium dehydroacetate	4418-26-2	Wako	Negative	Negative：100%	NI	JACT 4(3):123-159, 1985.
38	Sodium stearate	822-16-2	Wako	Negative	Negative：100%	NI	JACT 1(2):143-77, 1982.
39	Sorbitan oleate	1338-43-8	Wako	Negative	Negative：100%	NI	JACT 4(3):65-121, 1985.
40	Sorbitan sesquioleate	8007-43-0	Wako	Negative	Negative：100, 30%	NI	JACT 4(3):65-121, 1985.
41	Sorbitan stearate	1338-41-6	Wako	Negative	Negative：30%	NI	JACT 4(3):65-121, 1985.
42	Squalane	111-01-3	Wako	Negative	Negative：100%	NI	JACT 1(2):37-56, 1982.
43	Steareth-2	9005-00-9	Wako	Negative	Negative：60%	NI	JACT 7(6):881-910, 1988.
44	Steareth-20	9005-00-9	Wako	Negative	Negative：60%	NI	JACT 7(6):881-910, 1988.
45	Stearyl alcohol	112-92-5	Wako	Negative	Negative：100%	NI	JACT 4(5):1-29, 1985.
46	Triacetin	102-76-1	Wako	Negative	Negative：100%	NI	IJT 22(S2):1-10, 2003.
47	Triethylene glycol	112-27-6	Wako	Negative	Negative：100%	NI	IJT 25(5) :121-138,2006.
48	Zinc stearate	557-05-1	Wako	Negative	Negative：100%	NI	JACT 1(2):143-77, 1982.

Supplier means manufacturer of the material used in this study. The *in vivo* classification of positive or negative was based on the appearance or not of corneal damage, or an MAS value of 15 as a cut-off point, where reported MAS values are available. The classification was essentially based on whether or not corneal damage appeared after the application of 0.1 mL to rabbit eye without irrigation. However, where there were differences of test conditions, these were considered individually. For example, a case where corneal damage appeared after the application of 0.05 mL was judged as positive. In cases without data at 10% concentration, the assessment of positive or negative at the concentration of 10% was made on the basis of dose-response analysis of each ingredient.

Table 40 Results of 48 substances on the SIRC-CVS assay

No	Substance	Physical state of 2% in medium	Range finding test				Main test				Judgement
			Medium	Starting concn (μg/mL)	Physical state	Range of IC50 (μg/mL)	Medium	Starting concn (μg/mL)	Physical state	IC50 (μg/mL) ±SD	
1	2-Bromo-2-Nitropropane-1,3-Diol	Solution	Medium	10000	Solution	1<IC50<10	Medium	10	Solution	6.42±0.85	Positive
2	Benzalkonium chloride	Suspension	DMSO /Medium	1000	Solution	1<IC50<10	DMSO /Medium	10	Solution	3.47±0.47	Positive
3	Cetrimonium chloride	Solution	Medium	10000	Solution	IC50<1	Medium	10	Solution	0.56±0.16	Positive
4	Chlorhexidine digluconate (20% Solution)	Not suspended	DMSO /Medium	200 【1000】	Solution	2<IC50<20 【10<IC50<100】	DMSO /Medium	200 【1000】	Solution	7.92±3.92 【39.6±19.6】	Positive
5	Chlorophene	Not suspended	DMSO /Medium	100	Solution	10<IC50<100	DMSO /Medium	100	Suspension	25.6±9.1	Positive
6	Dioctyl sodium sulfosuccinate	Suspension	DMSO /Medium	1000	Solution	10<IC50<100	DMSO/Medium	100	Solution	81.3±4.8	Positive
7	Lauramide DEA	Suspension	DMSO/Medium	1000	Suspension	10<IC50<100	DMSO /Medium	100	Solution	18.3±4.1	Positive
8	Phenethyl alcohol	Suspension	DMSO/Medium	1000	Solution	1000<IC50	Medium	10000	Suspension	1830±1360	False negative
9	Stearalkonium chloride	Not suspended	Ethanol/Medium	100	Solution	1<IC50<10	Ethanol /Medium	10	Solution	2.66±0.56	Positive
10	TEA-Lauryl sulfate 【40% Solution】	Solution	Medium	4000 【10000】	Solution	40<IC50<400 【100<IC50<1000】	Medium	400 【1000】	Solution	117±3 【290±4】	Positive
11	Acetyl tributyl citrate	Not suspended	Ethanol /Medium	100	Solution	100<IC50	Medium	-	Not suspended	Could not be tested	NE
12	Benzophenone-1	Not suspended	DMSO/Medium	100	Suspension	10<IC50<100	DMSO /Medium	100	Suspension	29.3±8.0	False positive
13	Benzophenone-2	Not suspended	DMSO /Medium	100	Suspension	10<IC50<100	DMSO/Medium	100	Suspension	53.4±6.4	False positive
14	Butylene glycol	Solution	Medium	10000	Solution	10000<IC50	Medium	10000	Solution	10000<	Negative
15	Carnauba (Copernicia cerifera) wax	Not suspended	-	-	Not suspended	Could not be tested	-	-	-	Could not be tested	NE
16	Cetyl alcohol	Not suspended	DMSO /Medium	100	Suspension	10<IC50<100	DMSO /Medium	100	Suspension	25.1±12.1	False positive
17	Cetyl palmitate	Not suspended	-	-	Not suspended	Could not be tested	-	-	-	Could not be tested	NE
18	Decyl oleate	Not suspended	Ethanol /Medium	100	Suspension	100<IC50	Medium	-	Not suspended	Could not be tested	NE
19	Diazolidinyl urea	Solution	Medium	10000	Solution	1<IC50<10	Medium	100	Solution	11.5±7.7	False positive
20	Diethylhexyl adipate(=Octyl)	Not suspended	Ethanol /Medium	1000	Suspension	1000<IC50	Medium	-	Not suspended	Could not be tested	Negative #
21	Diisopropyl adipate	Not suspended	DMSO/Medium	1000	Suspension	100<IC50<1000	DMSO /Medium	1000	Suspension	633±16	Negative
22	Ethylhexyl palmitate (=Octyl)	Suspension	Ethanol /Medium	1000	Suspension	1000<IC50	Medium	10000	Suspension	10000<	Negative
23	Ethylhexyl stearate (=Octyl)	Not suspended	Ethanol /Medium	100	Suspension	100<IC50	Medium	-	Not suspended	Could not be tested	NE
24	Glyceryl stearate	Not suspended	-	-	Not suspended	Could not be tested	-	-	-	Could not be tested	NE
25	Hexylene glycol	Solution	Medium	10000	Solution	1000<IC50<10000	Medium	10000	Suspension	7500±600	Negative
26	Isocetyl stearate	Not suspended	Ethanol /Medium	1000	Suspension	1000<IC50	Medium	-	Not suspended	Could not be tested	Negative #
27	Isopropyl Myristate	Not suspended	Ethanol/Medium	1000	Suspension	1000<IC50	Medium	-	Not suspended	Could not be tested	Negative #
28	Isopropyl Palmitate	Not suspended	Ethanol /Medium	1000	Suspension	1000<IC50	Medium	-	Not suspended	Could not be tested	Negative #
29	Oleyl alcohol	Not suspended	Ethanol /Medium	100	Suspension	10<IC50<100	Ethanol /Medium	100	Suspension	41.9±13.3	False positive
30	PEG-2 stearate	Not suspended	DMSO /Medium	100	Solution	100<IC50	Medium	10000	Not suspended	Could not be tested	NE
31	PEG-40 stearate	Suspension	Medium	10000 〔5000〕	Suspension	100<IC50<1000	Medium	1000	Solution	230±79	False positive
32	Phytantriol	Not suspended	DMSO /Medium	1000	Suspension	10<IC50<100	DMSO /Medium	100	Suspension	37.2±11.8	False positive
33	Propylene carbonate	Solution	Medium	10000	Solution	1000<IC50<10000	Medium	10000	Solution	6050±490	Negative
34	Ricinus communis (Castor) seed oil	Not suspended	DMSO /Medium	100	Solution	100<IC50	Medium	-	Not suspended	Could not be tested	NE
35	Safflower (Carthamus tinctorius) oil	Not suspended	DMSO /Medium	1000	Solution	1000<IC50	Medium	-	Not suspended	Could not be tested	Negative #
36	Sesame (Sesamum indicum) oil	Not suspended	DMSO /Medium	1000	Solution	1000<IC50	Medium	-	Not suspended	Could not be tested	Negative #
37	Sodium dehydroacetate	Solution	Medium	10000	Solution	100<IC50<1000	Medium	1000	Solution	860±224	Negative
38	Sodium stearate	Suspension	Medium	10000 〔2500〕	Suspension	10<IC50<100	Medium	1000	Suspension	56.5±8.2	False positive

39	Sorbitan oleate	Suspension	DMSO /Medium	1000	Solution	1000<IC50	Medium	10000	Suspension	5170±1560	Negative
40	Sorbitan sesquioleate	Suspension	DMSO /Medium	1000	Solution	1000<IC50	Medium	10000	Suspension	10000<	Negative
41	Sorbitan stearate	Not suspended	-	-	Not suspended	Could not be tested	-	-	-	Could not be tested	NE
42	Squalane	Not suspended	DMSO /Medium	1000	Solution	1000<IC50	Medium	-	Not suspended	Could not be tested	Negative #
43	Steareth-2	Not suspended	Ethanol /Medium	100	Solution	10<IC50<100	Ethanol /Medium	100	Solution	22.4±5.4	False positive
44	Steareth-20	Solution	Medium	10000	Solution	10<IC50<100	Medium	100	Solution	16.5±8.3	False positive
45	Stearyl alcohol	Not suspended	-	-	Not suspended	Could not be tested	-	-	-	Could not be tested	NE
46	Triacetin	Solution	Medium	10000	Solution	1000<IC50<10000	Medium	10000	Solution	1780±720	Negative
47	Triethylene glycol	Solution	Medium	10000	Solution	10000<IC50	Medium	10000	Solution	10000<	Negative
48	Zinc stearate	Not suspended	-	-	Not suspended	Could not be tested	-	-	-	Could not be tested	NE
Negative reference	Tween 20	-	-	-	-	-	Medium	1000	Solution	501±33	Negative

#: It was judged as negative from results of range finding assay.
NE: It could not be evaluated.
【 】: The data was obtained from diluted agent.
〔 〕: The precipitation was appear at the concentration of 10000μg/mL in the culture of 72hr. The maximal concentrations without the precipitation were 5000ug/mL and 2500ug/mL in No31 and No38, respectively.

Table 41 Predicted irritancy of 48 substances based on the SIRC-CVS assay
(Concentration: 10%, Negative reference: Tween 20)

		In vitro (Classification by SIRC-CVS assay using Tween 20 as a reference substance for non-irritancy)		
		Positive	Negative	Could not be tested
In vivo (Classification by Draize eye test at 10% concn) Corneal damage or MAS over 15 was classified as positive.	Positive	2-Bromo-2-nitropropane-1,3-diol (6.42 ± 0.85) Benzalkonium chloride (3.47 ± 0.47) Cetrimonium chloride (0.56 ± 0.16) Chlorhexidine digluconate (7.92 ± 3.92) Chlorophene (25.6 ± 9.1) Dioctyl sodium sulfosuccinate (81.3 ± 4.8) Lauramide DEA (18.3 ± 4.1) Stearalkonium chloride (2.66 ± 0.56) TEA-Lauryl sulphate (117 ± 3) 9	Phenethyl alcohol (1830 ± 1360) 1	0
	Negative NI for GHS	Benzophenone-1 (29.3 ± 8.0) Benzophenone-2 (53.4 ± 6.4) Cetyl alcohol (25.1 ± 12.1) Diazolidinyl urea (11.5 ± 7.7) Oleyl alcohol (41.9 ± 13.3) PEG-40 stearate (230 ± 79) Phytantriol (37.2 ± 11.8) Sodium stearate (56.5 ± 8.2) Steareth-2 (22.4 ± 5.4) Steareth-20 (16.5 ± 8.3) 10	Butylene glycol (10000<) Diethylhexyl adipate (1000<) Diisopropyl adipate (633 ± 16) Ethylhexyl palmitate (10000<) Hexylene glycol (7500 ± 600) Isocetyl stearate (1000<) Isopropyl myristate (1000<) Isopropyl palmitate (1000<) Propylene carbonate (6050 ± 490) Safflower oil (1000<) Sesame oil (1000<) Sodium dehydroacetate (860 ± 224) Sorbitan oleate (5170 ± 1560) Sorbitan sesquioleate (10000<) Squalane (1000<) Triacetin (1780 ± 720) Triethylene glycol (10000<) 17	Acetyl tributyl citrate Carnauba wax Castor seed oil Cetyl palmitate Decyl oleate Ethylhexyl stearate Glyceryl stearate PEG-2 stearate Sorbitan stearate Stearyl alcohol Zinc stearate 11

The results of SIRC-CVS assay are shown as average ± standard deviation (n=3) of IC50 value in parenthesis. Tween 20 (IC50=501 ± 33 □g/ml) was used as a reference substance for non-irritancy. The 11 substances that were insufficiently soluble to be tested are also shown in this table.

Table 42 Predicted irritancy of 48 substances based on the SIRC-CVS assay
(Concentration: 10%, Negative reference: Sucrose fatty acid ester)

		In vitro (Classification by SIRC-CVS assay using Sucrose fatty acid ester as a reference substance for non-irritancy)		
		Positive	Negative	Could not be tested
In vivo (Classification by Draize eye test at 10% concn) Corneal damage or MAS over 15 was classified as positive.	Positive	2-Bromo-2-nitropropane-1,3-diol (6.42 ± 0.85) Benzalkonium chloride (3.47 ± 0.47) Cetrimonium chloride (0.56 ± 0.16) Chlorhexidine digluconate (7.92 ± 3.92) Chlorophene (25.6 ± 9.1) Dioctyl sodium sulfosuccinate (81.3 ± 4.8) Lauramide DEA (18.3 ± 4.1) Stearalkonium chloride (2.66 ± 0.56) TEA-Lauryl sulphate (117 ± 3) 9	Phenethyl alcohol (1830 ± 1360) 1	0
	Negative NI for GHS	Benzophenone-1 (29.3 ± 8.0) Benzophenone-2 (53.4 ± 6.4) Cetyl alcohol (25.1 ± 12.1) Diazolidinyl urea (11.5 ± 7.7) Oleyl alcohol (41.9 ± 13.3) PEG-40 stearate (230 ± 79) Phytantriol (37.2 ± 11.8) Sodium stearate (56.5 ± 8.2) Steareth-2 (22.4 ± 5.4) Steareth-20 (16.5 ± 8.3) 10	Butylene glycol (10000<) Diethylhexyl adipate (1000<) Diisopropyl adipate (633 ± 16) Ethylhexyl palmitate (10000<) Hexylene glycol (7500 ± 600) Isocetyl stearate (1000<) Isopropyl myristate (1000<) Isopropyl palmitate (1000<) Propylene carbonate (6050 ± 490) Safflower oil (1000<) Sesame oil (1000<) Sodium dehydroacetate (860 ± 224) Sorbitan oleate (5170 ± 1560) Sorbitan sesquioleate (10000<) Squalane (1000<) Triacetin (1780 ± 720) Triethylene glycol (10000<) 17	Acetyl tributyl citrate Carnauba wax Castor seed oil Cetyl palmitate Decyl oleate Ethylhexyl stearate Glyceryl stearate PEG-2 stearate Sorbitan stearate Stearyl alcohol Zinc stearate 11

The results of SIRC-CVS assay are shown as average ± standard deviation (n=3) of IC50 value in parenthesis. Sucrose fatty acid ester (IC50=250 □g/ml) was used as a reference substance for non-irritancy. The 11 substances that were insufficiently soluble to be tested are also shown in this table.

Table 43 Predicted irritancy of 48 substances based on the SIRC-CVS assay
(Concentration: 10%, Negative reference: Tween 20)
-GHS classification by considering pH-

		In vitro (Classification by SIRC-CVS assay using Tween 20 as a reference substance for non-irritancy)		
		Positive	Negative	Could not be tested
In vivo (Classification by Draize eye test at 10% concn) Corneal damage or MAS over 15 was classified as positive.	Positive	Calcium thioglycolate Sodium lauryl sulfate Diisopropanolamine Monoethanolamine Acid red 92 Sodium hydrogenated tallow L-glutamate Chlorhexidine gluconate (20% solution) Potassium laurate Polyoxyethylene octylphenylether (10 E.O.) Di (2-ethylhexyl) sodium sulfosuccinate Acetic acid Cetyltrimethylammonium bromide Benzalkonium chloride Stearyltrimethylammonium chloride Cetylpyridinium chloride Domiphen bromide 2-Bromo-2-nitropropane-1,3-diol ~~Benzalkonium chloride~~ Cetrimonium chloride Chlorhexidine digluconate Chlorophene Dioctyl sodium sulfosuccinate Lauramide DEA Stearalkonium chloride TEA-Lauryl sulphate 24	Lactic acid Benzyl alcohol Glycolic acid Butanol Phenethyl alcohol 5	0
	Negative NI for GHS	2-Ethylhexyl p-dimethylamino benzonate Polyethyleneglycol monolaurate (10 E.O.) Sodium polyoxyethylene laurylether sulfate (2 E.O.) (27% solution) Sodium N-lauryl sarcosinate (30% solution) Sucrose fatty acid ester Benzophenone-1 Benzophenone-2 Cetyl alcohol Diazolidinyl urea Oleyl alcohol PEG-40 stearate Phytantriol Sodium stearate Steareth-2 Steareth-20 15	Ethanol Glycerin Polyethylene glycol 400 Polyoxyethylene hydrogenated caster oil (60 E.O.) Polyoxyethylene sorbitan monooleate (20E.O.) Sodium salicylate Triethanolamine Isopropyl myristate Polyoxyethylene sorbitan monolaurate (20 E.O.) =Tween 20 Butylene glycol Diethylhexyl adipate Diisopropyl adipate Ethylhexyl palmitate Hexylene glycol Isocetyl stearate ~~Isopropyl myristate~~ Isopropyl palmitate Propylene carbonate Safflower oil Sesame oil Sodium dehydroacetate Sorbitan oleate Sorbitan sesquioleate Squalane Triacetin Triethylene glycol 25	Acetyl tributyl citrate Carnauba wax Castor seed oil Cetyl palmitate Decyl oleate Ethylhexyl stearate Glyceryl stearate PEG-2 stearate Sorbitan stearate Stearyl alcohol Zinc stearate 11

Table 44 Methods of the LDM-MTT assay

Test substance preparation	The MATREX kit was donated by Organogenesis Inc. for the first phase of the validation study, and by Toyobo Co. Ltd for the second and third phases. The kit consisted of LDMs, polyethylene ring, silicon sealant and assay medium, and included all requirements for the test. The solvents for diluting test substances were distilled water, 50% dimethyl sulfoxide and ethylene glycol in EC_{50} value measurement, while in the MATREX scoring method the solvent was distilled water only. In this case, if a substance was not soluble or could not be dispersed in water, it was carried out at only one dose level–100%.
Test kit and procedures	The MATREX kit was donated by Organogenesis Inc. for the first phase of the validation study, and by Toyobo Co. Ltd for the second and third phases. The kit consisted of LDMs, polyethylene ring, silicon sealant and assay medium, and included all requirements for the test. The solvents for diluting test substances were distilled water, 50% dimethyl sulfoxide and ethylene glycol in EC_{50} value measurement, while in the MATREX scoring method the solvent was distilled water only. In this case, if a substance was not soluble or could not be dispersed in water, it was carried out at only one dose level–100%. plate and 5 ml assay medium was added to the surface of the LDM for 30 min at room temperature to remove any residual conditioned medium from the LDM. Then, 5 ml the assay medium was aspirated and 1.5 ml of fresh assay medium was added underneath each LDM. The polyethylene ring was applied to the surface of the LDM using silicon sealant around the area of exposure. Then, 80 μl (or 80 mg in the case of a solid) test substance was applied to the surface. The LDM was exposed to the test substance for 24 hr at 37°C in a 5% CO_2 incubator. After incubation, the test substance was removed from the LDM by washing with the assay medium. The LDM was dipped in 1.5 ml MTT solution (0.333 mg MTT/1 ml assay medium) for 3–4 hr at 37°C. After exposure to MTT, the centre of the LDM tissue was excised using an 8 mm diameter skin biopsy punch. As an indicator of cell viability, MTT formazan formed by the reaction of MTT was extracted by exposure to 0.3 ml isopropanol containing 0.04 N HCl for 2 hr. The absorbance at 570 nm was measured after calibrating with the extraction solvent as a blank. Untreated controls were handled in the same manner, except that they were treated without the test substance.
EC50 value measurement	A preliminary range-finding test was performed with several concentrations of each test substance. The cell survival rate was calculated against untreated control value. According to the results of the preliminary test, the definitive test was carried out using five doses to obtain the EC_{50} value. The EC_{50} value for each test substance was estimated from a dose–response curve obtained.

The contents are the same as those reported by Ohuchi et al. (1999).

Table 45 Results of interlaboratory reproducibility on the LDM-MTT assay
(Concentration: 10%, Cut-off value: 4.15%)

Substance (Draize eye test was performed at 10% concentration)	MAS at 10%	GHS at 10%	IC50 of the LDM-MTT assay (%)							
			Lab. A	Lab. B	Lab. C	Lab. D	Lab. E	Lab. F	Lab. G	Average±SD (%)
Ethanol	0.0	NI	36	41	37.5	56	NT	NT	NT	43±9
2-Ethylhexyl p-dimethylamino benzonate	0.0	NI	100<	100<	100<	100<	100<	100<	100<	100<
Glycerin	0.0	NI	100<	100<	100<	100<	100<	100<	100<	100<
Polyethylene glycol 400	0.0	NI	100<	100<	85	78	100	67	82	67<
Polyoxyethylene hydrogenated caster oil (60 E.O.)	0.0	NI	36	26.5	21.5	NT	NT	NT	NT	28.0±7.4
Polyoxyethylene sorbitan monooleate (20E.O.)	0.0	NI	4.8	2.53	1.65	1.4	1.93	3.2	1.55	2.4±1.2
Sodium salicylate	0.0	NI	9.2	9.8	8.5	6.0	5.47	11.5	11.5	8.9±2.4
Triethanolamine	0.0	NI	7.6	4.1	6.2	8.4	NT	NT	NT	6.6±1.9
Isopropyl myristate	0.7	NI	100<	100<	100<	100<	100<	100<	100<	100<
Polyoxyethylene sorbitan monolaurate (20 E.O.)	0.7	NI	0.072	0.057	0.061	NT	NT	NT	NT	0.063±0.008
Polyethyleneglycol monolaurate (10 E.O.)	3.3	NI	0.064	0.06	0.058	NT	NT	NT	NT	0.061±0.003
Calcium thioglycolate	4.0	NI	1.4	6.4	6.0	7.7	2.15	7.0	1.5	4.6±2.8
m-Phenylenediamine (Lack of stability)	4.3	NI	0.56	3.4	0.145	0.72	0.47	0.45	0.4	0.88±1.13
Lactic acid	9.7	NI	0.31	0.27	0.285	0.26	NT	NT	NT	0.28±0.02
Sodium polyoxyethylene laurylether sulfate (2 E.O.) (27% solution)	10.0	NI	0.060	0.047	0.06	NT	NT	NT	NT	0.056±0.008
Sodium N-lauryl sarcosinate (30% solution)	10.3	NI	0.22	0.25	0.32	NT	NT	NT	NT	0.26±0.05
Sucrose fatty scid ester	11.0	NI	0.027	0.014	0.024	0.009	0.013	0.02	0.033	0.020±0.009
Diisopropanolamine	23.0	NI	1.2	1.1	0.92	0.88	NT	NT	NT	1.0±0.2
Sodium lauryl sulfate	15.0[$]	1or2A	0.017	0.015	0.018	NT	NT	NT	NT	0.017±0.002
Benzyl alcohol	23.0	1or2A	7.4	7.0	8.6	6.2	8.2	7.15	6.4	7.3±0.9
Monoethanolamine	23.3	2B	0.34	0.38	0.33	0.53	NT	NT	NT	0.40±0.09
Acid red 92	25.0	1or2A	0.0086	0.0062	0.0074	0.0038	0.0073	0.0008	0.018	0.0074±0.0054
Glycolic acid	25.0	2B	0.22	0.21	0.155	0.16	NT	NT	NT	0.19±0.03
Sodium hydrogenated tallow L-glutamate	26.7	1or2A	0.0018	0.00385	0.0041	NT	NT	NT	NT	0.0033±0.0013
Chlorhexidine gluconate (20% solution)	28.3	2A	0.061	0.037	0.0195	0.042	NT	NT	NT	0.040±0.017
Butanol	34.0	1or2A	8.6	6.0	12.3	9.6	NT	NT	NT	9.1±2.6
Potassium laurate	38.0	1or2A	0.17	0.23	0.13	0.13	NT	NT	NT	0.17±0.05
Polyoxyethylene octylphenylether (10 E.O.)	41.3	1or2A	0.034	0.0285	0.060	NT	NT	NT	NT	0.041±0.017
Di (2-ethylhexyl) sodium sulfosuccinate	57.0	1or2A	0.0066	0.0083	0.0068	0.0074	NT	NT	NT	0.0073±0.0008
Acetic acid	68.0	1or2A	0.23	0.24	0.215	0.96	NT	NT	NT	0.41±0.37
Cetyltrimethylammonium bromide	76.7	1or2A	0.0015	0.0014	0.0018	0.0022	NT	NT	NT	0.0017±0.0004
Benzalkonium chloride	78.0	1or2A	0.0018	0.0023	0.0016	NT	NT	NT	NT	0.0019±0.0004
Stearyltrimethylammonium chloride	91.3	1or2A	0.0030	0.0012	0.0013	0.0014	NT	NT	NT	0.0017±0.0009
Cetylpyridinium chloride	94.7	1	0.0027	0.0013	0.00265	0.0013	0.00124	0.00165	0.0026	0.0019±0.0007
Domiphen bromide	96.3	1	0.0018	0.0021	0.0070	0.0019	NT	NT	NT	0.0032±0.0025

The data were taken from Ohuchi et al. (1999). The cut off value of 4.15% was used for the classification in the LDM-MTT assay. As reported by Ohuchi et al. (1999), m-phenylenediamine was excluded from the subsequent analysis due to instability.
MAS: Maximal average score of the Draize eye test.
GHS category 1: Severe or corrosive irritant, 2A: Irritant, 2B: Irritant, NI: Non irritant.
1or2A: The Draize eye test results couldn't discriminate between 1 and 2A for no observation data on day 21. The observation was performed to day 14.
$: Sodium lauryl sulfate was evaluated as positive in the evaluation on the basis of MAS, because 2 of 3 individuals had the corneal damage of 15 and 10 (for the maximal corneal score), respectively.
SD: Standard deviation
NT: Not tested

Table 46 Results of interlaboratory reproducibility on the LDM-MTT assay
(Concentration: 10%, Cut-off value: 4.15%)
-GHS classification by considering pH-

Substance (Draize eye test was performed at 10% concentration)	MAS at 10%	GHS at 10%	IC50 of the LDM-MTT assay (%)							
			Lab. A	Lab. B	Lab. C	Lab. D	Lab. E	Lab. F	Lab. G	Average±SD (%)
Ethanol	0.0	NI	36	41	37.5	56	NT	NT	NT	43±9
2-Ethylhexyl p-dimethylamino benzonate	0.0	NI	100<	100<	100<	100<	100<	100<	100<	100<
Glycerin	0.0	NI	100<	100<	100<	100<	100<	100<	100<	100<
Polyethylene glycol 400	0.0	NI	100<	100<	85	78	100	67	82	67<
Polyoxyethylene hydrogenated caster oil (60 E.O.)	0.0	NI	36	26.5	21.5	NT	NT	NT	NT	28.0±7.4
Polyoxyethylene sorbitan monooleate (20E.O.)	0.0	NI	4.8	2.53	1.65	1.4	1.93	3.2	1.55	2.4±1.2
Sodium salicylate	0.0	NI	9.2	9.8	8.5	6.0	5.47	11.5	11.5	8.9±2.4
Triethanolamine	0.0	NI	7.6	4.1	6.2	8.4	NT	NT	NT	6.6±1.9
Isopropyl myristate	0.7	NI	100<	100<	100<	100<	100<	100<	100<	100<
Polyoxyethylene sorbitan monolaurate (20 E.O.)	0.7	NI	0.072	0.057	0.061	NT	NT	NT	NT	0.063±0.008
Polyethyleneglycol monolaurate (10 E.O.)	3.3	NI	0.064	0.06	0.058	NT	NT	NT	NT	0.061±0.003
m-Phenylenediamine (Lack of stability)	4.3	NI	0.56	3.4	0.145	0.72	0.47	0.45	0.4	0.88±1.13
Sodium polyoxyethylene laurylether sulfate (2 E.O.) (27% solution)	10.0	NI	0.060	0.047	0.06	NT	NT	NT	NT	0.056±0.008
Sodium N-lauryl sarcosinate (30% solution)	10.3	NI	0.22	0.25	0.32	NT	NT	NT	NT	0.26±0.05
Sucrose fatty scid ester	11.0	NI	0.027	0.014	0.024	0.009	0.013	0.02	0.033	0.020±0.009
Calcium thioglycolate	4.0	1*	1.4	6.4	6.0	7.7	2.15	7.0	1.5	4.6±2.8
Lactic acid	9.7	1*	0.31	0.27	0.285	0.26	NT	NT	NT	0.28±0.02
Sodium lauryl sulfate	15.0$	1or2A	0.017	0.015	0.018	NT	NT	NT	NT	0.017±0.002
Benzyl alcohol	23.0	1or2A	7.4	7.0	8.6	6.2	8.2	7.15	6.4	7.3±0.9
Diisopropanolamine	23.0	1*	1.2	1.1	0.92	0.88	NT	NT	NT	1.0±0.2
Monoethanolamine	23.3	1*	0.34	0.38	0.33	0.53	NT	NT	NT	0.40±0.09
Acid red 92	25.0	1or2A	0.0086	0.0062	0.0074	0.0038	0.0073	0.0008	0.018	0.0074±0.0054
Glycolic acid	25.0	1*	0.22	0.21	0.155	0.16	NT	NT	NT	0.19±0.03
Sodium hydrogenated tallow L-glutamate	26.7	1or2A	0.0018	0.00385	0.0041	NT	NT	NT	NT	0.0033±0.0013
Chlorhexidine gluconate (20% solution)	28.3	2A	0.061	0.037	0.0195	0.042	NT	NT	NT	0.040±0.017
Butanol	34.0	1or2A	8.6	6.0	12.3	9.6	NT	NT	NT	9.1±2.6
Potassium laurate	38.0	1or2A	0.17	0.23	0.13	0.13	NT	NT	NT	0.17±0.05
Polyoxyethylene octylphenylether (10 E.O.)	41.3	1or2A	0.034	0.0285	0.060	NT	NT	NT	NT	0.041±0.017
Di (2-ethylhexyl) sodium sulfosuccinate	57.0	1or2A	0.0066	0.0083	0.0068	0.0074	NT	NT	NT	0.0073±0.0008
Acetic acid	68.0	1or2A	0.23	0.24	0.215	0.96	NT	NT	NT	0.41±0.37
Cetyltrimethylammonium bromide	76.7	1or2A	0.0015	0.0014	0.0018	0.0022	NT	NT	NT	0.0017±0.0004
Benzalkonium chloride	78.0	1or2A	0.0018	0.0023	0.0016	NT	NT	NT	NT	0.0019±0.0004
Stearyltrimethylammonium chloride	91.3	1or2A	0.0030	0.0012	0.0013	0.0014	NT	NT	NT	0.0017±0.0009
Cetylpyridinium chloride	94.7	1	0.0027	0.0013	0.00265	0.0013	0.00124	0.00165	0.0026	0.0019±0.0007
Domiphen bromide	96.3	1	0.0018	0.0021	0.0070	0.0019	NT	NT	NT	0.0032±0.0025

The data were taken from Ohuchi et al. (1999). The cut off value of 4.15% was used for the classification in the LDM-MTT assay. As reported by Ohuchi et al. (1999), m-phenylenediamine was excluded from the subsequent analysis due to instability.
MAS: Maximal average score of the Draize eye test.
GHS category 1: Severe or corrosive irritant, 2A: Irritant, 2B: Irritant, NI: Non irritant.
1or2A: The Draize eye test results couldn't discriminate between 1 and 2A for no observation data on day 21. The observation was performed to day 14.
$: Sodium lauryl sulfate was evaluated as positive in the evaluation on the basis of MAS, because 2 of 3 individuals had the corneal damage of 15 and 10 (for the maximal corneal score), respectively.
SD: Standard deviation
NT: Not tested

Table 47 Results of interlaboratory reproducibility on the LDM-MTT assay
(Concentration: 10%, Negative reference: Triethanolamine)

Substance (Draize eye test was performed at 10% concentration)	MAS at 10%	GHS at 10%	IC50 of the LDM-MTT assay (%)							
			Lab. A	Lab. B	Lab. C	Lab. D	Lab. E	Lab. F	Lab. G	Average±SD (%)
Ethanol	0.0	NI	36	41	37.5	56	NT	NT	NT	43±9
2-Ethylhexyl p-dimethylamino benzonate	0.0	NI	100<	100<	100<	100<	100<	100<	100<	100<
Glycerin	0.0	NI	100<	100<	100<	100<	100<	100<	100<	100<
Polyethylene glycol 400	0.0	NI	100<	100<	85	78	100	67	82	67<
Polyoxyethylene hydrogenated caster oil (60 E.O.)	0.0	NI	36	26.5	21.5	NT	NT	NT	NT	28.0±7.4
Polyoxyethylene sorbitan monooleate (20E.O.)	0.0	NI	4.8	2.53	1.65	1.4	1.93	3.2	1.55	2.4±1.2
Sodium salicylate	0.0	NI	9.2	9.8	8.5	6.0	5.47	11.5	11.5	8.9±2.4
Triethanolamine	0.0	NI	7.6	4.1	6.2	8.4	NT	NT	NT	6.6±1.9
Isopropyl myristate	0.7	NI	100<	100<	100<	100<	100<	100<	100<	100<
Polyoxyethylene sorbitan monolaurate (20 E.O.)	0.7	NI	0.072	0.057	0.061	NT	NT	NT	NT	0.063±0.008
Polyethyleneglycol monolaurate (10 E.O.)	3.3	NI	0.064	0.06	0.058	NT	NT	NT	NT	0.061±0.003
Calcium thioglycolate	4.0	NI	1.4	6.4	6.0	7.7	2.15	7.0	1.5	4.6±2.8
m-Phenylenediamine (Lack of stability)	4.3	NI	0.56	3.4	0.145	0.72	0.47	0.45	0.4	0.88±1.13
Lactic acid	9.7	NI	0.31	0.27	0.285	0.26	NT	NT	NT	0.28±0.02
Sodium polyoxyethylene laurylether sulfate (2 E.O.) (27% solution)	10.0	NI	0.060	0.047	0.06	NT	NT	NT	NT	0.056±0.008
Sodium N-lauryl sarcosinate (30% solution)	10.3	NI	0.22	0.25	0.32	NT	NT	NT	NT	0.26±0.05
Sucrose fatty scid ester	11.0	NI	0.027	0.014	0.024	0.009	0.013	0.02	0.033	0.020±0.009
Diisopropanolamine	23.0	NI	1.2	1.1	0.92	0.88	NT	NT	NT	1.0±0.2
Sodium lauryl sulfate	15.0$	1or2A	0.017	0.015	0.018	NT	NT	NT	NT	0.017±0.002
Benzyl alcohol	23.0	1or2A	7.4	7.0	8.6	6.2	8.2	7.15	6.4	7.3±0.9
Monoethanolamine	23.3	2B	0.34	0.38	0.33	0.53	NT	NT	NT	0.40±0.09
Acid red 92	25.0	1or2A	0.0086	0.0062	0.0074	0.0038	0.0073	0.0008	0.018	0.0074±0.0054
Glycolic acid	25.0	2B	0.22	0.21	0.155	0.16	NT	NT	NT	0.19±0.03
Sodium hydrogenated tallow L-glutamate	26.7	1or2A	0.0018	0.00385	0.0041	NT	NT	NT	NT	0.0033±0.0013
Chlorhexidine gluconate (20% solution)	28.3	2A	0.061	0.037	0.0195	0.042	NT	NT	NT	0.040±0.017
Butanol	34.0	1or2A	8.6	6.0	12.3	9.6	NT	NT	NT	9.1±2.6
Potassium laurate	38.0	1or2A	0.17	0.23	0.13	0.13	NT	NT	NT	0.17±0.05
Polyoxyethylene octylphenylether (10 E.O.)	41.3	1or2A	0.034	0.0285	0.060	NT	NT	NT	NT	0.041±0.017
Di (2-ethylhexyl) sodium sulfosuccinate	57.0	1or2A	0.0066	0.0083	0.0068	0.0074	NT	NT	NT	0.0073±0.0008
Acetic acid	68.0	1or2A	0.23	0.24	0.215	0.96	NT	NT	NT	0.41±0.37
Cetyltrimethylammonium bromide	76.7	1or2A	0.0015	0.0014	0.0018	0.0022	NT	NT	NT	0.0017±0.0004
Benzalkonium chloride	78.0	1or2A	0.0018	0.0023	0.0016	NT	NT	NT	NT	0.0019±0.0004
Stearyltrimethylammonium chloride	91.3	1or2A	0.0030	0.0012	0.0013	0.0014	NT	NT	NT	0.0017±0.0009
Cetylpyridinium chloride	94.7	1	0.0027	0.0013	0.00265	0.0013	0.00124	0.00165	0.0026	0.0019±0.0007
Domiphen bromide	96.3	1	0.0018	0.0021	0.0070	0.0019	NT	NT	NT	0.0032±0.0025

The data were taken from Ohuchi et al. (1999). Triethanolamine was used as negative reference. In Lab. E-G that triethanolamine was not tested, 6.6% was used as the cut-off value. As reported by Ohuchi et al. (1999), m-phenylenediamine was excluded from the subsequent analysis due to instability.
MAS: Maximal average score of the Draize eye test.
GHS category 1: Severe or corrosive irritant, 2A: Irritant, 2B: Irritant, NI: Non irritant.
1or2A: The Draize eye test results couldn't discriminate between 1 and 2A for no observation data on day 21. The observation was performed to day 14.
$: Sodium lauryl sulfate was evaluated as positive in the evaluation on the basis of MAS, because 2 of 3 individuals had the corneal damage of 15 and 10 (for the maximal corneal score), respectively.
SD: Standard deviation
NT: Not tested

Table 48 Results of interlaboratory reproducibility on the LDM-MTT assay
(Concentration: 10%, Negative reference: Triethanolamine)
-GHS classification by considering pH-

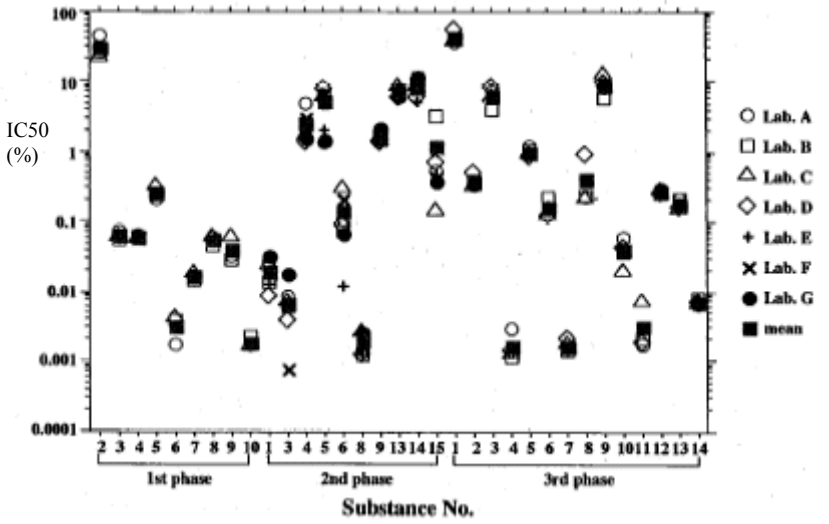
Substance (Draize eye test was performed at 10% concentration)	MAS at 10%	GHS at 10%	IC50 of the LDM-MTT assay (%)							
			Lab. A	Lab. B	Lab. C	Lab. D	Lab. E	Lab. F	Lab. G	Average±SD (%)
Ethanol	0.0	NI	36	41	37.5	56	NT	NT	NT	43±9
2-Ethylhexyl p-dimethylamino benzonate	0.0	NI	100<	100<	100<	100<	100<	100<	100<	100<
Glycerin	0.0	NI	100<	100<	100<	100<	100<	100<	100<	100<
Polyethylene glycol 400	0.0	NI	100<	100<	85	78	100	67	82	67<
Polyoxyethylene hydrogenated caster oil (60 E.O.)	0.0	NI	36	26.5	21.5	NT	NT	NT	NT	28.0±7.4
Polyoxyethylene sorbitan monooleate (20E.O.)	0.0	NI	4.8	2.53	1.65	1.4	1.93	3.2	1.55	2.4±1.2
Sodium salicylate	0.0	NI	9.2	9.8	8.5	6.0	5.47	11.5	11.5	8.9±2.4
Triethanolamine	0.0	NI	7.6	4.1	6.2	8.4	NT	NT	NT	6.6±1.9
Isopropyl myristate	0.7	NI	100<	100<	100<	100<	100<	100<	100<	100<
Polyoxyethylene sorbitan monolaurate (20 E.O.)	0.7	NI	0.072	0.057	0.061	NT	NT	NT	NT	0.063±0.008
Polyethyleneglycol monolaurate (10 E.O.)	3.3	NI	0.064	0.06	0.058	NT	NT	NT	NT	0.061±0.003
m-Phenylenediamine (Lack of stability)	4.3	NI	0.56	3.4	0.145	0.72	0.47	0.45	0.4	0.88±1.13
Sodium polyoxyethylene laurylether sulfate (2 E.O.) (27% solution)	10.0	NI	0.060	0.047	0.06	NT	NT	NT	NT	0.056±0.008
Sodium N-lauryl sarcosinate (30% solution)	10.3	NI	0.22	0.25	0.32	NT	NT	NT	NT	0.26±0.05
Sucrose fatty scid ester	11.0	NI	0.027	0.014	0.024	0.009	0.013	0.02	0.033	0.020±0.009
Calcium thioglycolate	4.0	1*	1.4	6.4	6.0	7.7	2.15	7.0	1.5	4.6±2.8
Lactic acid	9.7	1*	0.31	0.27	0.285	0.26	NT	NT	NT	0.28±0.02
Sodium lauryl sulfate	15.0$	1or2A	0.017	0.015	0.018	NT	NT	NT	NT	0.017±0.002
Benzyl alcohol	23.0	1or2A	7.4	7.0	8.6	6.2	8.2	7.15	6.4	7.3±0.9
Diisopropanolamine	23.0	1*	1.2	1.1	0.92	0.88	NT	NT	NT	1.0±0.2
Monoethanolamine	23.3	1*	0.34	0.38	0.33	0.53	NT	NT	NT	0.40±0.09
Acid red 92	25.0	1or2A	0.0086	0.0062	0.0074	0.0038	0.0073	0.0008	0.018	0.0074±0.0054
Glycolic acid	25.0	1*	0.22	0.21	0.155	0.16	NT	NT	NT	0.19±0.03
Sodium hydrogenated tallow L-glutamate	26.7	1or2A	0.0018	0.00385	0.0041	NT	NT	NT	NT	0.0033±0.0013
Chlorhexidine gluconate (20% solution)	28.3	2A	0.061	0.037	0.0195	0.042	NT	NT	NT	0.040±0.017
Butanol	34.0	1or2A	8.6	6.0	12.3	9.6	NT	NT	NT	9.1±2.6
Potassium laurate	38.0	1or2A	0.17	0.23	0.13	0.13	NT	NT	NT	0.17±0.05
Polyoxyethylene octylphenylether (10 E.O.)	41.3	1or2A	0.034	0.0285	0.060	NT	NT	NT	NT	0.041±0.017
Di (2-ethylhexyl) sodium sulfosuccinate	57.0	1or2A	0.0066	0.0083	0.0068	0.0074	NT	NT	NT	0.0073±0.0008
Acetic acid	68.0	1or2A	0.23	0.24	0.215	0.96	NT	NT	NT	0.41±0.37
Cetyltrimethylammonium bromide	76.7	1or2A	0.0015	0.0014	0.0018	0.0022	NT	NT	NT	0.0017±0.0004
Benzalkonium chloride	78.0	1or2A	0.0018	0.0023	0.0016	NT	NT	NT	NT	0.0019±0.0004
Stearyltrimethylammonium chloride	91.3	1or2A	0.0030	0.0012	0.0013	0.0014	NT	NT	NT	0.0017±0.0009
Cetylpyridinium chloride	94.7	1	0.0027	0.0013	0.00265	0.0013	0.00124	0.00165	0.0026	0.0019±0.0007
Domiphen bromide	96.3	1	0.0018	0.0021	0.0070	0.0019	NT	NT	NT	0.0032±0.0025

The data were taken from Ohuchi et al. (1999). Triethanolamine was used as negative reference. In Lab. E-G that triethanolamine was not tested, 6.6% was used as the cut-off value. As reported by Ohuchi et al. (1999), m-phenylenediamine was excluded from the subsequent analysis due to instability.
MAS: Maximal average score of the Draize eye test.
GHS category 1: Severe or corrosive irritant, 2A: Irritant, 2B: Irritant, NI: Non irritant.
1or2A: The Draize eye test results couldn't discriminate between 1 and 2A for no observation data on day 21. The observation was performed to day 14.
$: Sodium lauryl sulfate was evaluated as positive in the evaluation on the basis of MAS, because 2 of 3 individuals had the corneal damage of 15 and 10 (for the maximal corneal score), respectively.
SD: Standard deviation
NT: Not tested

Table 49 Results of interlaboratory reproducibility on the LDM-MTT assay
(Remainging substances)

Substance (Draize eye test was not performed at 10% concentration)	MAS as is	GHS as is	IC50 of the LDM-MTT assay (%)							
			Lab. A	Lab. B	Lab. C	Lab. D	Lab. E	Lab. F	Lab.G	Average±SD
Isotonic sodium chloride solution	0.0	NI	100<	100<	100<	NT	NT	NT	NT	10000<
Silicic anhydride	2.7	NI	100<	100<	100<	100<	000<	100<	100<	100<
Methyl p-hydroxybenzoate	8.7	NI	1.6	1.66	1.6	1.4	1.36	1.75	2.2	1.7±0.3
Distearyldimethylammonium chloride	96.3	1	0.11	0.072	0.295	0.092	0.0125	0.22	0.07	0.12±0.10

Fig. 9 Interlaboratory variability in the LDM-MTT assay



Substance no. and substance name

1st phase -2	Polyoxyethylene hydrogenated caster oil (60 E.O.)
1st phase -3	Polyoxyethylene sorbitan monolaurate (20 E.O.)
1st phase -4	Polyethyleneglycol monolaurate (10 E.O.)
1st phase -5	Sodium N-lauryl sarcosinate (30% solution)
1st phase-6	Sodium hydrogenated tallow L-glutamate
1st phase-7	Sodium lauryl sulfate
1st phase-8	Sodium polyoxyethylene laurylether sulfate (2 E.O.) (27% solution)
1st phase-9	Polyoxyethylene octylphenylether (10 E.O.)
1st phase-10	Benzalkonium chloride
2nd phase-1	Sucrose fatty scid ester
2nd phase-3	Acid red 92
2nd phase-4	Polyoxyethylene sorbitan monooleate (20E.O.)
2nd phase-5	Calcium thioglycolate
2nd phase-6	Distearyldimethylammonium chloride
2nd phase-8	Cetylpyridinium chloride
2nd phase-9	Methyl p-hydroxybenzoate
2nd phase-13	Benzyl alcohol
2nd phase-14	Sodium salicylate
2nd phase-15	m-Phenylenediamine
3rd phase-1	Ethanol
3rd phase-2	Monoethanolamine
3rd phase-3	Triethanolamine
3rd phase-4	Stearyltrimethylammonium chloride
3rd phase-5	Diisopropanolamine
3rd phase-6	Potassium laurate
3rd phase-7	Cetyltrimethylammonium bromide
3rd phase-8	Acetic acid
3rd phase-9	Butanol
3rd phase-10	Chlorhexidine gluconate (20% solution)
3rd phase-11	Domiphen bromide
3rd phase-12	Lactic acid
3rd phase-13	Glycolic acid
3rd phase-14	Di (2-ethylhexyl) sodium sulfosuccinate

The figure is the same as that reported by Ohuchi et al (1999). IC50 values obtained were plotted on the figure. The following substances which did not inhibit MTT conversion by 50% when tested at full strength were excluded: S1-1, S2-2, S2-7, S2-10, S2-11, S2-12. Participation: first phase-three laboratories; second phase-seven laboratories; third phase-four laboratories.

Table 50 Rank correlation coefficient between the average IC50 of all laboratories and the IC50 of each laboratory in the LDM-MTT assay

	Lab. A	Lab. B	Lab. C	Lab. D	Lab. E	Lab. F	Lab. G
Rank correlation coefficent	0.996	0.997	0.993	0.995	0.995	0.998	0.991

The data are extracted from the table reported by Ohuchi et al (1999).

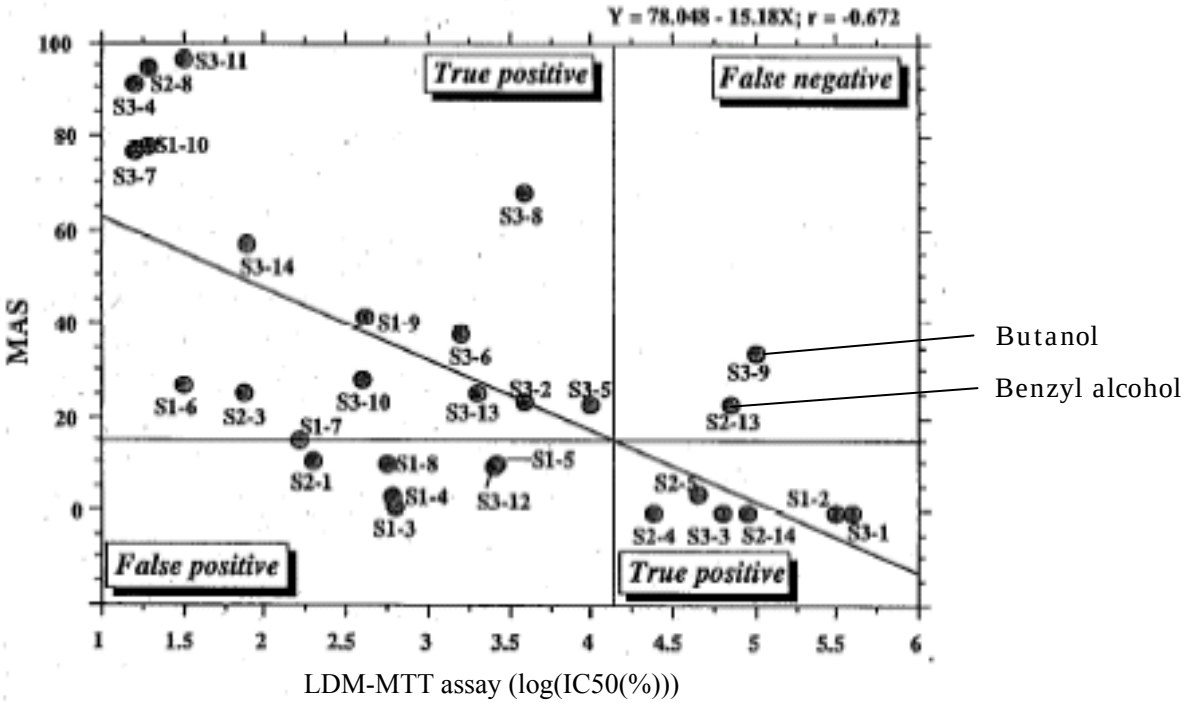
Table 51 Correlation of the results obtained by alternative methods and Draize eye test

Methods	Analysis using all data			Analysis excluding specific classes of chemicals			
		Correlation coefficients				Correlation coefficients	
	N	Pearson's linear	Spearman's rank	class###	N	Pearson's linear	Spearman's rank
Chorioallantoic membranc							
HET–CAM	52	0.688	0.802	1	46	0.702	0.831
				2	6	0.779	0.714
CAM–TB	55	0.718	0.838	1	48	0.801	0.863
				2	7	0.926	0.964
Red blood cells							
RBC	17	–0.631	0.643	3	16	–0.651	0.674
Haemoglobin							
RDC_{50}	8##	0.906	0.714				
1%RDR	23##	0.671	0.579				
1% λ max	31##	0.791	0.697				
Artificial skin models							
SKIN™ (ZK 1100)#	30	–0.694	0.680	4	20	–0.842	
MATREX™#	30	–0.672	0.832	4	20	–0.754	
Normal cells from rabbit cornea							
CornePack*#	28	–0.538	0.588	4	21	–0.731	0.787
Cell lines from rabbit cornea							
SIRC–CVS#	29	–0.805	0.779	4	22	–0.924	0.945
SIRC–NRU#	30	–0.816	0.787	4	23	–0.916	0.931
Cell lines from the other mammals							
HeLa–MTT#	29	–0.799	0.745	4	22	–0.922	0.926
CHL–CVS#	29	–0.729	0.703	4	22	–0.864	0.880
EYTEX™	38	0.313					

#: log (EC_{50}) were correlated with Draize scores (maximal average total score). ##: include the data of substances of the first validation, for which the experiments were conducted afterwards, during the second and the third validations. ###: 1: liquid sample only, 2: powder sample only; 3: excluded strong alkali and acid samples; 4: excluded alcohol (lower mono-ol), strong acids and strong alkalis.

The data are the same as those of Ohno et al. (1999). The LDM-MTT assay is shown as "MATREX™" in the figure.

Fig. 10 Relationship between the LDM-MTT assay and the Draize eye test



The figure is the same as that reported by Ohuchi et al (1999).

Substance no.	and substance name
S1-1	Isotonic sodium chloride solution
S1-2	Polyoxyethylene hydrogenated caster oil (60 E.O.)
S1-3	Polyoxyethylene sorbitan monolaurate (20 E.O.)
S1-4	Polyethyleneglycol monolaurate (10 E.O.)
S1-5	Sodium N-lauryl sarcosinate (30% solution)
S1-6	Sodium hydrogenated tallow L-glutamate
S1-7	Sodium lauryl sulfate
S1-8	Sodium polyoxyethylene laurylether sulfate (2 E.O.) (27% solution)
S1-9	Polyoxyethylene octylphenylether (10 E.O.)
S1-10	Benzalkonium chloride
S2-1	Sucrose fatty scid ester
S2-2	Glycerin
S2-3	Acid red 92
S2-4	Polyoxyethylene sorbitan monooleate (20E.O.)
S2-5	Calcium thioglycolate
S2-6	Distearyldimethylammonium chloride
S2-7	2-Ethylhexyl p-dimethylamino benzonate
S2-8	Cetylpyridinium chloride
S2-9	Methyl p-hydroxybenzoate
S2-10	Isopropyl myristate
S2-11	Polyethylene glycol 400
S2-12	Silicic anhydride
S2-13	Benzyl alcohol
S2-14	Sodium salicylate
S2-15	m-Phenylenediamine
S3-1	Ethanol
S3-2	Monoethanolamine
S3-3	Triethanolamine
S3-4	Stearyltrimethylammonium chloride
S3-5	Diisopropanolamine
S3-6	Potassium laurate
S3-7	Cetyltrimethylammonium bromide
S3-8	Acetic acid
S3-9	Butanol
S3-10	Chlorhexidine gluconate (20% solution)
S3-11	Domiphen bromide
S3-12	Lactic acid
S3-13	Glycolic acid
S3-14	Di (2-ethylhexyl) sodium sulfosuccinate

Table 52 Predicted irritancy of test samples based on the LDM-MTT assay
(Concentration: 10%, Cut-off value: 4.15%)

		In vitro (Classification by the LDM-MTT assay)	
		Positive	Negative
In vivo (Classification by GHS)	1, 2Aor 2B	Sodium lauryl sulfate Monoethanolamine Acid red 92 Glycolic acid Sodium hydrogenated tallow L-glutamate Chlorhexidine gluconate (20% solution) Potassium laurate Polyoxyethylene octylphenylether (10 E.O.) Di (2-ethylhexyl) sodium sulfosuccinate Acetic acid Cetyltrimethylammonium bromide Benzalkonium chloride Stearyltrimethylammonium chloride Cetylpyridinium chloride Domiphen bromide 15	Benzyl alcohol Butanol 2
	NI	Polyoxyethylene sorbitan monooleate (20E.O.) Polyoxyethylene sorbitan monolaurate (20 E.O.) Polyethyleneglycol monolaurate (10 E.O.) Lactic acid Sodium polyoxyethylene laurylether sulfate (2 E.O.) (27% solution) Sodium N-lauryl sarcosinate (30% solution) Sucrose fatty scid ester Diopropanolamine 8	Ethanol 2-Ethylhexyl p-dimethylamino benzonate Glycerin Polyethylene glycol 400 Polyoxyethylene hydrogenated caster oil (60 E.O.) Sodium salicylate Triethanolamine Isopropyl myristate Calcium thioglycolate 9

Table 53 Predicted irritancy of test samples based on the LDM-MTT assay
(Concentration: 10%, Cut-off value: 4.15%)
-GHS classification by considering pH-

		In vitro (Classification by the LDM-MTT assay)	
		Positive	Negative
In vivo (Classification by GHS)	1, 2Aor 2B	Lactic acid Sodium lauryl sulfate Diisopropanolamine Monoethanolamine Acid red 92 Glycolic acid Sodium hydrogenated tallow L-glutamate Chlorhexidine gluconate (20% solution) Potassium laurate Polyoxyethylene octylphenylether (10 E.O.) Di (2-ethylhexyl) sodium sulfosuccinate Acetic acid Cetyltrimethylammonium bromide Benzalkonium chloride Stearyltrimethylammonium chloride Cetylpyridinium chloride Domiphen bromide 17	Calcium thioglycolate Benzyl alcohol Butanol 3
	NI	Polyoxyethylene sorbitan monooleate (20E.O.) Polyoxyethylene sorbitan monolaurate (20 E.O.) Polyethyleneglycol monolaurate (10 E.O.) Sodium polyoxyethylene laurylether sulfate (2 E.O.) (27% solution) Sodium N-lauryl sarcosinate (30% solution) Sucrose fatty scid ester 6	Ethanol 2-Ethylhexyl p-dimethylamino benzonate Glycerin Polyethylene glycol 400 Polyoxyethylene hydrogenated caster oil (60 E.O.) Sodium salicylate Triethanolamine Isopropyl myristate 8

Table 54 Predicted irritancy of test samples based on the LDM-MTT assay
(Concentration: 10%, Negative reference: Triethanolamine)

		In vitro (Classification by the LDM-MTT assay)	
		Positive	Negative
In vivo (Classification by GHS)	1, 2Aor 2B	Sodium lauryl sulfate Monoethanolamine Acid red 92 Glycolic acid Sodium hydrogenated tallow L-glutamate Chlorhexidine gluconate (20% solution) Potassium laurate Polyoxyethylene octylphenylether (10 E.O.) Di (2-ethylhexyl) sodium sulfosuccinate Acetic acid Cetyltrimethylammonium bromide Benzalkonium chloride Stearyltrimethylammonium chloride Cetylpyridinium chloride Domiphen bromide 15	Benzyl alcohol Butanol 2
	NI	Polyoxyethylene sorbitan monooleate (20E.O.) Polyoxyethylene sorbitan monolaurate (20 E.O.) Polyethyleneglycol monolaurate (10 E.O.) Lactic acid Sodium polyoxyethylene laurylether sulfate (2 E.O.) (27% solution) Sodium N-lauryl sarcosinate (30% solution) Sucrose fatty scid ester Diopropanolamine 8	Ethanol 2-Ethylhexyl p-dimethylamino benzonate Glycerin Polyethylene glycol 400 Polyoxyethylene hydrogenated caster oil (60 E.O.) Sodium salicylate Triethanolamine Isopropyl myristate Calcium thioglycolate 9

Table 55 Predicted irritancy of test samples based on the LDM-MTT assay
(Concentration: 10%, Negative reference: Triethanolamine)
-GHS classification by considering pH-

		In vitro (Classification by the LDM-MTT assay)	
		Positive	Negative
In vivo (Classification by GHS)	1, 2Aor 2B	Calcium thioglycolate Lactic acid Sodium lauryl sulfate Diisopropanolamine Monoethanolamine Acid red 92 Glycolic acid Sodium hydrogenated tallow L-glutamate Chlorhexidine gluconate (20% solution) Potassium laurate Polyoxyethylene octylphenylether (10 E.O.) Di (2-ethylhexyl) sodium sulfosuccinate Acetic acid Cetyltrimethylammonium bromide Benzalkonium chloride Stearyltrimethylammonium chloride Cetylpyridinium chloride Domiphen bromide 18	Benzyl alcohol Butanol 2
	NI	Polyoxyethylene sorbitan monooleate (20E.O.) Polyoxyethylene sorbitan monolaurate (20 E.O.) Polyethyleneglycol monolaurate (10 E.O.) Sodium polyoxyethylene laurylether sulfate (2 E.O.) (27% solution) Sodium N-lauryl sarcosinate (30% solution) Sucrose fatty scid ester 6	Ethanol 2-Ethylhexyl p-dimethylamino benzonate Glycerin Polyethylene glycol 400 Polyoxyethylene hydrogenated caster oil (60 E.O.) Sodium salicylate Triethanolamine Isopropyl myristate 8

Table 56 Forty-eight substances (Concentration: 10%)

No	Substance	CAS	Supplier (*in vitro* test)	*in vivo* data reported previously		Estimated GHS at 10% concn	Reference
				Classification at 10% concn	Classification at the applied concn		
1	2-Bromo-2-nitropropane-1,3-diol	52-51-7	Fluorochem	Positive	Positive：100, 20, 10, 5% Negative：2, 0.5%	1, 2A or 2B	JACT 3(3):139-155,1984. JEPT 4(4):47-61, 1980.
2	Benzalkonium chloride	8001-54-5	Wako	Positive	Positive：2, 1, 0.5% Negative：0.1, 0.01%	1, 2A or 2B	JACT 8(4):589-625, 1989.
3	Cetrimonium chloride	112-02-7	Wako	Positive	Positive：2.5, 1.2, 0.5% Negative：0.1%	1, 2A or 2B	IJT 16(S3):195-220,1997.
4	Chlorhexidine digluconate	18472-51-0	Wako	Positive	Positive：20, 2% Negative：0.05%	1, 2A or 2B	JACT 12(3):201-23, 1993.
5	Chlorophene	120-32-1	Wako	Positive	Positive：100, 3% Negative：1, 0.3%	1, 2A or 2B	IJT 23(S1):1-27 2004.
6	Dioctyl sodium sulfosuccinate	577-11-7	Alfa Aesar	Positive	Positive：10% Negative：2, 0.5%	1, 2A or 2B	IJT 17(S4):1-20, 1998.
7	Lauramide DEA	120-40-1	Wako	Positive	Positive：20, 10%	1, 2A or 2B	JACT 5(5):415-54, 1986.
8	Phenethyl alcohol	60-12-8	Wako	Positive	Positive：100, 15, 5% Negative：0.3%	1, 2A or 2B	JACT 9(2):165-83, 1990.
9	Stearalkonium chloride	122-19-0	Wako	Positive	Positive：25, 4, 2.5% Negative：0.5%	1, 2A or 2B	JACT 1(2):57-69, 1982.
10	TEA-Lauryl sulfate	139-96-8	Wako	Positive	Positive：20, 10, 5, 2.5, 1.25%	1, 2A or 2B	JACT 1(4):143-67, 1982.
11	Acetyl tributyl citrate	77-90-7	Wako	Negative	Negative：100%	NI	IJT 21(S2):1-17, 2002.
12	Benzophenone-1	131-56-6	Wako	Negative	Positive：100% Negative：16, 8, 4%	NI	JACT 2(5):35-77, 1983.
13	Benzophenone-2	131-55-5	Wako	Negative	Positive：100% Negative：16, 8, 4%	NI	JACT 2(5):79-84, 1983.
14	Butylene glycol	107-88-0	Wako	Negative	Negative：100, 10%	NI	Hifu 26(5):1065-1074, 1984.
15	Carnauba wax	8015-86-9	Wako	Negative	Negative：50%	NI	JACT 3(3):1-41, 1984.
16	Cetyl alcohol	36653-82-4	Wako	Negative	Negative：100%	NI	JACT 7(3):359-413, 1988.
17	Cetyl palmitate	540-10-3	Wako	Negative	Negative：100%	NI	JACT 1(2):13-35, 1982.
18	Decyl oleate	3687-46-5	Wako	Negative	Negative：100%	NI	JACT 1(2):85-95, 1982.
19	Diazolidinyl urea	78491-02-8	MP Biomedicals	Negative	Negative：30%	NI	JACT 9(2):229-45, 1990.
20	Diethylhexyl adipate	103-23-1	Wako	Negative	Negative：100%	NI	JACT 3(3):101-30, 1984.
21	Diisopropyl adipate	6938-94-9	Wako	Negative	Negative：100%	NI	JACT 3(3):101-30, 1984.
22	Ethylhexyl palmitate	29806-73-3	Wako	Negative	Negative：100%	NI	JACT 1(2):13-35, 1982.
23	Ethylhexyl stearate	22047-49-0	Wako	Negative	Negative：100%	NI	JACT 4(5):107-46, 1985.
24	Glyceryl stearate	11099-07-3	Wako	Negative	Negative：100%	NI	JACT 1(4):169-192, 1982.
25	Hexylene glycol	107-41-5	Wako	Negative	Positive：100% Negative：25%	NI	JACT 4(5):223-48, 1985.
26	Isocetyl stearate	25339-09-7	Wako	Negative	Negative：100%	NI	JACT 4(5):107-46, 1985.
27	Isopropyl myristate	110-27-0	TCI	Negative	Negative：100%	NI	JACT 1(4):55-80, 1982.
28	Isopropyl palmitate	142-91-6	Wako	Negative	Negative：100%	NI	JACT 1(2):13-35, 1982.
29	Oleyl alcohol	143-28-2	Wako	Negative	Negative：100%	NI	JACT 4(5):1-29, 1985.
30	PEG-2 stearate	106-11-6	Wako	Negative	Negative：100%	NI	JACT 2(7):17-60, 1983.
31	PEG-40 stearate	9004-99-4	Wako	Negative	Negative：100%	NI	JACT 2(7):17-60, 1983.
32	Phytantriol	74563-64-7	Wako	Negative	Positive：100, 23% Negative：10, 3%	NI	IJT 26(Suppl. 1):107-117, 2007.
33	Propylene carbonate	108-32-7	Wako	Negative	Negative：100, 17.5, 10.5%	NI	JACT 6(1):23-51, 1987.
34	Castor seed oil	8001-79-4	Wako	Negative	Negative：100%	NI	JACT 7(6):721-739, 1988.
35	Safflower oil	8001-23-8	Wako	Negative	Negative：100%	NI	JACT 4(5):171-97, 1985.
36	Sesame (Sesamum indicum) oil	8008-74-0	Wako	Negative	Negative：100%	NI	JACT 12(3):261-77, 1993.
37	Sodium dehydroacetate	4418-26-2	Wako	Negative	Negative：100%	NI	JACT 4(3):123-159, 1985.
38	Sodium stearate	822-16-2	Wako	Negative	Negative：100%	NI	JACT 1(2):143-77, 1982.
39	Sorbitan oleate	1338-43-8	Wako	Negative	Negative：100%	NI	JACT 4(3):65-121, 1985.
40	Sorbitan sesquioleate	8007-43-0	Wako	Negative	Negative：100, 30%	NI	JACT 4(3):65-121, 1985.
41	Sorbitan stearate	1338-41-6	Wako	Negative	Negative：30%	NI	JACT 4(3):65-121, 1985.
42	Squalane	111-01-3	Wako	Negative	Negative：100%	NI	JACT 1(2):37-56, 1982.
43	Steareth-2	9005-00-9	Wako	Negative	Negative：60%	NI	JACT 7(6):881-910, 1988.
44	Steareth-20	9005-00-9	Wako	Negative	Negative：60%	NI	JACT 7(6):881-910, 1988.
45	Stearyl alcohol	112-92-5	Wako	Negative	Negative：100%	NI	JACT 4(5):1-29, 1985.
46	Triacetin	102-76-1	Wako	Negative	Negative：100%	NI	IJT 22(S2):1-10, 2003.
47	Triethylene glycol	112-27-6	Wako	Negative	Negative：100%	NI	IJT 25(5) :121-138,2006.
48	Zinc stearate	557-05-1	Wako	Negative	Negative：100%	NI	JACT 1(2):143-77, 1982.

Supplier means manufacturer of the material used in this study. The *in vivo* classification of positive or negative was based on the appearance or not of corneal damage, or an MAS value of 15 as a cut-off point, where reported MAS values are available. The classification was essentially based on whether or not corneal damage appeared after the application of 0.1 mL to rabbit eye without irrigation. However, where there were differences of test conditions, these were considered individually. For example, a case where corneal damage appeared after the application of 0.05 mL was judged as positive. In cases without data at 10% concentration, the assessment of positive or negative at the concentration of 10% was made on the basis of dose-response analysis of each ingredient.

Table 57 Results of 48 substances in the LDM-MTT assay
(Concentration: 10%, Negative reference: Triethanolamine)

No	Substance	Draize eye test at 10% concn	Estimated GHS at 10% concn	LDM-MTT assay		
				Medium	IC50 (%)	Results
1	2-Bromo-2-Nitropropane-1,3-Diol	Positive	1, 2A or 2B	DW	<1	Positive
2	Benzalkonium chloride	Positive	1, 2A or 2B	DW	<1	Positive
3	Cetrimonium chloride	Positive	1, 2A or 2B	DW	<1	Positive
4	Chlorhexidine digluconate	Positive	1, 2A or 2B	DW	<1	Positive
5	Chlorophene	Positive	1, 2A or 2B	EG	<1	Positive
6	Dioctyl sodium sulfosuccinate	Positive	1, 2A or 2B	DW	<1	Positive
7	Lauramide DEA	Positive	1, 2A or 2B	DW	<1	Positive
8	Phenethyl alcohol	Positive	1, 2A or 2B	50%DMSO	2.7	Positive
9	Stearalkonium chloride	Positive	1, 2A or 2B	DW	<1	Positive
10	TEA-Lauryl sulfate	Positive	1, 2A or 2B	DW	<1	Positive
11	Acetyl tributyl citrate	Negative	NI	-	100	Negative
12	Benzophenone-1	Negative	NI	50%DMSO	<1	Positive
13	Benzophenone-2	Negative	NI	50%DMSO	<1	Positive
14	Butylene glycol	Negative	NI	-	100	Negative
15	Carnauba (Copernicia cerifera) wax	Negative	NI	-	100	Negative
16	Cetyl alcohol	Negative	NI	LP	10<	Negative
17	Cetyl palmitate	Negative	NI	LP	10<	Negative
18	Decyl oleate	Negative	NI	-	100	Negative
19	Diazolidinyl urea	Negative	NI	DW	<1	Positive
20	Diethylhexyl adipate(=Octyl)	Negative	NI	-	100	Negative
21	Diisopropyl adipate	Negative	NI	LP	8.6	Negative
22	Ethylhexyl palmitate (=Octyl)	Negative	NI	-	100	Negative
23	Ethylhexyl stearate (=Octyl)	Negative	NI	-	100	Negative
24	Glyceryl stearate	Negative	NI	-	100	Negative
25	Hexylene glycol	Negative	NI	DW	10<	Negative
26	Isocetyl stearate	Negative	NI	-	100	Negative
27	Isopropyl Myristate	Negative	NI	-	100	Negative
28	Isopropyl Palmitate	Negative	NI	-	100	Negative
29	Oleyl alcohol	Negative	NI	-	100	Negative
30	PEG-2 stearate	Negative	NI	-	100	Negative
31	PEG-40 stearate	Negative	NI	50%DMSO	1.3	Positive
32	Phytantriol	Negative	NI	50%DMSO	1.9	Positive
33	Propylene carbonate	Negative	NI	DW	10<	Negative
34	Ricinus communis (Castor) seed oil	Negative	NI	-	100	Negative
35	Safflower (Carthamus tinctorius) oil	Negative	NI	-	100	Negative
36	Sesame (Sesamum indicum) oil	Negative	NI	-	100	Negative
37	Sodium dehydroacetate	Negative	NI	DW	10<	Negative
38	Sodium stearate	Negative	NI	DW	2.1	Positive
39	Sorbitan oleate	Negative	NI	-	100	Negative
40	Sorbitan sesquioleate	Negative	NI	-	100	Negative
41	Sorbitan stearate	Negative	NI	-	100	Negative
42	Squalane	Negative	NI	-	100	Negative
43	Steareth-2	Negative	NI	DW	10<	Negative
44	Steareth-20	Negative	NI	DW	<1	Positive
45	Stearyl alcohol	Negative	NI	-	100	Negative
46	Triacetin	Negative	NI	DW	10<	Negative
47	Triethylene glycol	Negative	NI	-	100	Negative
48	Zinc stearate	Negative	NI	-	100	Negative
Negative 基準	Triethanolamine	Negative	NI	DW	4.6	Negative

The results of LDM-MTT assay are shown as average (n=2-3) of IC50 value.

Table 58 Predicted irritancy of 48 substances in the LDM-MTT assay

		In vitro (Classification by LDM-MTT assay using triethanolamine as a reference substance for non-irritancy)	
		Positive	Negative
In vivo (Classicication by Draize eye test at 10% concn) Corneal damage or MAS over 15 was classified as positive.	Positive 1,2Aor2B in GHS	2-Bromo-2-nitropropane-1,3-diol Benzalkonium chloride Cetrimonium chloride Chlorhexidine digluconate Chlorophene Dioctyl sodium sulfosuccinate Lauramide DEA Stearalkonium chloride TEA-Lauryl sulphate 9	Phenethyl alcohol 1
	Negative NI in GHS	Benzophenone-1 Benzophenone-2 Diazolidinyl urea PEG-40 stearate Phytantriol Sodium stearate Steareth-20 7	Acetyl tributyl citrate Butylene glycol Carnauba wax Castor seed oil Cetyl alcohol Cetyl palmitate Decyl oleate Diethylhexyl adipate Diisopropyl adipate Ethylhexyl palmitate Ethylhexyl stearate Glyceryl stearate Hexylene glycol Isocetyl stearate Isopropyl Myristate Isopropyl Palmitate Oleyl alcohol PEG-2 stearate Propylene carbonate Safflower oil Sesame oil Sodium dehydroacetate Sorbitan oleate Sorbitan sesquioleate Sorbitan stearate Squalane Steareth-2 Stearyl alcohol Triacetin Triethylene glycol Zinc stearate 31

Triethanolamine (IC50=4.6%) was used as a reference substance for non-irritancy.

Table 59 Predicted irritancy of test samples based on the LDM-MTT assay
(Concentration: 10%, Negative reference: Triethanolamine)
-GHS classification by considering pH-

		In vitro (Classification by the LDM-MTT assay)	
		Positive	Negative
In vivo (Classification by GHS)	1, 2Aor 2B	Calcium thioglycolate Lactic acid Sodium lauryl sulfate Diisopropanolamine Monoethanolamine Acid red 92 Glycolic acid Sodium hydrogenated tallow L-glutamate Chlorhexidine gluconate (20% solution) Potassium laurate Polyoxyethylene octylphenylether (10 E.O.) Di (2-ethylhexyl) sodium sulfosuccinate Acetic acid Cetyltrimethylammonium bromide Benzalkonium chloride Stearyltrimethylammonium chloride Cetylpyridinium chloride Domiphen bromide 2-Bromo-2-nitropropane-1,3-diol ~~Benzalkonium chloride~~ ~~Cetrimonium chloride~~ Chlorhexidine digluconate Chlorophene Dioctyl sodium sulfosuccinate Lauramide DEA Stearalkonium chloride TEA-Lauryl sulphate 25	Benzyl alcohol Butanol Phenethyl alcohol 3
	NI	Polyoxyethylene sorbitan monooleate (20E.O.) Polyoxyethylene sorbitan monolaurate (20 E.O.) Polyethyleneglycol monolaurate (10 E.O.) Sodium polyoxyethylene laurylether sulfate (2 E.O.) (27% solution) Sodium N-lauryl sarcosinate (30% solution) Sucrose fatty scid ester Benzophenone-1 Benzophenone-2 Diazolidinyl urea PEG-40 stearate Phytantriol Sodium stearate Steareth-20 13	Ethanol 2-Ethylhexyl p-dimethylamino benzonate Glycerin Polyethylene glycol 400 Polyoxyethylene hydrogenated caster oil (60 E.O.) Sodium salicylate Triethanolamine Isopropyl myristate Acetyl tributyl citrate Butylene glycol Carnauba wax Castor seed oil Cetyl alcohol Cetyl palmitate Decyl oleate Diethylhexyl adipate Diisopropyl adipate Ethylhexyl palmitate Ethylhexyl stearate Glyceryl stearate Hexylene glycol Isocetyl stearate ~~Isopropyl Myristate~~ Isopropyl Palmitate Oleyl alcohol PEG-2 stearate Propylene carbonate Safflower oil Sesame oil Sodium dehydroacetate Sorbitan oleate Sorbitan sesquioleate Sorbitan stearate Squalane Steareth-2 Stearyl alcohol Triacetin Triethylene glycol Zinc stearate 38

Table 60 Relationship between IC_{50} in the LDM-MTT assay and concentration evaluated as non irritant in the Draize eye test.

Test substance	Three-dimensional dermal model IC_{50} (%)	Draize eye irritation test results				
		Concentration evaluated as non irritant (MAS<5)	MAS at each applied concentration			
			100%	10%	1%	0.1%
Isotonic sodium chloride solution	100	100	0	NT	NT	NT
2-Ethylhexyl p-dimethylaminobenzoate	100	100	0	0	NT	NT
Isopropyl myristate	100	100	0	0.7	NT	NT
Silicic anhydride	100	100	2.7	NT	NT	NT
Glycerin	100	100	4.7	0	NT	NT
Polyethylene glycol 400	67-100	100	4	0	NT	NT
Polyoxyethylene sorbitan monooleate (20E.O.)	2.4	100	4.7	0	NT	NT
Sodium salicylate	8.9	10	83.7	0	NT	NT
Triethanolamine	6.6	10	8.0	0	NT	NT
Calcium thioglycolate	4.6	10	79.7	4.0	NT	NT
Polyoxyethylene sorbitan monolaurate (20 E.O.)	0.063	10≤	NT	0.7	NT	NT
Polyethyleneglycol monolaurate (10 E.O.)	0.061	10≤	NT	3.3	NT	NT
Acid red 92	0.0074	1	71.0	25.0	0.7	NT
Cetylpyridinium chloride	0.0019	0.1	NT	94.7	34.7	2.7
Ethanol	43	10	32.7	0	NT	NT
Polyoxyethylene hydrogenated caster oil (60E.O)	28.0	10≤	NT	0	NT	NT
Benzyl alcohol	7.3	1	31.0	23.0	0	NT

The figure is the same as that reported by Hagino et al (2008).The data were taken from Ohno et al. (1999) and Ohuchi et al. (1999). The IC_{50} in LDM-MTT assay was the mean of data from 3-7 laboratories. The result of IC_{50} for polyethylene glycol 400 was 100% in 3 laboratories and 67, 78, 82, 85% in the other 4 laboratories. “Not tested” is shown as NT. No conclusion could be reached for ethanol, polyoxyethylene hydorogenated caster oil (60 E.O.) or benzyl alcohol, because of the large concentration intervals in the Draize eye test.

Table 61 Prediction of eye irritancy at various concentrations in the LDM-MTT assay

		In vitro (Classification by LDM-MTT assay using a viability of 50% as cut-off point)	
		Positive	Negative
In vivo (Estimated classification by GHS)	1, 2A or 2B	Calcium thioglycolate (100, 10%) Lactic acid (100, 10%) Sodium lauryl sulfate (10%) Benzyl alcohol (100, 10%) Diisopropanolamine (10%) Monoethanolamine (10%) Acid red 92 (100, 10%) Glycolic acid (10%) Sodium hydrogenated tallow L-glutamate (10%) Chlorhexidine gluconate (20% solution) (10%) Butanol (10%) Potassium laurate (10%) Polyoxyethylene octylphenylether (10 E.O.) (10%) Di (2-ethylhexyl) sodium sulfosuccinate (10%) Acetic acid (10%) Cetyltrimethylammonium bromide (10%) Benzalkonium chloride (10%) Stearyltrimethylammonium chloride (10%) Cetylpyridinium chloride (10, 1%) Domiphen bromide (10%) Sucrose fatty acid ester (100%) Ethanol (100%) Sodium salicylate (100%) Distearyldimethylammonium chloride (100%) 29	0
	NI	Polyoxyethylene sorbitan monooleate (20E.O.) (100, 10%) Sodium salicylate (10%) Triethanolamine (100, 10%) Polyoxyethylene sorbitan monolaurate (20 E.O.) (10%) Polyethyleneglycol monolaurate (10 E.O.) (10%) Sodium polyoxyethylene laurylether sulfate (2 E.O.) (27% solution) (10%) Sodium N-lauryl sarcosinate (30% solution) (10%) Sucrose fatty scid ester (10%) Methyl p-hydroxybenzoate (100%) Acid red 92 (1%) Cetylpyridinium chloride (0.1%) 13	Ethanol (10%) 2-Ethylhexyl p-dimethylamino benzonate (100, 10%) Glycerin (100, 10%) Polyethylene glycol 400 (10%) Polyoxyethylene hydrogenated caster oil (60 E.O.) (10%) Isopropyl myristate (100, 10%) Isotonic sodium chloride solution (100%) Silicic anhydride (100%) Benzyl alcohol (1%) 12

PEG 400 (100%) could not be classified (IC50=67-100%).

Table 62 Fifty-nine test substances

No	Substance	CAS	Supplier (*in vitro* test)	Estimated classification of GHS at the applied concn by using *in vivo* data reported previously	Reference
1	2-Bromo-2-nitropropane-1,3-diol	52-51-7	Fluorochem	1, 2A or 2B：100, 20, 10, 5% NI：2, 0.5%	JACT 3(3):139-155,1984. JEPT 4(4):47-61, 1980.
2	Benzalkonium chloride	8001-54-5	Wako	1, 2A or 2B：2, 1, 0.5%　NI：0.1, 0.01%	JACT 8(4):589-625, 1989.
3	Cetrimonium chloride	112-02-7	Wako	1, 2A or 2B：2.5, 1.2, 0.5%　NI：0.1%	IJT 16(S3):195-220,1997.
4	Chlorhexidine digluconate	18472-51-0	Wako	1, 2A or 2B：20, 2%　NI：0.05%	JACT 12(3):201-23, 1993.
5	Chlorophene	120-32-1	Wako	1, 2A or 2B：100, 3%　NI：1, 0.3%	IJT 23(S1):1-27 2004.
6	Dioctyl sodium sulfosuccinate	577-11-7	Alfa Aesar	1, 2A or 2B：10%　NI：2, 0.5%	IJT 17(S4):1-20, 1998.
7	Lauramide DEA	120-40-1	Wako	1, 2A or 2B：20, 10%	JACT 5(5):415-54, 1986.
8	Phenethyl alcohol	60-12-8	Wako	1, 2A or 2B：100, 15, 5%　NI：0.3%	JACT 9(2):165-83, 1990.
9	Stearalkonium chloride	122-19-0	Wako	1, 2A or 2B：25, 4, 2.5%　NI：0.5%	JACT 1(2):57-69, 1982.
10	TEA-Lauryl sulfate	139-96-8	Wako	1, 2A or 2B：20, 10, 5, 2.5, 1.25%	JACT 1(4):143-67, 1982.
11	Acetyl tributyl citrate	77-90-7	Wako	NI：100%	IJT 21(S2):1-17, 2002.
12	Benzophenone-1	131-56-6	Wako	1, 2A or 2B：100%　NI：16, 8, 4%	JACT 2(5):35-77, 1983.
13	Benzophenone-2	131-55-5	Wako	1, 2A or 2B：100%　NI：16, 8, 4%	JACT 2(5):79-84, 1983.
14	Butylene glycol	107-88-0	Wako	NI：100, 10%	Hifu 26(5):1065-1074, 1984.
15	Carnauba wax	8015-86-9	Wako	NI：50%	JACT 3(3):1-41, 1984.
16	Cetyl alcohol	36653-82-4	Wako	NI：100%	JACT 7(3):359-413, 1988.
17	Cetyl palmitate	540-10-3	Wako	NI：100%	JACT 1(2):13-35, 1982.
18	Decyl oleate	3687-46-5	Wako	NI：100%	JACT 1(2):85-95, 1982.
19	Diazolidinyl urea	78491-02-8	MP Biomedicals	NI：30%	JACT 9(2):229-45, 1990.
20	Diethylhexyl adipate	103-23-1	Wako	NI：100%	JACT 3(3):101-30, 1984.
21	Diisopropyl adipate	6938-94-9	Wako	NI：100%	JACT 3(3):101-30, 1984.
22	Ethylhexyl palmitate	29806-73-3	Wako	NI：100%	JACT 1(2):13-35, 1982.
23	Ethylhexyl stearate	22047-49-0	Wako	NI：100%	JACT 4(5):107-46, 1985.
24	Glyceryl stearate	11099-07-3	Wako	NI：100%	JACT 1(4):169-192, 1982.
25	Hexylene glycol	107-41-5	Wako	1, 2A or 2B：100%　NI：25%	JACT 4(5):223-48, 1985.
26	Isocetyl stearate	25339-09-7	Wako	NI：100%	JACT 4(5):107-46, 1985.
27	Isopropyl myristate	110-27-0	TCI	NI：100%	JACT 1(4):55-80, 1982.
28	Isopropyl palmitate	142-91-6	Wako	NI：100%	JACT 1(2):13-35, 1982.
29	Oleyl alcohol	143-28-2	Wako	NI：100%	JACT 4(5):1-29, 1985.
30	PEG-2 stearate	106-11-6	Wako	NI：100%	JACT 2(7):17-60, 1983.
31	PEG-40 stearate	9004-99-4	Wako	NI：100%	JACT 2(7):17-60, 1983.
32	Phytantriol	74563-64-7	Wako	1, 2A or 2B：100, 23%　NI：10, 3%	IJT 26(Suppl. 1):107-117, 2007.
33	Propylene carbonate	108-32-7	Wako	NI：100, 17.5, 10.5%	JACT 6(1):23-51, 1987.
34	Castor seed oil	8001-79-4	Wako	NI：100%	JACT 7(6):721-739, 1988.
35	Safflower oil	8001-23-8	Wako	NI：100%	JACT 4(5):171-97, 1985.
36	Sesame (Sesamum indicum) oil	8008-74-0	Wako	NI：100%	JACT 12(3):261-77, 1993.
37	Sodium dehydroacetate	4418-26-2	Wako	NI：100%	JACT 4(3):123-159, 1985.
38	Sodium stearate	822-16-2	Wako	NI：100%	JACT 1(2):143-77, 1982.
39	Sorbitan oleate	1338-43-8	Wako	NI：100%	JACT 4(3):65-121, 1985.
40	Sorbitan sesquioleate	8007-43-0	Wako	NI：100, 30%	JACT 4(3):65-121, 1985.
41	Sorbitan stearate	1338-41-6	Wako	NI：30%	JACT 4(3):65-121, 1985.
42	Squalane	111-01-3	Wako	NI：100%	JACT 1(2):37-56, 1982.
43	Steareth-2	9005-00-9	Wako	NI：60%	JACT 7(6):881-910, 1988.
44	Steareth-20	9005-00-9	Wako	NI：60%	JACT 7(6):881-910, 1988.
45	Stearyl alcohol	112-92-5	Wako	NI：100%	JACT 4(5):1-29, 1985.
46	Triacetin	102-76-1	Wako	NI：100%	IJT 22(S2):1-10, 2003.
47	Triethylene glycol	112-27-6	Wako	NI：100%	IJT 25(5) :121-138,2006.
48	Zinc stearate	557-05-1	Wako	NI：100%	JACT 1(2):143-77, 1982.
49	Benzethonium chloride	121-54-0	TCI	NI：0.5%	JACT 4(5):65-106, 1985.
50	Butoxyethanol	111-76-2	Wako	1, 2A or 2B：100, 15%　NI：5%	JACT 15(6):462-526, 1996.
51	Chloroxylenol	88-04-0	Wako	1, 2A or 2B：100, 30%	JACT 4(5):147-69, 1985.
52	Methoxyisopropyl acetate	108-65-6	Wako	1, 2A or 2B：100%	IJT 27(S2), 2008.
53	Phenoxyethanol	122-99-6	Wako	1, 2A or 2B：100%　NI：2.2%	JACT 9(2):259-77, 1990.
54	Phenyl methyl pyrazolone	89-25-8	Wako	NI：0.66%	JACT 11(4):475-88, 1992.
55	Resorcinol	108-46-3	Wako	1, 2A or 2B：100%	JACT 5(3):167-203, 1986.
56	Sodium hexametaphosphate	10124-56-8	Wako	NI：0.2%	IJT 20(S3):75-89, 2001.
57	Sodium lauroyl sarcosinate	137-16-6	Wako	NI：5%	IJT 20(S1):1-14, 2001.
58	Sodium naphthalenesulfonate	532-02-5	Wako	1, 2A or 2B：100%　NI：2%	IJT 22(Suppl. 2:37-44,)2003.

Supplier means manufacturer of the material used in this study. The *in vivo* classification of positive or negative was based on the appearance or not of corneal damage, or an MAS value of 15 as a cut-off point, where reported MAS values are available. The classification was essentially based on whether or not corneal damage appeared after the application of 0.1 mL to rabbit eye without irrigation. However, where there were differences of test conditions, these were considered individually. For example, a case where corneal damage appeared after the application of 0.05 mL was judged as positive. In cases without data at 10% concentration, the assessment of positive or negative at the concentration of 10% was made on the basis of dose-response analysis of each ingredient.

Table 63 Prediction of eye irritancy at various concentrations in the LDM-MTT assay

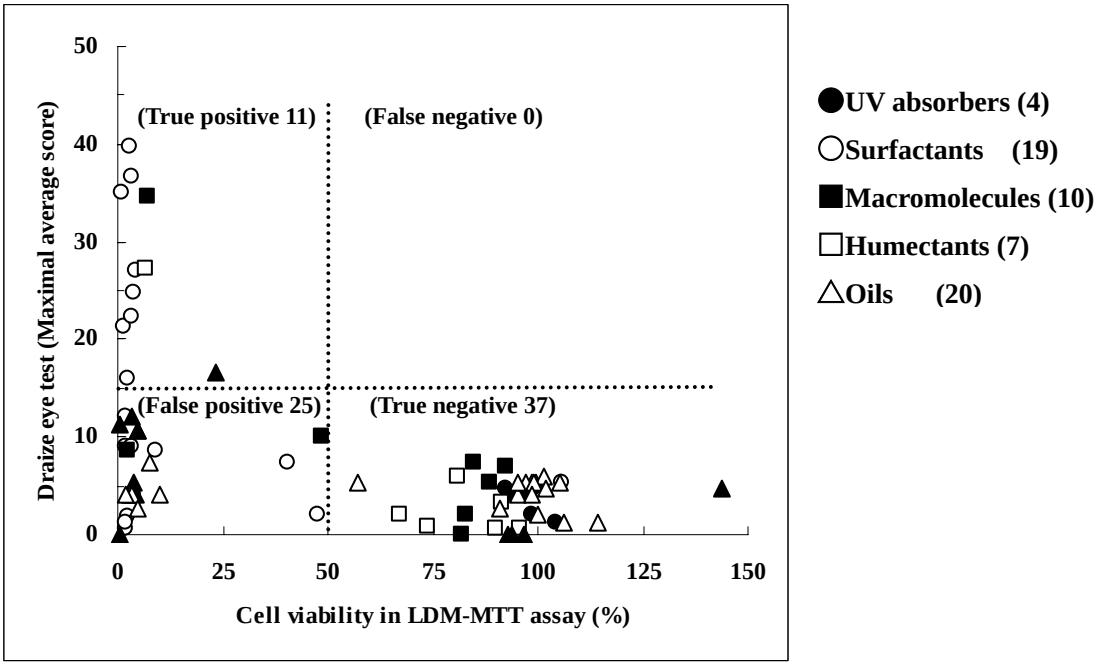
		In vitro (Classification by LDM-MTT assay using a viability of 50% as cut-off point)	
		Positive	Negative
In vivo (Estimated classification by GHS)	1, 2A or 2B	2-Bromo-2-nitropropane-1,3-diol (100, 20, 10, 5%) Benzalkonium chloride (2, 1, 0.5%) Benzophenone-1 (100%) Benzophenone-2 (100%) Butoxyethanol (100, 15%) Cetrimonium chloride (2.5, 1.2, 0.5%) Chlorhexidine digluconate (20, 2%) Chlorophene (100, 3%) Chloroxylenol (100, 30%) Dioctyl sodium sulfosuccinate (10%) Hexylene glycol (100%) Lauramide DEA (20, 10%) Methoxyisopropyl acetate (100%) Phenethyl alcohol (100%) Phenethyl alcohol (15, 5%) Phenoxyethanol (100%) Phytantriol (100, 23%) Resorcinol (100%) Sodium naphthalenesulfonate (100%) Stearalkonium chloride (25, 4, 2.5%) TEA-Lauryl sulfate (20, 10, 5, 2.5, 1.25%) Triisopropanolamine (100%) 42	0
	NI	2-Bromo-2-nitropropane-1,3-diol (2, 0.5%) Benzalkonium chloride (0.1, 0.01%) Benzethonium chloride (0.5%) Benzophenone-1 (16, 8, 4%) Benzophenone-2 (16, 8, 4%) Cetrimonium chloride (0.1%) Cetyl alcohol (100%) Cetyl palmitate (100%) Chlorhexidine digluconate (0.05%) Chlorophene (1, 0.3%) Diazolidinyl urea (30%) Diisopropyl adipate (100%) Dioctyl sodium sulfosuccinate (2, 0.5%) PEG-40 stearate (100%) Phytantriol (10, 3%) Propylene carbonate (100%) Sodium dehydroacetate (100%) Sodium lauroyl sarcosinate (5%) Sodium naphthalenesulfonate (2%) Sodium stearate (100%) Stearalkonium chloride (0.5%) Steareth-2 (60%) Steareth-20 (60%) Triacetin (100%) 33	Acetyl tributyl citrate (100%) Buthoxyethanol (5%) Butylene glycol (100, 10%) Carnauba wax (50%) Decyl oleate (100%) Diethylhexyl adipate (100%) Ethylhexyl palmitate (100%) Ethylhexyl stearate (100%) Glyceryl stearate (100%) Hexylene glycol (25%) Isocetyl stearate (100%) Isopropyl myristate (100%) Isopropyl palmitate (100%) Oleyl alcohol (100%) PEG-2 stearate (100%) Phenethyl alcohol (0.3%) Phenoxyethanol (2.2%) Phenyl methyl pyrazolone (0.66%) Propylene carbonate (17.5, 10.5%) Castor seed oil (100%) Safflower oil (100%) Sesame oil (100%) Sodium hexametaphosphate (0.2%) Sorbitan oleate (100%) Sorbitan sesquioleate (100, 30%) Sorbitan stearate (30%) Squalane (100%) Stearyl alcohol (100%) Triethylene glycol (100%) Zinc stearate (100%) 33

LDM-MTT assay was performed at the concentration at which a reported *in vivo* result was previously obtained. The concentrations of substance are shown in parenthesis, as substances are classified as true positive, true negative, false positive or false negative.

Table 64 Seventy-three substances

Category	Nos of Positive	Nos of Negative	Total
UV absorbers	0	4	4
Surfactants	8	11	19
Macromolecules	1	9	10
Oils	0	20	20
Humectants	1	6	7
Medicants	1	12	13
Total	11	62	73

Fig. 11 The relationship between LDM-MTT assay and Draize eye test results for cosmetic ingredients



The classification in the Draize eye test was based on MAS 15 as the cut-off point. That in the LDM-MTT assay was based on a viability of 50% as the cut-off point. The number of substances classified as true positive, true negative, false positive and false negative is shown in each area in the figure.

Table 65 Sixty substances

No.	Substance	CAS	GHS
1	1-Decanol	112-30-1	NI
2	2,4-Dichloro-5-sulfamoylbenzoic acid	2736-23-4	NI
3	2-Aminophenol	95-55-6	NI
4	2-Mercaptopyrimidine	1450-85-7	NI
5	2-methylpentane	107-83-5	NI
6	3,3-Dimethylpentane	562-49-2	NI
7	3-Methoxy-1,2-propanediol	623-39-2	NI
8	3-methylhexane	589-34-4	NI
9	Aluminum Hydroxide	21645-51-2	NI
10	Diisobutyl Ketone	108-83-8	NI
11	Ethyl acetate	141-78-6	NI
12	Ethyl trimethyl acetate	3938-95-2	NI
13	Ethylenediaminetetraacetic acid dipotassium salt dehydrate	25102-12-9	NI
14	Gluconolactone	90-80-2	NI
15	Glycerol	56-81-5	NI
16	Iminodibenzyl	494-19-9	NI
17	Iso-octyl acrylate	29590-42-9	NI
18	Methyl amyl ketone	110-43-0	NI
19	Methyl cyclopentane	96-37-7	NI
20	Methyl isobutyl ketone	108-10-1	NI
21	n,n-Dimethylguanidine sulfate	598-65-2	NI
22	n-Butyl acetate	123-86-4	NI
23	Phenothiazine	92-84-2	NI
24	Polyethylene glycol 400	25322-68-3	NI
25	Potassium tetrafluoroborate	14075-53-7	NI
26	Toluene	108-88-3	NI
27	Tween 20	9005-64-5	NI
28	Xylene	1330-20-7	NI
29	1-Octanol	111-87-5	2B
30	2, 6-dichlorobenzoyl chloride	4659-45-4	2A
31	2-Ethyl-1-hexanol	104-76-7	2A
32	2-Methyl-1-pentanol	105-30-6	2
33	Acetone	67-64-1	2A
34	Benzalkonium chloride (10%)	71-36-3	1
35	Butanol	79-92-5	2A
36	Camphen	111-87-5	2
37	Cetylpyridinium bromide (6%)	140-72-7	1
38	Chlorhexidine	55-56-1	1
39	Cyclohexanol	108-93-0	1
40	Dibenzoyl-L-tartaric acid (100%)	2743-38-6	1
41	Dibenzyl phosphate	1623-08-1	2A
42	Diethylethanolamine	100-37-8	1
43	Ethanol	64-17-5	2A
44	Ethyl-2-methylacetoacetate	609-14-3	2B
45	Isopropanol	67-63-0	2A
46	Lactic Acid 100% (liquid)	50-21-5	1
47	Maneb	12427-38-2	2
48	m-Dinitrobenzene	99-65-0	2
49	Methoxyethyl acrylate	3121-61-7	1
50	Methyl acetate	79-20-9	2A
51	Methyl cyanoacetate	105-34-0	2A
52	Methyl ethyl ketone (MEK)	78-93-3	2A
53	n-Hexanol	111-27-3	2
54	Promethazine Hydrochloride	58-33-3	1
55	Quinacrine	69-05-6	1
56	Sodium hydroxide (1%)	1310-73-2	2B
57	Sodium monochloroacetate	3926-62-3	2
58	Tetrahydrofuran	109-99-9	1
59	Tetraoctylammonium bromide	14866-33-2	1
60	Triton X-100 (5%)	9002-93-1	2A

Table 66 Prediction of eye irritancy at various concentrations in the LDM-MTT assay

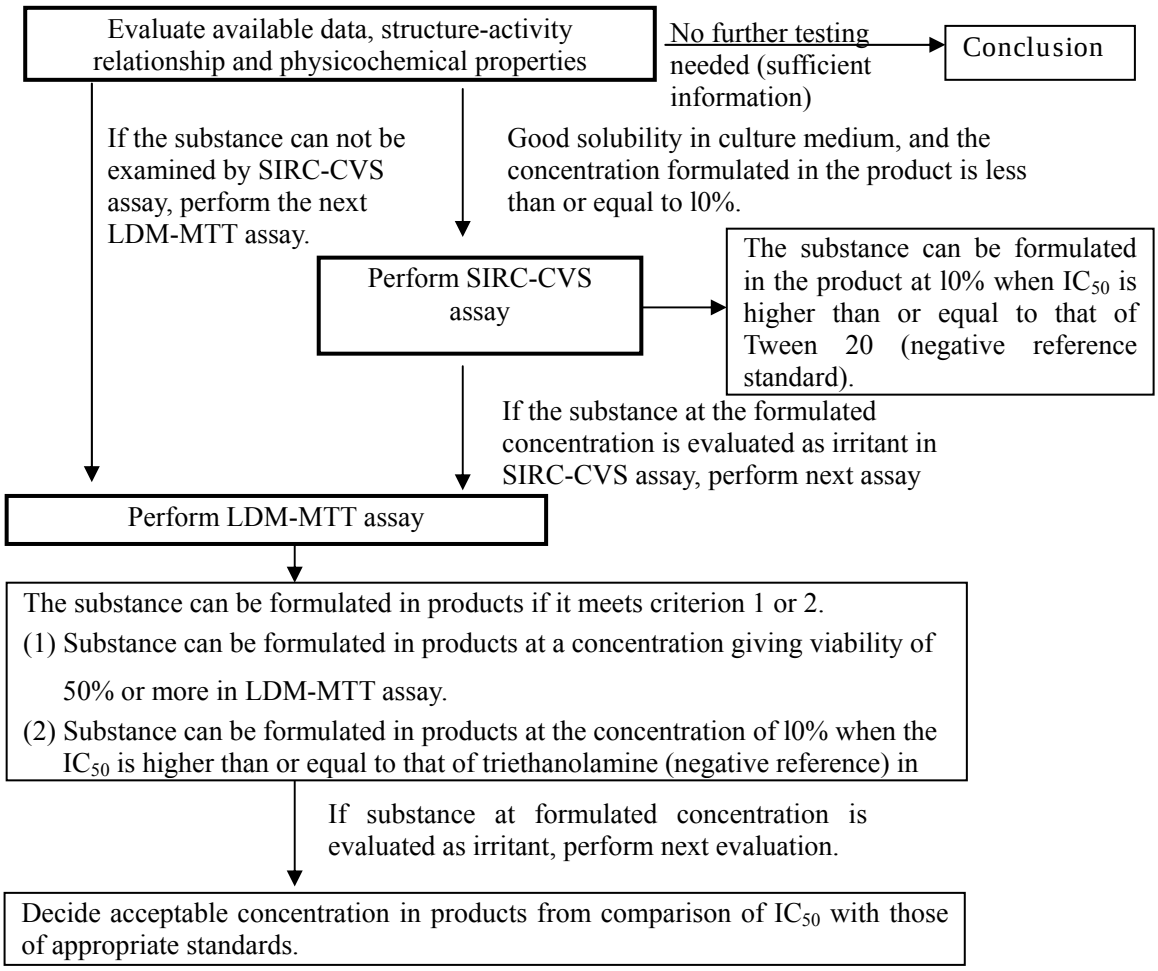
		In vitro (Classification by LDM-MTT assay using a viability of 50% as cut-off point)	
		Positive	Negative
In vivo (Estimated classification by GHS)	1, 2A or 2B	1-Octanol 2, 6-dichlorobenzoyl chloride 2-Ethyl-1-hexanol 2-Methyl-1-pentanol Acetone Benzalkonium chloride（10%) Butanol Camphen Cetylpyridinium bromide(6%) Chlorhexidine Cyclohexanol Dibenzoyl-L-tartaric acid Dibenzyl phosphate Diethylethanolamine Ethanol Ethyl-2-methylacetoacetate Isopropanol Lactic Acid Maneb m-Dinitrobenzene Methoxyethyl acrylate Methyl acetate Methyl cyanoacetate Methyl ethyl ketone n-Hexanol Promethazine Hydrochloride Quinacrine Sodium hydroxide(1%) Sodium monochloroacetate Tetrahydrofuran Tetraoctylammonium bromide Triton X-100(5%) 32	0
	NI	1-Decanol 2,4-Dichloro-5-sulfamoylbenzoic acid 2-Aminophenol 3,3-Dimethylpentane Diisobutyl Ketone Ethyl acetate Ethyl trimethyl acetate Ethylenediaminetetraacetic acid dipotassium salt dihydrate Gluconolactone Iminodibenzyl Iso-octyl acrylate Methyl amyl ketone Methyl cyclopentane Methyl isobutyl ketone n,n-Dimethylguanidine sulfate n-Butyl acetate Polyethylene glycol 400 Potassium tetrafluoroborate Toluene Tween 20 Xylene 21	2-Mercaptopyrimidine 2-methylpentane 3-Methoxy-1,2-propanediol 3-methylhexane Aluminum Hydroxide Glycerol Phenothiazine 7

Table 67 Prediction of eye irritancy at various concentrations in the LDM-MTT assay

		In vitro (Classification by LDM-MTT assay using a viability of 50% as cut-off point)	
		Positive	Negative
In vivo (Estimated classification by GHS)	1, 2A or 2B	Calcium thioglycolate (100, 10%) Lactic acid (100, 10%) Sodium lauryl sulfate (10%) Benzyl alcohol (100, 10%) Diisopropanolamine (10%) Monoethanolamine (10%) Acid red 92 (100, 10%) Glycolic acid (10%) Sodium hydrogenated tallow L-glutamate (10%) Chlorhexidine gluconate (20% solution) (10%) Butanol (10%) Potassium laurate (10%) Polyoxyethylene octylphenylether (10 E.O.) (10%) Di (2-ethylhexyl) sodium sulfosuccinate (10%) Acetic acid (10%) Cetyltrimethylammonium bromide (10%) Benzalkonium chloride (10%) Stearyltrimethylammonium chloride (10%) Cetylpyridinium chloride (10, 1%) Domiphen bromide (10%) Sucrose fatty acid ester (100%) Ethanol (100%) Sodium salicylate (100%) Distearyldimethylammonium chloride (100%) 29 2-Bromo-2-nitropropane-1,3-diol (100, 20, 10, 5%) Benzalkonium chloride (2, 1, 0.5%) Benzophenone-1 (100%) Benzophenone-2 (100%) Butoxyethanol (100, 15%) Cetrimonium chloride (2.5, 1.2, 0.5%) Chlorhexidine diaiuconate (20, 2%) Chlorophene (100, 3%) Chloroxylenol (100, 30%) Dioctyl sodium sulfosuccinate (10%) Hexylene glycol (100%) Lauramide DEA (20, 10%) Methoxyisopropyl acetate (100%) Phenethyl alcohol (100%) Phenethyl alcohol (15, 5%) Phenoxyethanol (100%) Phytantriol (100, 23%) Resorcinol (100%) Sodium naphthalenesulfonate (100%) Stearalkonium chloride (25, 4, 2.5%) TEA-Lauryl sulfate (20, 10, 5, 2.5, 1.25%) Triisopropanolamine (100%) 42 Cosmetic inaredients 11 1-Octanol (100%) 2, 6-dichlorobenzoyl chloride (100%) 2-Ethyl-1-hexanol (100%) 2-Methyl-1-pentanol (100%) Acetone (100%) Benzalkonium chloride (10%) Butanol (100%) Camphen (100%) Cetylpyridinium bromide(6%) Chlorhexidine (100%) Cyclohexanol (100%) Dibenzoyl-L-tartaric acid (100%) Dibenzyl phosphate (100%) Diethylethanolamine (100%) Ethanol (100%) Ethyl-2-methylacetoacetate (100%) Isopropanol (100%) Lactic Acid (100%) Maneb (100%) m-Dinitrobenzene (100%) Methoxyethyl acrylate (100%) Methyl acetate (100%) Methyl cyanoacetate (100%) Methyl ethyl ketone (100%) n-Hexanol (100%) Promethazine Hydrochloride (100%) Quinacrine (100%) Sodium hydroxide(1%) Sodium monochloroacetate (100%) Tetrahydrofuran (100%) Tetraoctylammonium bromide (100%) Triton X-100(5%) 32 **114**	**0**
	NI	Polyoxyethylene sorbitan monooleate (20E.O.) (100, 10%) Sodium salicylate (10%) Triethanolamine (100, 10%) Polyoxyethylene sorbitan monolaurate (20 E.O.) (10%) Polyethyleneglycol monolaurate (10 E.O.) (10%) Sodium polyoxyethylene laurylether sulfate (2 E.O.) (27% solution) (10%) Sodium N-lauryl sarcosinate (30% solution) (10%) Sucrose fatty scid ester (10%) Methyl p-hydroxybenzoate (100%) Acid red 92 (1%) Cetylpyridinium chloride (0.1%) 13 2-Bromo-2-nitropropane-1,3-diol (2, 0.5%) Benzalkonium chloride (0.1, 0.01%) Benzethonium chloride (0.5%) Benzophenone-1 (16, 8, 4%) Benzophenone-2 (16, 8, 4%) Cetrimonium chloride (0.1%) Cetyl alcohol (100%) Cetyl palmitate (100%) Chlorhexidine digluconate (0.05%) Chlorophene (1, 0.3%) Diazolidinyl urea (30%) Diisopropyl adipate (100%) Dioctyl sodium sulfosuccinate (2, 0.5%) PEG-40 stearate (100%) Phytantriol (10, 3%) Propylene carbonate (100%) Sodium dehydroacetate (100%) Sodium lauroyl sarcosinate (5%) Sodium naphthalenesulfonate (2%) Sodium stearate (100%) Stearalkonium chloride (0.5%) Steareth-2 (60%) Steareth-20 (60%) Triacetin (100%) 33 Cosmetic ingredients 25 1-Decanol (100%) 2,4-Dichloro-5-sulfamoylbenzoic acid (100%) 2-Aminophenol (100%) 3,3-Dimethylpentane (100%) Diisobutyl Ketone (100%) Ethyl acetate (100%) Ethyl trimethyl acetate (100%) Ethylenediaminetetraacetic acid dipotassium salt dihydrate (100%) Gluconolactone (100%) Iminodibenzyl (100%) Iso-octyl acrylate (100%) Methyl amyl ketone (100%) Methyl cyclopentane (100%) Methyl isobutyl ketone (100%) n,n-Dimethylguanidine sulfate (100%) n-Butyl acetate (100%) Polyethylene glycol 400 (100%) Potassium tetrafluoroborate (100%) Toluene (100%) Tween 20 (100%) Xylene (100%) 21 **92**	Ethanol (10%) 2-Ethylhexyl p-dimethylamino benzonate (100, 10%) Glycerin (100, 10%) Polyethylene glycol 400 (10%) Polyoxyethylene hydrogenated caster oil (60 E.O.) (10%) Isopropyl myristate (100, 10%) Isotonic sodium chloride solution (100%) Silicic anhydride (100%) Benzyl alcohol (1%) 12 Acetyl tributyl citrate (100%) Buthoxyethanol (5%) Butylene glycol (100, 10%) Carnauba wax (50%) Decyl oleate (100%) Diethylhexyl adipate (100%) Ethylhexyl palmitate (100%) Ethylhexyl stearate (100%) Glyceryl stearate (100%) Hexylene glycol (25%) Isocetyl stearate (100%) Isopropyl myristate (100%) Isopropyl palmitate (100%) Oleyl alcohol (100%) PEG-2 stearate (100%) Phenethyl alcohol (0.3%) Phenoxyethanol (2.2%) Phenyl methyl pyrazolone (0.66%) Propylene carbonate (17.5, 10.5%) Castor seed oil (100%) Safflower oil (100%) Sesame oil (100%) Sodium hexametaphosphate (0.2%) Sorbitan oleate (100%) Sorbitan sesquioleate (100, 30%) Sorbitan stearate (30%) Squalane (100%) Stearyl alcohol (100%) Triethylene glycol (100%) Zinc stearate (100%) 33 Cosmetic ingredients 37 2-Mercaptopyrimidine 2-methylpentane 3-Methoxy-1,2-propanediol 3-methylhexane Aluminum Hydroxide Glycerol Phenothiazine 7 **89**

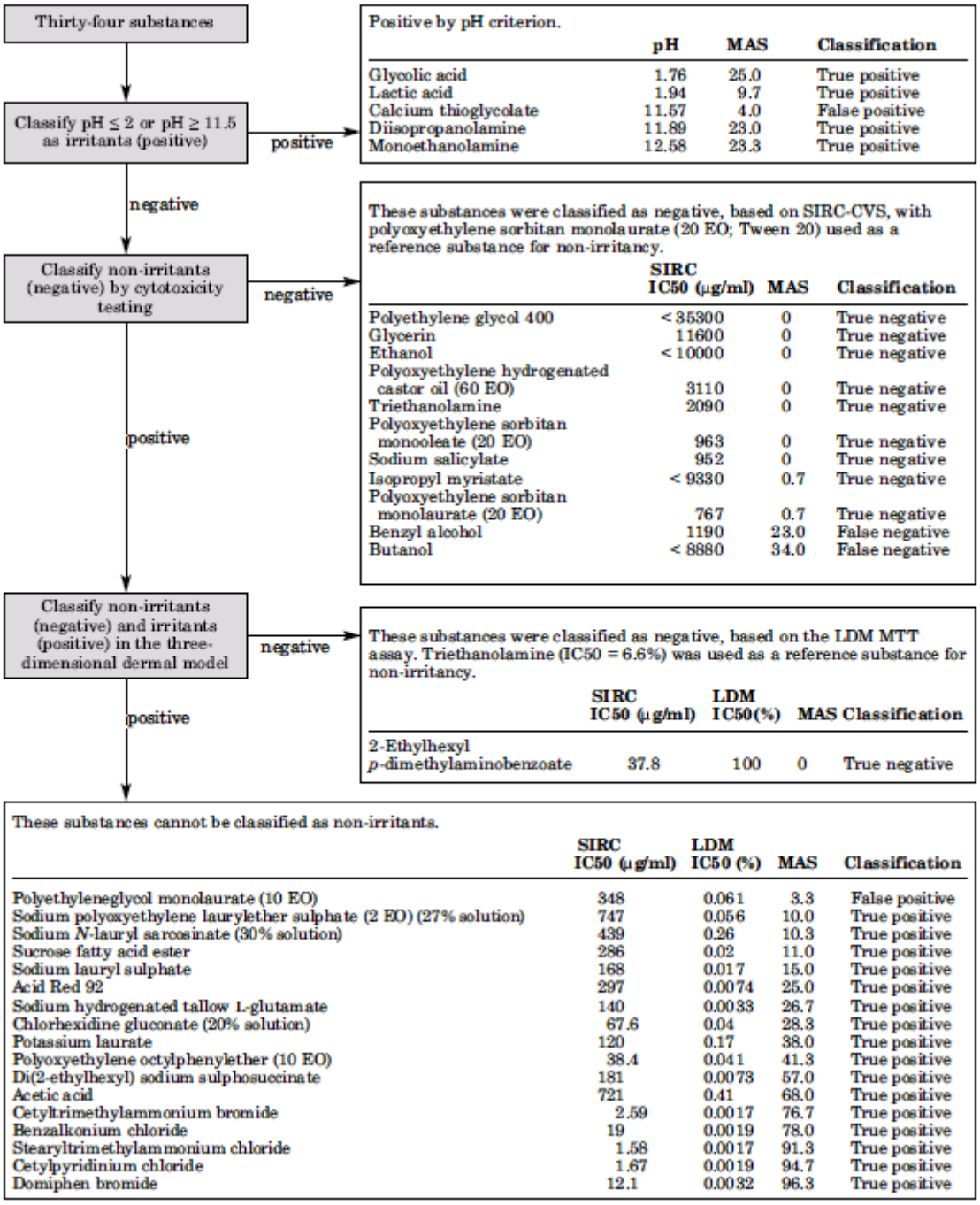
Thirty-two of 92 false positives were samples applied without dilution. Though these could not be negligible in the viewpoint of labelling of chemicals, the influence is relatively small for the evaluation of the cosmetic ingredients, that are virtually used with dilution.

Fig. 12 Schematic illustration of the tier evaluation using SIRC-CVS assay and LDM-MTT assay for the identification of non irritating ingredients.



The figure is the same as that reported by Hagino et al (2008).

Fig. 13 Verification of the tier evaluation method using monolayer cell culture and three-dimensional dermal model for the identification of non irritating ingredients.



Positive by pH criterion.

	pH	MAS	Classification
Glycolic acid	1.76	25.0	True positive
Lactic acid	1.94	9.7	True positive
Calcium thioglycolate	11.57	4.0	False positive
Diisopropanolamine	11.89	23.0	True positive
Monoethanolamine	12.58	23.3	True positive

These substances were classified as negative, based on SIRC-CVS, with polyoxyethylene sorbitan monolaurate (20 EO; Tween 20) used as a reference substance for non-irritancy.

	SIRC IC50 (μg/ml)	MAS	Classification
Polyethylene glycol 400	< 35300	0	True negative
Glycerin	11600	0	True negative
Ethanol	< 10000	0	True negative
Polyoxyethylene hydrogenated castor oil (60 EO)	3110	0	True negative
Triethanolamine	2090	0	True negative
Polyoxyethylene sorbitan monooleate (20 EO)	963	0	True negative
Sodium salicylate	952	0	True negative
Isopropyl myristate	< 9330	0.7	True negative
Polyoxyethylene sorbitan monolaurate (20 EO)	767	0.7	True negative
Benzyl alcohol	1190	23.0	False negative
Butanol	< 8880	34.0	False negative

These substances were classified as negative, based on the LDM MTT assay. Triethanolamine (IC50 = 6.6%) was used as a reference substance for non-irritancy.

	SIRC IC50 (μg/ml)	LDM IC50(%)	MAS	Classification
2-Ethylhexyl *p*-dimethylaminobenzoate	37.8	100	0	True negative

These substances cannot be classified as non-irritants.

	SIRC IC50 (μg/ml)	LDM IC50 (%)	MAS	Classification
Polyethyleneglycol monolaurate (10 EO)	348	0.061	3.3	False positive
Sodium polyoxyethylene laurylether sulphate (2 EO) (27% solution)	747	0.056	10.0	True positive
Sodium *N*-lauryl sarcosinate (30% solution)	439	0.26	10.3	True positive
Sucrose fatty acid ester	286	0.02	11.0	True positive
Sodium lauryl sulphate	168	0.017	15.0	True positive
Acid Red 92	297	0.0074	25.0	True positive
Sodium hydrogenated tallow L-glutamate	140	0.0033	26.7	True positive
Chlorhexidine gluconate (20% solution)	67.6	0.04	28.3	True positive
Potassium laurate	120	0.17	38.0	True positive
Polyoxyethylene octylphenylether (10 EO)	38.4	0.041	41.3	True positive
Di(2-ethylhexyl) sodium sulphosuccinate	181	0.0073	57.0	True positive
Acetic acid	721	0.41	68.0	True positive
Cetyltrimethylammonium bromide	2.59	0.0017	76.7	True positive
Benzalkonium chloride	19	0.0019	78.0	True positive
Stearyltrimethylammonium chloride	1.58	0.0017	91.3	True positive
Cetylpyridinium chloride	1.67	0.0019	94.7	True positive
Domiphen bromide	12.1	0.0032	96.3	True positive

The figure is the same as that reported by Hagino et al (2008). The data were taken from Ohno et al. (1999), Tani et al. (1999) and Ohuchi et al. (1999). Non irritants (= negative) was defined here as those having MAS of 5 or less in the Draize eye test. Eye irritancy (=MAS) of 10% solutions of the substances was predicted based on the IC_{50} in the two models after classification according to pH. The figure was the same as that reported by Hagino et al (2008).

Table 68 Predicted irritancy according to in vitro tier system consisting of SIRC-CVS assay and LDM-MTT assay
(Concentration: 10%, Negative reference: Tween 20 in the SIRC-CVS assay and Triethanolamine in the LDM-MTT assay)

		In vitro (Classification by SIRC-CVS assay using Tween 20 as a reference substance for non-irritancy)	
		Positive	Negative
In vivo (Classification by GHS)	1, 2Aor 2B	Sodium lauryl sulfate Monoethanolamine Acid red 92 Sodium hydrogenated tallow L-glutamate Chlorhexidine gluconate (20% solution) Potassium laurate Polyoxyethylene octylphenylether (10 E.O.) Di (2-ethylhexyl) sodium sulfosuccinate Acetic acid Cetyltrimethylammonium bromide Benzalkonium chloride Stearyltrimethylammonium chloride Cetylpyridinium chloride Domiphen bromide 14	Benzyl alcohol* Glycolic acid* Butanol* 3
	NI	Polyethyleneglycol monolaurate (10 E.O.) Sodium polyoxyethylene laurylether sulfate (2 E.O.) (27% solution) Sodium N-lauryl sarcosinate (30% solution) Sucrose fatty acid ester Diisopropanolamine 5	Ethanol* Glycerin* Polyethylene glycol 400* Polyoxyethylene hydrogenated caster oil (60 E.O.)* Polyoxyethylene sorbitan monooleate (20E.O.)* Sodium salicylate* Triethanolamine* Isopropyl myristate* Polyoxyethylene sorbitan monolaurate (20 E.O.)=Tween 20* Lactic acid* 2-Ethylhexyl p-dimethylamino benzonate Calcium thioglycolate 12

*: It was classified as negative by the SIRC-CVS assay.

Table 69 Predicted irritancy according to in vitro tier system consisting of SIRC-CVS assay and LDM-MTT assay
(Concentration: 10%, Negative reference: Tween 20 in the SIRC-CVS assay and Triethanolamine in the LDM-MTT assay)
-GHS classification by considering pH-

		In vitro (Classification by SIRC-CVS assay using Tween 20 as a reference substance for non-irritancy)	
		Positive	Negative
In vivo (Classification by GHS)	1, 2Aor 2B	Calcium thioglycolate Sodium lauryl sulfate Diisopropanolamine Monoethanolamine Acid red 92 Sodium hydrogenated tallow L-glutamate Chlorhexidine gluconate (20% solution) Potassium laurate Polyoxyethylene octylphenylether (10 E.O.) Di (2-ethylhexyl) sodium sulfosuccinate Acetic acid Cetyltrimethylammonium bromide Benzalkonium chloride Stearyltrimethylammonium chloride Cetylpyridinium chloride Domiphen bromide 16	Lactic acid* Benzyl alcohol* Glycolic acid* Butanol* 4
	NI	Polyethyleneglycol monolaurate (10 E.O.) Sodium polyoxyethylene laurylether sulfate (2 E.O.) (27% solution) Sodium N-lauryl sarcosinate (30% solution) Sucrose fatty acid ester 4	Ethanol* Glycerin* Polyethylene glycol 400* Polyoxyethylene hydrogenated caster oil (60 E.O.)* Polyoxyethylene sorbitan monooleate (20E.O.)* Sodium salicylate* Triethanolamine* Isopropyl myristate* Polyoxyethylene sorbitan monolaurate (20 E.O.) =Tween 20* 2-Ethylhexyl p-dimethylamino benzonate 10

*: It was classified as negative by the SIRC-CVS assay.

Table 70 Predicted irritancy in LDM-MTT assay of 19 substances positive in SIRC-CVS assay and 11 with poor solubility in culture medium.
(Concentration: 10%, Negative reference: Tween 20 in the SIRC-CVS assay and Triethanolamine in the LDM-MTT assay)

		In vitro (Classification by LDM-MTT assay using triethanolamine as a reference substance for non-irritancy)		
		Positive	Negative	
In vivo (Classification by Draize eye test at 10% concn) Corneal damage or MAS over 15 was classified as positive.	Positive 1,2Aor2B in GHS	2-Bromo-2-Nitropropane-1,3-Diol Benzalkonium chloride Cetrimonium chloride Chlorhexidine digluconate Chlorophene Dioctyl sodium sulfosuccinate Lauramide DEA Stearalkonium chloride TEA-Lauryl sulfate 9	0	
	Negative NI in GHS	Benzophenone-1 Benzophenone-2 Diazolidinyl urea PEG-40 stearate Phytantriol Sodium stearate Steareth-20 7	Acetyl tributyl citrate Carnauba wax Cetyl alcohol Cetyl palmitate Decyl oleate Ethylhexyl stearate Glyceryl stearate 14	Oleyl alcohol PEG-2 stearate Castor seed oil Sorbitan stearate Steareth-2 Stearyl alcohol Zinc stearate

*: It was classified as negative by the SIRC-CVS assay.

Table 71 Predicted irritancy according to in vitro tier system consisting of SIRC-CVS assay and LDM-MTT assay
(Concentration: 10%, Negative reference: Tween 20 in the SIRC-CVS assay and Triethanolamine in the LDM-MTT assay)

		In vitro (Classification by LDM-MTT assay using triethanolamine as a reference substance for non-irritancy)	
		Positive	Negative
In vivo (Classification by Draize eye test at 10% concn) Corneal damage or MAS over 15 was classified as positive.	Positive 1,2Aor2B in GHS	2-Bromo-2-Nitropropane-1,3-Diol Benzalkonium chloride Cetrimonium chloride Chlorhexidine digluconate Chlorophene Dioctyl sodium sulfosuccinate Lauramide DEA Stearalkonium chloride TEA-Lauryl sulfate 9	Phenethyl alcohol* 1
	Negative NI in GHS	Benzophenone-1 Benzophenone-2 Diazolidinyl urea PEG-40 stearate Phytantriol Sodium stearate Steareth-20 7	Butylene glycol* Diethylhexyl adipate* Diisopropyl adipate* Ethylhexyl palmitate* Hexylene glycol* Isocetyl stearate* Isopropyl myristate* Isopropyl palmitate* Propylene carbonate* Safflower oil* Sesame oil* Sodium dehydroacetate* Sorbitan oleate* Sorbitan sesquioleate* Squalane* Triacetin* Triethylene glycol* Acetyl tributyl citrate Carnauba wax Cetyl alcohol Cetyl palmitate Decyl oleate Ethylhexyl stearate Glyceryl stearate Oleyl alcohol PEG-2 stearate Castor seed oil Sorbitan stearate Steareth-2 Stearyl alcohol Zinc stearate 31

*: It was classified as negative by the SIRC-CVS assay.

Table 72 Predicted irritancy according to in vitro tier system consisting of SIRC-CVS assay and LDM-MTT assay
(Concentration: 10%, Negative reference: Tween 20 in the SIRC-CVS assay and Triethanolamine in the LDM-MTT assay)
-GHS classification by considering pH-

		In vitro (Classification by SIRC-CVS assay using Tween 20 as a reference substance for non-irritancy)	
		Positive	Negative
In vivo (Classification by GHS)	1, 2Aor 2B	Calcium thioglycolate Sodium lauryl sulfate Diisopropanolamine Monoethanolamine Acid red 92 Sodium hydrogenated tallow L-glutamate Chlorhexidine gluconate (20% solution) Potassium laurate Polyoxyethylene octylphenylether (10 E.O.) Di (2-ethylhexyl) sodium sulfosuccinate Acetic acid Cetyltrimethylammonium bromide Benzalkonium chloride Stearyltrimethylammonium chloride Cetylpyridinium chloride Domiphen bromide 2-Bromo-2-Nitropropane-1,3-Diol ~~Benzalkonium chloride~~ Cetrimonium chloride Chlorhexidine digluconate Chlorophene Dioctyl sodium sulfosuccinate Lauramide DEA Stearalkonium chloride TEA-Lauryl sulfate 24	Lactic acid* Benzyl alcohol* Glycolic acid* Butanol* Phenethyl alcohol* 5
	NI	Polyethyleneglycol monolaurate (10 E.O.) Sodium polyoxyethylene laurylether sulfate (2 E.O.) (27% solution) Sodium N-lauryl sarcosinate (30% solution) Sucrose fatty acid ester Benzophenone-1 Benzophenone-2 Diazolidinyl urea PEG-40 stearate Phytantriol Sodium stearate Steareth-20 11	Ethanol* Glycerin* Polyethylene glycol 400* Polyoxyethylene hydrogenated caster oil (60 E.O.)* Polyoxyethylene sorbitan monooleate (20E.O.)* Sodium salicylate* Triethanolamine* Isopropyl myristate* Polyoxyethylene sorbitan monolaurate (20 E.O.) =Tween 20* 2-Ethylhexyl p-dimethylamino benzonate Butylene glycol* Diethylhexyl adipate* Diisopropyl adipate* Ethylhexyl palmitate* Hexylene glycol* Isocetyl stearate* ~~Isopropyl myristate*~~ Isopropyl palmitate* Propylene carbonate* Safflower oil* Sesame oil* Sodium dehydroacetate* Sorbitan oleate* Sorbitan sesquioleate* Squalane* Triacetin* Triethylene glycol* Acetyl tributyl citrate Carnauba wax Cetyl alcohol Cetyl palmitate Decyl oleate Ethylhexyl stearate Glyceryl stearate Oleyl alcohol PEG-2 stearate Castor seed oil Sorbitan stearate Steareth-2 Stearyl alcohol Zinc stearate 40

*: It was classified as negative by the SIRC-CVS assay.

Table 73 Abbriviations

CV	Coefficient of variation
CVS	Crystal violet staining
EC50	>> IC50 (EC50 is the same as IC50 here)
GHS	Globally harmonized system of classification and labelling of chemicals
IC50	Concentration that inhibits the viability of the cell to 50% of control
LDM	Living dermal model
MAS	Maximal average score
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide
NRU	Neutral red uptake
SD	Standard deviation
SIRC	Statens Seruminstitut Rubbit Cornea
SOP	Standard operating procedure

日本語の参考資料

表 1 Draize 眼粘膜刺激性試験から得られる情報と対応する代替法

ドレイズ試験からの情報	CAM	赤血球	皮膚モデル	SIRC	ヒト培養細胞	動物培養細胞	EYTEX
① 角膜混濁							
a 膜実質（コラーゲン）の変性	▲	×	○	×	×	×	○
b コラーゲンの膨潤（上皮・内皮の障害度に依存）	▲	×	○	×	×	×	○
c 上皮細胞の変性・剥離（細胞毒性による）	▲	▲	○	○	○	○	×
② 虹彩							
a 経角膜吸収と虹彩損傷性	×	×	×	×	×	×	×
b 対光反射	×	×	×	×	×	×	×
③ 結膜							
a 発赤（炎症性血管拡張）	○	×	▲	▲	▲	▲	×
b 浮腫（炎症性の浮腫）	▲	×	▲	▲	▲	▲	×
c 分泌物（涙液の過剰分泌・炎症性浸潤反応）	×	×	×	×	×	×	×
④ 経過観察からの情報							
a 修復性	▲	×	▲	▲	▲	▲	×
b 遅発性の有無	▲	▲	▲	▲	▲	▲	×
⑤ ドレイズの観察項目にない情報							
a 角膜潰瘍（角膜上皮の損傷・欠落）	×	×	×	×	×	×	×
b 角膜の凹凸（乾燥性・回地形成）	×	×	×	×	×	×	×
c 洗浄による障害の軽減性	○	×	○	▲	▲	▲	×
d 瘙みの評価（行動観察・瞬目回数・閉眼）	×	×	×	×	×	×	×
e 物理的刺激による障害の検出（不溶性物質）	×	×	×	×	×	×	×

注（バリデーション開始前の文献に基づく評価で，○：導入可能。▲：導入には検討の必要。×：導入不可能）

金子 (1996) による表を引用

表 2 Draize 試験のスコアリング

Ⅰ 角膜	
A 不透明度：混濁の程度（もっとも混濁した領域を読み取る）	
不透明度なし	0
虹彩を明視できる程度の散在からび慢性の不透明化	1
虹彩の細部がわずかにぼやけて見える	2
虹彩の細部が観察できないが、瞳孔の大きさはかろうじて識別できる	3
虹彩が透視できない	4
B 角膜損傷域	
正常	0
0＜A＜1/4	1
1/4≦A＜1/2	2
1/2≦A＜3/4	3
3/4≦A	4
評点：A×B×5（最大値：80）	
Ⅱ 虹彩（A）	
正常	0
皺壁形成亢進、充血、腫脹、角膜周囲の充血（いずれか1つ、あるいは全て、若しくは組み合わせ）が見られるが、対光反射は認められる（緩除反応陽性）。	1
対光反射消失、出血、広範囲の破壊（いずれか1つ、あるいは全て）が見られる。	2
評点：A×5 （最大値：10）	
Ⅲ 結膜	
A 発赤（角膜及び虹彩を除く瞼、球結膜）	
正常	0
充血亢進	1
広範囲かつ深紅色となり、血管の識別困難	2
全域の深紅色化	3
B 結膜浮腫	
正常	0
腫脹亢進（瞬瞼を含む）	1
眼瞼の部分的外反を伴う腫脹	2
腫脹を伴う 1/2 程度の眼瞼閉鎖	3
腫脹を伴う 1/2 以上の眼瞼閉鎖	4
C 分泌物	
正常	0
常量以上の分泌物（正常な動物の内眥に見られる少量は含まない）	1
眼瞼及び眼瞼に接する被毛を湿潤	2
眼瞼及び眼の周囲を相当範囲湿潤	3
評点：（A＋B＋C）×2 （最大値：20）	

表 3 GHS（世界調和ｼｽﾃﾑ）による判定基準

GHS の区分	in vivo の試験（Draize 試験）結果による判定	既存の分類による判定
区分1	・少なくとも 1 匹の動物で角膜、虹彩、あるいは結膜に可逆的とは思われない障害を出現、あるいは処置後 21 日目でも障害が完全には回復しない場合。 ・3匹中2匹以上で処置後 24, 48, 72 時間目での評点の平均値が角膜混濁指標では3以上、虹彩指標では 1.5 より大きかった場合。	Severe あるいは Corrosive（非常に強い刺激性または腐食性 AOI 80 以上に相当）と分類された物質は区分1に分類（但し、非可逆的病変が観察されない場合は刺激性（区分2A）と判定）
区分 2A	・3 匹の動物を用いて実施した Draize 試験で2匹以上に処置後 24, 48, 72 時間目での評点の平均値が角膜混濁では1以上、虹彩炎では 1 以上、結膜発赤では2以上、結膜浮腫では2以上の場合。かつ、21日間の観察期間中に完全に回復する。	Moderate（強い刺激性　AOI 30-80 に相当）と分類された物質は区分2Aに分類
区分 2B	・3 匹の動物を用いて実施した Draize 試験で2匹以上に処置後 24, 48, 72 時間目での評点の平均値が角膜混濁では1以上、虹彩炎では 1 以上、結膜発赤では2以上、結膜浮腫では2以上の場合。かつ7日以内に回復する	Mild と分類された物質は区分2Bに分類

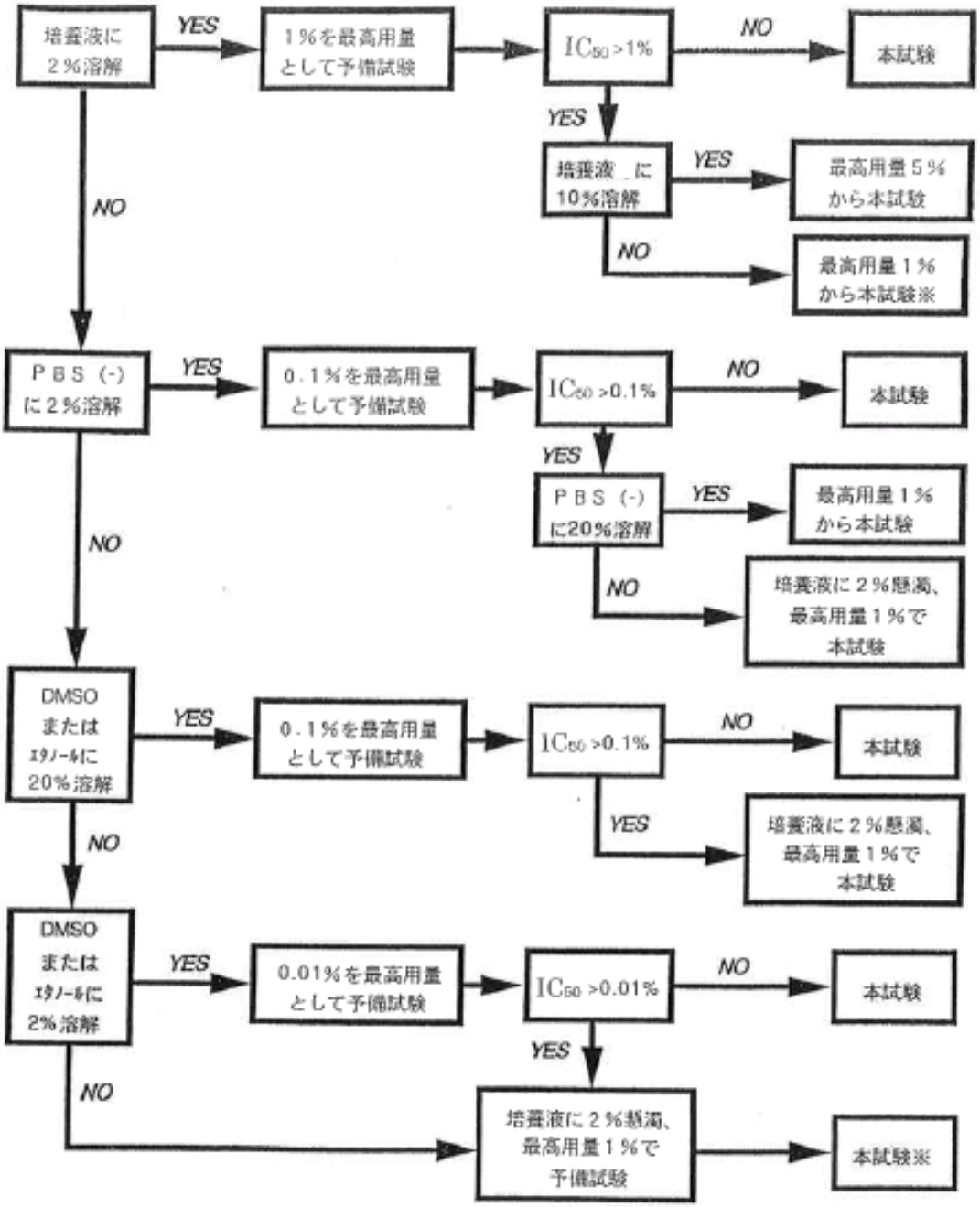
試験を行う前に、化学物質の眼に対する重篤な損傷性または眼刺激性を判定するのに、いくつかの要因を考慮するべきである。人および動物で蓄積された経験からは、眼に対する作用に直接関連する情報が得られるので、それが分析の第一段階に置かれるべきである。また、構造的に関連している化合物から有害性決定に十分な情報が得られる例もある。同様に、pH≦2 および≧11.5 など極端な pH は、特に有意な緩衝能力をともなっている場合は、眼に対する重篤な損傷作用があることを示唆している。そのような物質は眼に有意な作用を生じると予測される。皮膚腐食性物質について、局所的な作用である眼への試験を行うことを回避するために、眼に対する重篤な損傷性／刺激性を考えるに先立って、皮膚腐食性の可能性について評価しておかなければならない。有効性が確認され、承認されている in vitro 代替試験を用いて分類決定をおこなってもよい。

【出典：経済産業省 HP, http://www.meti.go.jp/policy/chemical_management/GHS/text/part3.3.htm ，2007 年 12 月 7 日ｱｸｾｽ. 】

表 4 化粧品･医薬部外品製造販売ガイドブック 2006 に掲載されている試験方法の例
―眼刺激性試験―

試験動物	原則として若齢成熟白色ウサギ
動物数	原則として1群3匹以上
用量	原則として 0.1mL（液体）又は 100mg（固体）
投与方法	片方の眼の下眼瞼を眼球より穏やかに引き離し，結膜嚢内に投与し，上下眼瞼を約 1 秒間穏やかに合わせる。他方の眼は未処置のまま残し，無処置対照眼とする。眼刺激性を示す物質は点眼後に洗眼を行う。
観察	原則として 1、 24、 48、72 及び 96 時間後に眼の観察を行う。持続性の角膜障害等が認められた場合には、その経過及び可逆性の有無について観察を続ける。

図 1 細胞毒性試験における被験物質の調製手順



小島 (1999) による図を引用

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