Appendix 8.14 Reexamination of predictive capacity and applicability domain by using appropriate in vivo data

Evaluation of Draize eye test reference data was done by Barroso et al after the completion of this validation test. We examined predictive capacity and applicability domain except for chemical evaluated as "Should not be used" in single in vivo data. Table 1 shows one chemical excluded from analysis due to precipitation in in vitro test, one chemical excluded due to overlap and chemicals excluded due to inappropriate in vivo data. Twenty two chemicals were excluded from 120 chemicals and 98 chemicals were used for the analysis of predictive capacity.

Table 2 shows the predictive capacity of the SIRC-CVS: TEA test using in vitro and in vivo data of 98 chemicals. The SIRC-CVS: TEA test method demonstrated an accuracy of 50% (49/98), a sensitivity of 55% (27/49), and a specificity of 47% (22/47). There was little difference in predictive capacity before and after exclusion of chemicals with inappropriate in vivo data.

Further analysis was conducted to reduce false negatives by delimiting the applicability domain to certain chemical classes and properties of interest. Table 3 shows one chemical excluded from analysis due to precipitation in in vitro test, one chemical excluded due to overlap, chemicals excluded due to inappropriate in vivo data and chemicals excluded due to purity of less than 80%. Thirty three chemicals were excluded from 120 chemicals and 87 chemicals were used for analysis. Alcohols (The number of hydroxyl group≤2), esters, ethers, ketones, heterocyclic compounds, and carboxylic acid (containing salt) with a molecular weight of less than 180 as exclusion condition were used for the selection of the applicability domain in consideration of decreasing false negative, as shown in Table 4. Forty one out of 87 chemicals were excluded, and 46 chemicals were used for the analysis of predictive capacity. Table 5 shows the predictive capacity of the SIRC-CVS: TEA test using in vitro data and in vivo data of 46 chemicals. The SIRC-CVS:TEA test method demonstrated an accuracy of 57% (26/46), a sensitivity of 88% (14/16), and a specificity of 40% (12/30). False negative rate was improved to 12.5% (2/16). They suggest that the predictive capacity of the SIRC-CVS:TEA test can be improved by delimiting the applicability domain. Toluene was one of the two false negatives and was > Category 2B per TSCA in vivo data, but was classified no category, meaning “negative” per ECETOC in vivo data. Because 3,3-dithiodipropionic acid is a strong acid, it is evaluated as positive by prior information.

It was concluded that the SIRC-CVS:TEA test was useful alternative to the Draize eye test for distinguishing test chemicals that are ocular non irritants.

Table 1 Twenty two test chemicals excluded from the analysis of the predictive capacity

Code No	Chemical Name	Reason for exclusion from analysis
P2-002	2,5-Dimethylhexaediol	Inappropriate in vivo data
P2-016	1-Naphthaleneacetic acid	Inappropriate in vivo data
P3-026	Methylthioglycolate	Inappropriate in vivo data
P3-032	Disodium 4,4'-bis(2-sulfonatostyryl)biphenyl	Inappropriate in vivo data
P3-039	1,2,4-Triazole,sodium salt	Inappropriate in vivo data
P3-041	Benzenamine,4,4'-(4-aimino-3-methylphenyl) (4-imino-3-methyl-2,5-cyclohexadien-1-ylidene) methyl-2-methy HCL	Inappropriate in vivo data
P3-047	2-Benzyloxyethanol	Inappropriate in vivo data
P3-051	Myristyl alcohol	Inappropriate in vivo data
P3-052	Hexyl cinnamic aldehyde	Inappropriate in vivo data
P3-054	Monoethanolamine	Inappropriate in vivo data
P3-058	Methoxyethyl acrylate	Inappropriate in vivo data
P3-065	2-Methylbutyric acid	Inappropriate in vivo data
P3-066	Calcium thioglycolate trihydrate	Inappropriate in vivo data (and in vitro data excluded by precipitation)
P3-067	Citric acid	Inappropriate in vivo data
P3-068	Potassium sorbate	Inappropriate in vivo data
P3-071	n-Lauroylsarcosine sodium salt	Inappropriate in vivo data
P3-072	Sodium lauryl sulfate	Inappropriate in vivo data
P3-090	Cetylpyridinium bromide	Inappropriate in vivo data
P3-093	Sodium hydroxide	Inappropriate in vivo data
P3-094	Glycolic acid	Inappropriate in vivo data
P3-095	3,3-Dithiodipropionic acid	Overlap (P3-095 was the same as P3-023)
P3-096	Sucrose fatty acid ester	Inappropriate in vivo data

Table 2 Predictive capacity of SIRC-CVS:TEA test

N=98	+ (SIRC-CVS)	− (SIRC-CVS)
+ (in vivo) GHS 1,2B 2A	27 P2-004 Ammonium nitrate P2-011 Sodium oxalate P2-015 Isobutyraldehyde P2-018 Ethyl 2,6-dichloro-5-fluoro-beta-oxo-3-pyridinepropionate P2-019 Camphene P3-016 3-Chloropropionitrile P3-019 Diethyl toluamide P3-021 Sodium chloroacetate P3-022 2,4,11,13-Tetraazatetra (Chlorohexidine glucocinate) P3-024 2-Amino-3-hydroxy pyridine P3-027 3-(2-Aminoethylamino)propyl]trimethoxysilane P3-028 Tetraethylene glycol P3-029 Dodecanoic acid P3-030 1,2-Benzisothiazol-3(2H)-one P3-031 2-Hydroxy-1,4-naphthoquinone P3-040 4,4'-(4,5,6,7-Tetrabromo-1,1-dioxido-3H-2,1-benzoxathiole-3,3-diyl)bis[2,6-dibromophenol] P3-045 (3R,4R)-4-Acetoxy-3-[(R)-(tert-butyldimethylsilyloxy)ethyl]-2-azetidinone P3-046 1-Octanol P3-049 Isobutyl alcohol P3-053 n-Butanal P3-055 m-Phenylenediamine P3-061 Imidazole P3-070 Distearyldimethylammonium chloride P3-073 Triton X-100 (5%) P3-075 Promethazine hydrochloride P3-076 2-Ethyl-1-hexanol P3-091 Triton X-100	24 P2-003 1-(2-Propoxy-1-methylethoxy)-2-propanol P2-009 Propylene glycol propyl ether P2-020 Cyclopentanol P3-017 2-Methyl-1-pentanol P3-018 Ethyl-2-methylacetoacetate P3-020 4-Nitrobenzoic acid P3-023 3,3-Dithiodipropionic acid P3-025 Sodium benzoate P3-033 Gamma-Butyrolactone P3-044 Isopropyl acetoacetate P3-048 Butanol P3-050 Isopropyl alcohol P3-059 Methyl acetate P3-060 Methyl cyanoacetate P3-062 Pyridine P3-069 Sodium salicylate P3-078 Cyclohexanol P3-079 Ethanol P3-080 n-Hexanol P3-083 Toluene P3-084 Acetone P3-087 Methyl ethyl ketone (2-butanone) P3-099 Benzyl alcohol P3-100 Lactic acid
− (in vivo) GHS NC	25 P2-001 Piperonylbutoxide P2-006 3,4,4'-Trichlorocarbanilide P2-007 1-Bromohexane P2-010 Ethyl thioglycolate P2-013 1-Bromo-4-chlorobutane P2-014 Sodium hydrogensulfite P2-017 Propyl 4-hydroxybenzoate P3-001 2-Ethoxyethyl methacrylate P3-003 Dipropyl disulfide P3-004 1-Bromo-octane P3-006 Dioctyl ether P3-007 3-Phenoxybenzyl alcohol P3-008 Glycidyl methacrylate P3-011 6-Hydroxy-2,4,5-triaminopyrimidine Sulfate P3-015 3,4-Dimethoxy benzaldehyde P3-035 4-(Methylmercapto)benzaldehyde P3-036 1,9-Decaine P3-042 1-(9H-Carbozol-4-yloxy)-3-[[2-(2-methoxy phenoxy)ethyl] amino]-2-propanol P3-043 3-Methyl-1,5-di(2,4-xylyl)-1,3,5-Triazapenta-1,4-dien P3-074 2-Ethylhexyl p-dimethyl-amino benzoate P3-081 3,3-Dimethylpentane P3-082 Methyl cyclopentane P3-092 Tween20 P3-097 Methyl para-Hydroxybenzoate P3-098 Silic acid	22 P2-005 Potassium tetrafluoroborate P2-008 4,4'-Methylenebis(2,6-di-tert-butylphenol) P2-012 2-Phospho-L-ascorbic acid trisodium salt P3-002 Iso-octylthioglycolate P3-005 2-(2-Ethoxyethoxy)ethanol P3-009 2-Ethylhexylthioglycolate P3-010 n,n-Dimethylguanidine sulfate P3-012 Polyethylene hydrogenated caster oil (40E.O.) P3-013 2,2'-Methylene-bis-(6-(2Hbenzotriazol-2-yl) -4-(1,1,3,3-tetramethylbutyl)phenol) P3-014 Cellulose 2-(2-hydroxy-3-(trimethylammonio)propoxy) ethyl ether chloride P3-034 1-Methylpropyl benzene P3-037 2,4-Dimethyl-3-pentanol P3-038 1-Ethyl-3-methylimidazolium ethylsulfate P3-056 Ethyl acetate P3-057 Isopropyl myristate P3-063 Isopropyl bromide P3-064 Cyclohexanone P3-077 3-Methoxy-1.2-propanediol P3-085 Gluconolactone P3-086 Methyl amyl ketone (2-heptanol) P3-088 Methyl isobutyl ketone(4-methyl 2-pentanol) P3-089 Glycerol

Table 3 Thirty three test chemicals excluded from the analysis of the predictive capacity and the applicability domain

Code	Chemical Name	Reason for exclusion from analysis
P2-002	2,5-Dimethylhexaediol	Inappropriate in vivo data
P2-014	Sodium hydrogensulfite	Purity<80%
P2-016	1-Naphthaleneacetic acid	Inappropriate in vivo data
P3-012	Polyethylene hydrogenated caster oil (40E.O.)	Purity<80%
P3-014	Cellulose 2-(2-hydroxy-3-(trimethylammonio)propoxy) ethyl ether chloride	Purity<80%
P3-022	2,4,11,13-Tetraazatetra (Chlorohexidine glucocinate)	Purity<80%
P3-026	Methylthioglycolate	Inappropriate in vivo data
P3-028	Tetraethylene glycol	Purity<80%
P3-032	Disodium 4,4'-bis(2-sulfonatostyryl)biphenyl	Inappropriate in vivo data
P3-039	1,2,4-Triazole,sodium salt	Inappropriate in vivo data
P3-041	Benzenamine,4,4'-(4-aimino-3-methylphenyl)(4-imino-3-methyl-2,5-cyclohexadien-1-ylidene)methyl-2-methy HCL	Inappropriate in vivo data
P3-047	2-Benzyloxyethanol	Inappropriate in vivo data
P3-051	Myristyl alcohol	Inappropriate in vivo data
P3-052	Hexyl cinnamic aldehyde	Inappropriate in vivo data
P3-054	Monoethanolamine	Inappropriate in vivo data
P3-058	Methoxyethyl acrylate	Inappropriate in vivo data
P3-063	Isopropyl bromide	Inappropriate in vivo data
P3-065	2-Methylbutyric acid	Inappropriate in vivo data
P3-066	Calcium thioglycolate trihydrate	Inappropriate in vivo data (and in vitro data excluded by precipitation)
P3-067	Citric acid	Inappropriate in vivo data
P3-068	Potassium sorbate	Inappropriate in vivo data
P3-070	Distearyldimethylammonium chloride	Inappropriate in vivo data
P3-071	n-Lauroylsarcosine sodium salt	Inappropriate in vivo data
P3-072	Sodium lauryl sulfate	Inappropriate in vivo data
P3-073	Triton X-100 (5%)	Purity<80%
P3-090	Cetylpyridinium bromide	Inappropriate in vivo data
P3-091	Triton X-100	Inappropriate in vivo data
P3-092	Tween20	Purity<80%
P3-093	Sodium hydroxide	Inappropriate in vivo data
P3-094	Glycolic acid	Inappropriate in vivo data
P3-095	3,3-Dithiodipropionic acid	Overlap (P3-095 was the same as P3-023)
P3-096	Sucrose fatty acid ester	Inappropriate in vivo data , Purity<80%
P3-098	Silic acid	Purity<80%

Table 4 Eighty seven test chemicals classified on the basis of applicability domain

Code	Chemical Name	Within(1)or outside(0) applicability domain
P2-001	Piperonylbutoxide	1
P2-004	Ammonium nitrate	1
P2-005	Potassium tetrafluoroborate	1
P2-006	3,4,4'-Trichlorocarbanilide	1
P2-007	1-Bromohexane	1
P2-008	4,4'-Methylenebis(2,6-di-tert-butylphenol)	1
P2-012	2-Phospho-L-ascorbic acid trisodium salt	1
P2-013	1-Bromo-4-chlorobutane	1
P2-015	Isobutyraldehyde	1
P2-017	Propyl 4-hydroxybenzoate	1
P2-018	Ethyl 2,6-dichloro-5-fluoro-beta-oxo-3-pyridinepropionate	1
P2-019	Camphene	1
P3-002	Iso-octylthioglycolate	1
P3-003	Dipropyl disulfide	1
P3-004	1-Bromo-octane	1
P3-006	Dioctyl ether	1
P3-007	3-Phenoxybenzyl alcohol	1
P3-009	2-Ethylhexylthioglycolate	1
P3-010	n,n-Dimethylguanidine sulfate	1
P3-011	6-Hydroxy-2,4,5-triaminopyrimidine Sulfate	1
P3-013	2,2'-Methylene-bis-(6-(2Hbenzotriazol-2-yl) -4-（1,1,3,3-tetramethylbutyl)phenol)	1
P3-015	3,4-Dimethoxy benzaldehyde	1
P3-016	3-Chloropropionitrile	1
P3-019	Diethyl toluamide	1
P3-023	3,3-Dithiodipropionic acid	1
P3-027	3-(2-Aminoethylamino)propyl]trimethoxysilane	1
P3-029	Dodecanoic acid	1
P3-031	2-Hydroxy-1,4-naphthoquinone	1
P3-034	1-Methylpropyl benzene	1
P3-035	4-(Methylmercapto)benzaldehyde	1
P3-036	1,9-Decaine	1
P3-038	1-Ethyl-3-methylimidazolium ethylsulfate	1
P3-040	4,4'-(4,5,6,7-Tetrabromo-1,1-dioxido-3H-2,1 -benzoxathiole-3,3-diyl)bis[2,6-dibromophenol]	1
P3-042	1-(9H-Carbozol-4-yloxy)-3 -[[2-(2-methoxy phenoxy)ethyl] amino]-2-propanol	1
P3-043	3-Methyl-1,5-di(2,4-xylyl)-1,3,5-Triazapenta-1,4-dien	1
P3-045	(3R,4R)-4-Acetoxy-3-[(R) -(tert-butyldimethylsilyloxy)ethyl]-2-azetidinone	1
P3-053	n-Butanal	1
P3-055	m-Phenylenediamine	1
P3-057	Isopropyl myristate	1
P3-074	2-Ethylhexyl p-dimethyl-amino benzoate	1
P3-075	Promethazine hydrochloride	1
P3-081	3,3-Dimethylpentane	1
P3-082	Methyl cyclopentane	1
P3-083	Toluene	1
P3-085	Gluconolactone	1
P3-089	Glycerol	1
P2-003	1-(2-Propoxy-1-methylethoxy)-2-propanol	0
P2-009	Propylene glycol propyl ether	0
P2-010	Ethyl thioglycolate	0
P2-011	Sodium oxalate	0
P2-020	Cyclopentanol	0
P3-001	2-Ethoxyethyl methacrylate	0
P3-005	2-(2-Ethoxyethoxy)ethanol	0
P3-008	Glycidyl methacrylate	0

P3-017	2-Methyl-1-pentanol	0
P3-018	Ethyl-2-methylacetoacetate	0
P3-020	4-Nitrobenzoic acid	0
P3-021	Sodium chloroacetate	0
P3-024	2-Amino-3-hydroxy pyridine	0
P3-025	Sodium benzoate	0
P3-030	1,2-Benzisothiazol-3(2H)-one	0
P3-033	Gamma-Butyrolactone	0
P3-037	2,4-Dimethyl-3-pentanol	0
P3-044	Isopropyl acetoacetate	0
P3-046	1-Octanol	0
P3-048	Butanol	0
P3-049	Isobutyl alcohol	0
P3-050	Isopropyl alcohol	0
P3-056	Ethyl acetate	0
P3-059	Methyl acetate	0
P3-060	Methyl cyanoacetate	0
P3-061	Imidazole	0
P3-062	Pyridine	0
P3-064	Cyclohexanone	0
P3-069	Sodium salicylate	0
P3-076	2-Ethyl-1-hexanol	0
P3-077	3-Methoxy-1.2-propanediol	0
P3-078	Cyclohexanol	0
P3-079	Ethanol	0
P3-080	n-Hexanol	0
P3-084	Acetone	0
P3-086	Methyl amyl ketone (2-heptanol)	0
P3-087	Methyl ethyl ketone (2-butanone)	0
P3-088	Methyl isobutyl ketone(4-methyl 2-pentanol)	0
P3-097	Methyl para-Hydroxybenzoate	0
P3-099	Benzyl alcohol	0
P3-100	Lactic acid	0

Table 5 Predictive capacity of SIRC-CVS test except for test chemicals such as alcohols, esters, ethers, ketones, heterocyclic compounds, and carboxylic acids with a molecular weight of less than180

N=46	＋ (SIRC-CVS)	－ (SIRC-CVS)
＋ (in vivo) GHS 1,2B 2A	14 P2-004 Ammonium nitrate P2-015 Isobutyraldehyde P2-018 Ethyl 2,6-dichloro-5-fluoro-beta-oxo-3-pyridinepropionate P2-019 Camphene P3-016 3-Chloropropionitrile P3-019 Diethyl toluamide P3-027 3-(2-Aminoethylamino)propyl]trimethoxysilane P3-029 Dodecanoic acid P3-031 2-Hydroxy-1,4-naphthoquinone P3-040 4,4'-(4,5,6,7-Tetrabromo-1,1-dioxido-3H-2,1-benzoxathiole-3,3-diyl)bis[2,6-dibromophenol] P3-045 (3R,4R)-4-Acetoxy-3-[(R)-(tert-butyldimethylsilyloxy)ethyl]-2-azetidinone P3-053 n-Butanal P3-055 m-Phenylenediamine P3-075 Promethazine hydrochloride	2 P3-023 3,3-Dithiodipropionic acid P3-083 Toluene
－ (in vivo) GHS NC	18 P2-001 Piperonylbutoxide P2-006 3,4,4'-Trichlorocarbanilide P2-007 1-Bromohexane P2-013 1-Bromo-4-chlorobutane P2-017 Propyl 4-hydroxybenzoate P3-003 Dipropyl disulfide P3-004 1-Bromo-octane P3-006 Dioctyl ether P3-007 3-Phenoxybenzyl alcohol P3-011 6-Hydroxy-2,4,5-triaminopyrimidine Sulfate P3-015 3,4-Dimethoxy benzaldehyde P3-035 4-(Methylmercapto)benzaldehyde P3-036 1,9-Decaine P3-042 1-(9H-Carbozol-4-yloxy)-3-[[2-(2-methoxy phenoxy)ethyl] amino]-2-propanol P3-043 3-Methyl-1,5-di(2,4-xylyl)-1,3,5-Triazapenta-1,4-dien P3-074 2-Ethylhexyl p-dimethyl-amino benzoate P3-081 3,3-Dimethylpentane P3-082 Methyl cyclopentane	12 P2-005 Potassium tetrafluoroborate P2-008 4,4'-Methylenebis(2,6-di-tert-butylphenol) P2-012 2-Phospho-L-ascorbic acid trisodium salt P3-002 Iso-octylthioglycolate P3-009 2-Ethylhexylthioglycolate P3-010 n,n-Dimethylguanidine sulfate P3-013 2,2'-Methylene-bis-(6-(2Hbenzotriazol-2-yl) -4-(1,1,3,3-tetramethylbutyl)phenol) P3-034 1-Methylpropyl benzene P3-038 1-Ethyl-3-methylimidazolium ethylsulfate P3-057 Isopropyl myristate P3-085 Gluconolactone P3-089 Glycerol

References

Barroso, J. et al.(2016) Cosmetics Europe compilation of historical serious eye damage/eye irritation in vivo data analysed by drivers of classification to support the selection of chemicals for development and evaluation of alternative method

Appendix 8.15 Physicochemical explanation of applicability domain

A study to establish a physiochemical explanation of the applicability domain resulted in the following criteria for exclusion that reduces false negatives to a similar level.

(1) pKa

・Chemicals with an acid dissociation constant (pKa) of 4 or less

・Organic salts consisting of weak acid and strong base (=Alkaline)

(The pKa of the weak acid is 3 or more, and the strong base is “sodium”, “potassium” and so on)

(2) Log P

・Chemicals with a distribution coefficient (log P) of greater than -1.5 and less than 2

#Basis of these criteria

Conditions of the SIRC-CVS test differ from in vivo. The test chemical is immersed in a buffer solution, which we think inhibits effects from hydrogen ions or hydroxide ions. The acid disassociation constant is a quantitative index of the strength of an acid in solution, and the smaller the pKa value, the stronger the acid. Chemicals with pKa of 4 or less should be excluded from applicability domain. Furthermore, organic salts consisting of weak acid and strong base (=Alkaline) may take false negative in the SIRC-CVS test on the basis of the above reason. Therefore, they should be excluded from applicability domain.

Examining the quantitative structure–activity relationship (QSAR) for the ocular irritation potential of 53 chemicals, Cronin et al focused on the partition coefficient (log P; equal to log Kow) and found that some amphiphilic chemicals are ocular irritants, as shown in Fig. 1. Conversely, this tendency was not found in non-irritants, as shown in Fig.2. A chemical with a low log P value will have excellent solubility in water but poor cellular membrane permeability. Conversely, a chemical with a high log P value will have both excellent lipid solubility and excellent membrane permeability. When conducting in vivo tests for ocular irritation, however, a layer of aqueous lacrimal fluid covering the cornea prevents the test chemical from coming in direct contact with the cornea. Chemicals with intermediate log P values are amphiphilic, capable of permeating both an aqueous layer of lacrimal fluid and lipid cellular membranes, and thereby affecting cells and cornea alike. But since amphiphilic ocular irritants (active ingredients) generally do not exhibit cytotoxicity at the level of concentration (0.5% or less) used in the SIRC-CVS test method, they yield false negative results.

#Reexamination of predictive capacity and applicability domain

In determining the applicability domain, we looked at 98 chemicals after the exclusion of 22 test chemicals (as shown in table 1 of appendix 8.14) from 120 chemicals tested in the validation study.

Ninty two chemicals were obtained after excluding 4 chemicals with an acid dissociation constant (pKa) of 4 or less (Table 1) and 2 organic salts consisting of weak acid and strong base (Table 2). Furthermore, 52 substances were obtained after excluding chemicals with a distribution coefficient (log P) of greater than -1.5 and less than 2 (Table 3). Table 4 shows the predictive capacity of SIRC-CVS: TEA test under this applicability domain. The SIRC-CVS:TEA test method demonstrated an accuracy of 58% (30/52), a sensitivity of 94% (15/16), and a specificity of 42% (15/36). False negative rate was improved to 6% (1/16). They suggest that the predictive capacity of the SIRC-CVS:TEA test can be improved by delimiting the applicability domain. Toluene was one of the two false negatives and was > Category 2B per TSCA in vivo data, but was classified No Category, meaning “negative,” per ECETOC in vivo data. Chemicals exhibiting false positive is considered that they has a possibility of having an effect on the eye. They are considered to be negative in vivo because they are discharged from the rabbit eye usually in about 5 minutes in vivo. Takahashi et al reported that rabbits excreted about 80% of applied materials from the conjunctival sac in 3-4 min. If they contact with the cornea sufficiently without being discharged in about 5 minutes from the eye, they may have an effect on the rabbit eye.

It was concluded that the SIRC-CVS:TEA test was useful alternative to the Draize eye test for distinguishing test chemicals that are ocular non irritants. A study to establish a physiochemical explanation of the applicability domain results in the better criteria of applicability domain.

References

1) Cronin, M.T.D, Basketter, D.A. and York, M.(1994) A quantitative structure-activity relationship (QSAR) investigation of a Draize eye irritation database, Toxicol. in Vitro, Vol.8, No.1, 21-28.
2) Takahashi, Y., Koike, M., Honda, H., Ito, Y., Sakaguchi, H. (2008) Development of the short time exposure (STE) test: An in vitro eye irritation test using SIRC cells, Toxicol. in Vitro, Vol.22, 760-770.

Fig. 1: Log P of some ocular irritants

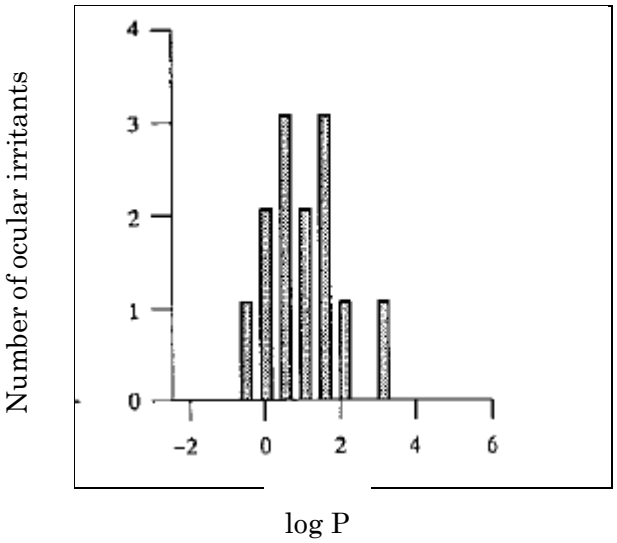

Fig 2: Log P of some ocular non-irritants

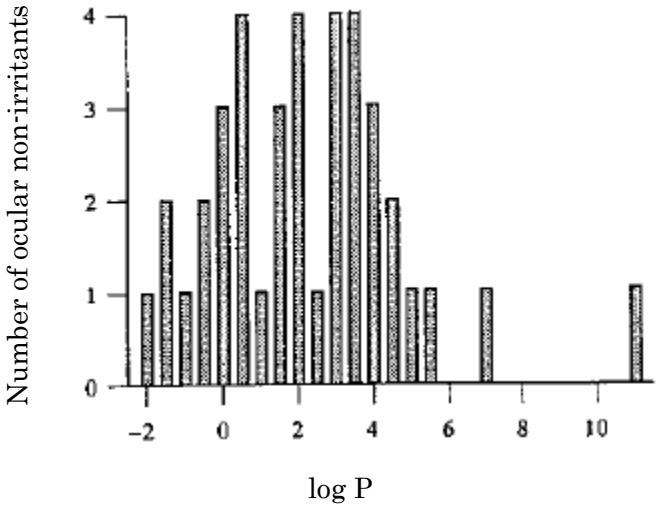

Table 1 Four test chemicals evaluated as eye irritants due to pKa value of 4 or less

Code No	Chemical Name	Substances with pKa value of 4 or less (Most Acidic Temp: 25°C by SciFinder)
P3-020	4-Nitrobenzoic acid	3.42 ±0.10
P3-023	3,3-Dithiodipropionic acid	3.94 ±0.10
P3-060	Methyl cyanoacetate	2.75 ±0.10
P3-100	Lactic acid	3.91 ±0.11

Excluding the above four chemicals, chemicals to be analyzed were decreased from 98 to 94.

Table 2 Two test chemicals evaluated as eye irritants due to organic salts containing strong base and weak acid with pKa of 3 or more

Code No	Chemical Name	pKa value (Most Acidic Temp: 25°C by SciFinder)
P3-025	Sodium benzoate	pKa of benzoic acid is 4.20±0.10
P3-069	Sodium salicylate	pKa of salicylic acid is 3.01±0.10

Excluding the above two chemicals, chemicals to be analyzed were decreased from 94 to 92.

Table 3 Ninety two test chemicals classified by log P

Code	Chemical Name	Log P (Log Kow KOWWIN v.1.68 estimate, EPI Suite)	Within(1)or outside(0) applicability domain
P2-001	Piperonylbutoxide	4.29	1
P2-004	Ammonium nitrate	-4.39	1
P2-005	Potassium tetrafluoroborate	-0.78	1
P2-006	3,4,4'-Trichlorocarbanilide	4.90	1
P2-007	1-Bromohexane	3.63	1
P2-008	4,4'-Methylenebis(2,6-di-tert-butylphenol)	8.99	1
P2-011	Sodium oxalate	-7.00	1
P2-012	2-Phospho-L-ascorbic acid trisodium salt	-9.96	1
P2-013	1-Bromo-4-chlorobutane	2.90	1
P2-014	Sodium hydrogensulfite	-7.51	1
P2-017	Propyl 4-hydroxybenzoate	2.98	1
P2-018	Ethyl 2,6-dichloro-5-fluoro-beta-oxo-3-pyridine propionate	2.01	1
P2-019	Camphene	4.35	1
P3-002	Iso-octylthioglycolate	3.68	1
P3-003	Dipropyl disulfide	3.84	1
P3-004	1-Bromo-octane	4.61	1
P3-005	2-(2-Ethoxyethoxy)ethanol	-0.69	1
P3-006	Dioctyl ether	6.94	1
P3-007	3-Phenoxybenzyl alcohol	3.13	1
P3-009	2-Ethylhexylthioglycolate	3.68	1
P3-011	6-Hydroxy-2,4,5-triaminopyrimidine Sulfate	-4.92	1
P3-012	Polyethylene hydrogenated caster oil (40E.O.)	17.71	1
P3-013	2,2'-Methylene-bis-(6-(2Hbenzotriazol-2-yl) -4- (1,1,3,3-tetramethylbutyl)phenol)	12.46	1
P3-019	Diethyl toluamide	2.26	1
P3-021	Sodium chloroacetate	-3.47	1
P3-027	3-(2-Aminoethylamino)propyl]trimethoxy silane	-1.67	1
P3-029	Dodecanoic acid	5.00	1
P3-034	1-Methylpropyl benzene	3.94	1
P3-035	4-(Methylmercapto)benzaldehyde	2.31	1
P3-036	1,9-Decaine	4.98	1
P3-037	2,4-Dimethyl-3-pentanol	2.13	1
P3-040	4,4'-(4,5,6,7-Tetrabromo-1,1-dioxido-3H-2,1-benzoxathiole-3,3-diyl)bis[2,6-dibromo phenol]	10.33	1
P3-042	1-(9H-Carbozol-4-yloxy)-3-[[2-(2-methoxy phenoxy)ethyl] amino]-2-propanol	3.05	1
P3-043	3-Methyl-1,5-di(2,4-xylyl)-1,3,5-Triazape nta-1,4-dien	5.55	1
P3-046	1-Octanol	2.81	1
P3-057	Isopropyl myristate	7.17	1
P3-063	Isopropyl bromide	2.08	1
P3-070	Distearyldimethylammonium chloride	12.52	1
P3-073	Triton X-100 (5%)	4.86	1
P3-074	2-Ethylhexyl p-dimethyl-amino benzoate	5.77	1
P3-075	Promethazine hydrochloride	2.97	1

P3-076	2-Ethyl-1-hexanol	2.73	1
P3-077	3-Methoxy-1.2-propanediol	-1.15	1
P3-081	3,3-Dimethylpentane	3.67	1
P3-082	Methyl cyclopentane	3.10	1
P3-083	Toluene	2.54	1
P3-085	Gluconolactone	-1.98	1
P3-089	Glycerol	-1.65	1
P3-091	Triton X-100	4.86	1
P3-092	Tween20	-2.03	1
P3-097	Methyl para-Hydroxybenzoate	2.00	1
P3-098	Silic acid	-1.50	1
P2-003	1-(2-Propoxy-1-methylethoxy) -2-propanol	0.64	0
P2-009	Propylene glycol propyl ether	0.49	0
P2-010	Ethyl thioglycolate	0.81	0
P2-015	Isobutyraldehyde	0.74	0
P2-020	Cyclopentanol	1.15	0
P3-001	2-Ethoxyethyl methacrylate	1.49	0
P3-008	Glycidyl methacrylate	0.81	0
P3-010	n,n-Dimethylguanidine sulfate	No data	0
P3-014	Cellulose 2-(2-hydroxy-3-(trimethylammonio)propoxy) ethyl ether chloride	No data	0
P3-015	3,4-Dimethoxy benzaldehyde	1.36	0
P3-016	3-Chloropropionitrile	0.60	0
P3-017	2-Methyl-1-pentanol	1.75	0
P3-018	Ethyl-2-methylacetoacetate	0.21	0
P3-022	2,4,11,13-Tetraazatetra (Chlorohexidine glucocinate)	-0.33	0
P3-024	2-Amino-3-hydroxy pyridine	0.05	0
P3-028	Tetraethylene glycol	0.29	0
P3-030	1,2-Benzisothiazol-3(2H)-one	0.64	0
P3-031	2-Hydroxy-1,4-naphthoquinone	0.78	0
P3-033	Gamma-Butyrolactone	-0.31	0
P3-038	1-Ethyl-3-methylimidazolium ethylsulfate	No data	0
P3-044	Isopropyl acetoacetate	0.21	0
P3-045	(3R,4R)-4-Acetoxy-3-[(R)-(tert-butyldimethylsilyloxy)ethyl]-2-azetidinone	No data	0
P3-048	Butanol	0.84	0
P3-049	Isobutyl alcohol	0.77	0
P3-050	Isopropyl alcohol	0.28	0
P3-053	n-Butanal	0.82	0
P3-055	m-Phenylenediamine	-0.39	0
P3-056	Ethyl acetate	0.86	0
P3-059	Methyl acetate	0.37	0
P3-061	Imidazole	0.06	0
P3-062	Pyridine	0.80	0
P3-064	Cyclohexanone	1.13	0
P3-078	Cyclohexanol	1.64	0
P3-079	Ethanol	-0.14	0
P3-080	n-Hexanol	1.82	0
P3-084	Acetone	-0.24	0
P3-086	Methyl amyl ketone (2-heptanol)	1.73	0

P3-087	Methyl ethyl ketone (2-butanone)	0.26	0
P3-088	Methyl isobutyl ketone (4-methyl 2-pentanol)	1.16	0
P3-099	Benzyl alcohol	1.08	0

Table 4 Predictive capacity of SIRC-CVS test

N=52	＋ (SIRC-CVS)	－ (SIRC-CVS)
＋ (in vivo) GHS 1,2B 2A	15 P2-004 Ammonium nitrate P2-011 Sodium oxalate P2-018 Ethyl 2,6-dichloro-5-fluoro-beta-oxo-3-pyridinepropionate P2-019 Camphene P3-019 Diethyl toluamide P3-021 Sodium chloroacetate P3-027 3-(2-Aminoethylamino)propyl]trimethoxysilane P3-029 Dodecanoic acid P3-040 4,4'-(4,5,6,7-Tetrabromo-1,1-dioxido-3H-2,1-benzoxathiole-3,3-diyl)bis[2,6-dibromophenol] P3-046 1-Octanol P3-070 Distearyldimethylammonium chloride P3-073 Triton X-100 (5%) P3-075 Promethazine hydrochloride P3-076 2-Ethyl-1-hexanol P3-091 Triton X-100	1 P3-083 Toluene
－ (in vivo) GHS NC	21 P2-001 Piperonylbutoxide P2-006 3,4,4'-Trichlorocarbanilide P2-007 1-Bromohexane P2-013 1-Bromo-4-chlorobutane P2-014 Sodium hydrogensulfite P2-017 Propyl 4-hydroxybenzoate P3-003 Dipropyl disulfide P3-004 1-Bromo-octane P3-006 Dioctyl ether P3-007 3-Phenoxybenzyl alcohol P3-011 6-Hydroxy-2,4,5-triaminopyrimidine Sulfate P3-035 4-(Methylmercapto)benzaldehyde P3-036 1,9-Decaine P3-042 1-(9H-Carbozol-4-yloxy)-3-[[2-(2-methoxy phenoxy)ethyl]amino]-2-propanol P3-043 3-Methyl-1,5-di(2,4-xylyl)-1,3,5-Triazapenta-1,4-dien P3-074 2-Ethylhexyl p-dimethyl-amino benzoate P3-081 3,3-Dimethylpentane P3-082 Methyl cyclopentane P3-092 Tween20 P3-097 Methyl para-Hydroxybenzoate P3-098 Silic acid	15 P2-005 Potassium tetrafluoroborate P2-008 4,4'-Methylenebis(2,6-di-tert-butylphenol) P2-012 2-Phospho-L-ascorbic acid trisodium salt P3-002 Iso-octylthioglycolate P3-005 2-(2-Ethoxyethoxy)ethanol P3-009 2-Ethylhexylthioglycolate P3-012 Polyethylene hydrogenated caster oil (40E.O.) P3-013 2,2'-Methylene-bis-(6-(2Hbenzotriazol-2-yl) -4-(1,1,3,3-tetramethylbutyl)phenol) P3-034 1-Methylpropyl benzene P3-037 2,4-Dimethyl-3-pentanol P3-057 Isopropyl myristate P3-063 Isopropyl bromide P3-077 3-Methoxy-1.2-propanediol P3-085 Gluconolactone P3-089 Glycerol

Appendix 8.16 Physicochemical explanation of applicability domain by using the additional data from Shiseido

A study to establish a physiochemical explanation of the applicability domain resulted in the following criteria for exclusion that reduces false negatives to a similar level. They were also obtained by using the additional data from Shiseido.

(1) pKa

・Chemicals with an acid dissociation constant (pKa) of 4 or less

・Organic salts consisting of weak acid and strong base (=Alkaline)

(The pKa of the weak acid is 3 or more, and the strong base is “sodium”, “potassium” and so on)

(2) Log P

・Chemicals with a distribution coefficient (log P) of greater than -1.5 and less than 2

The predictive capacity of SIRC-CVS:TEA test was analyzed by the additional data from Shiseido. Shiseido’s data were taken from the report used in the peer review by JaCVAM eye irritation test evaluating committee in 2009-2011, and their data sheets was checked during the peer review.

Table 1 shows whether or not 46 chemicals falls within the scope of applicability domain when classified by Log P.

Table 2 shows the predicative capacity when examined with 46 chemical substances before being classified by Log P. The SIRC-CVS:TEA test method demonstrated an accuracy of 63% (29/46), a sensitivity of 81% (17/21), and a specificity of 48% (12/25). False negative rate was 19% (4/21).

Table 3 shows the predictive capacity of SIRC-CVS: TEA test under this applicability domain classified by Log P. The SIRC-CVS:TEA test method demonstrated an accuracy of 65% (20/31), a sensitivity of 100% (11/11), and a specificity of 45% (9/20). False negative rate was 0% (0/11). They suggest that the predictive capacity of the SIRC-CVS:TEA test can be improved by delimiting the applicability domain.

It was concluded that the SIRC-CVS:TEA test was useful alternative to the Draize eye test for distinguishing test chemicals that are ocular non irritants. A study to establish a physiochemical explanation of the applicability domain results in the better criteria of applicability domain.

Table 1

Code	Chemical name	CAS	Log P (Log Kow KOWWIN v.1.68 estimate, EPI Suite)	Applicable (1) or unapplicable (0)	in vitro	in vivo
1	Butylene glycol	107-88-0	-0.29	0	N	N
2	Propylene carbonate	108-32-7	0.08	0	N	N
3	2,4-Pentanediol	625-69-4	0.13	0	N	N
4	Resorcinol	108-46-3	1.03	0	P	P
5	Butoxyethanol	111-76-2	0.57	0	N	P
6	Hexylene glycol	107-41-5	0.58	0	N	P
7	Phenethyl alcohol	1960/12/8	1.57	0	P	P
8	Methoxyisopropyl acetate	108-65-6	0.52	0	N	P
9	6-Methyl purine	2004/3/7	-0.27	0	P	P
10	Phenoxyethanol	122-99-6	1.10	0	N	P
11	Di-iso-butyl ketone	108-83-8	2.56	1	N	N
12	Triethylene glycol	112-27-6	-1.75	1	N	N
13	Chloroxylenol	88-04-0	3.25	1	P	P
14	2,4-Difluoronitrobenzene	446-35-5	2.21	1	P	N
15	iso-Octyl acrylate	29590-42-9	4.09	1	P	N
16	Sodium dehydroacetate	4418-26-2	-0.32	0	P	N
17	Triisopropanolamine	122-20-3	-1.22	0	P	P
18	2-Bromo-2-Nitropropane-1,3-Diol	52-51-7	-0.64	0	P	P
19	2-(n-Dodecylthio)ethanol	1462-55-1	5.35	1	P	N
20	Benzophenone-1	131-56-6	2.96	1	P	P
21	Triacetin	102-76-1	0.36	0	P	N
22	Chlorophene	120-32-1	4.18	1	P	P
23	Sodium naphthalenesulfonate	532-02-5	-1.78	1	P	P
24	Diisopropyl adipate	6938-94-9	3.20	1	P	N
25	tetra-Aminopyrimidine sulfate	5392-28-9	-5.37	1	P	N
26	Cetyl alcohol	36653-82-4	6.73	1	P	N
27	Benzophenone-2	131-55-5	2.78	1	P	P
28	Oleyl alcohol	143-28-2	7.50	1	P	N
29	Benzalkonium chloride	8001-54-5	2.93	1	P	P
30	Lauramide DEA	120-40-1	2.89	1	P	P
31	Isopropyl Palmitate	142-91-6	8.16	1	N	N
32	Sodium stearate	822-16-2	4.13	1	P	N
33	Cetrimonium chloride	112-02-7	3.18	1	P	P
34	Phytantriol	74563-64-7	6.36	1	P	P
35	Ethylhexyl palmitate	29806-73-3	10.61	1	N	N
36	Diethylhexyl adipate	103-23-1	8.12	1	N	N
37	TEA-Lauryl sulfate 40% solution	139-96-8	0.55	0	P	P
38	Squalane	111-01-3	14.63	1	N	N
39	Stearalkonium chloride	122-19-0	5.87	1	P	P
40	Sorbitan oleate	1338-43-8	5.89	1	P	N
41	Dioctyl sodium sulfosuccinate	577-11-7	3.95	1	P	P
42	Isocetyl stearate	25339-09-7	15.52	1	N	N
43	PEG-40 stearate	9004-99-3	6.16	1	P	N
44	Safflower (Carthamus tinctorius) oil	8001-23-8	22.65	1	N	N
45	Sesame (Sesamum indicum) oil	8008-74-0	22.80	1	N	N
46	Sorbitan sesquioleate	8007-43-0	13.83	1	P	N

P: 1,2B or 2A in GHS

N: NC in GHS

Table 2 Predictive capacity of SIRC-CVS:TEA test evaluated in Shiseido's additional data

N=46	+ (SIRC-CVS)	− (SIRC-CVS)
+ (in vivo) GHS 1,2B 2A	17 Resorcinol Phenethyl alcohol 6-Methyl purine Chloroxylenol Triisopropanolamine 2-Bromo-2-Nitropropane-1,3-Diol Benzophenone-1 Chlorophene Sodium naphthalenesulfonate Benzophenone-2 Benzalkonium chloride Lauramide DEA Cetrimonium chloride Phytantriol TEA-Lauryl sulfate 40% solution Stearalkonium chloride Dioctyl sodium sulfosuccinate	4 Butoxyethanol Hexylene glycol Methoxyisopropyl acetate Phenoxyethanol
− (in vivo) GHS NC	13 2,4-Difluoronitrobenzene iso-Octyl acrylate Sodium dehydroacetate 2-(n-Dodecylthio)ethanol Triacetin Diisopropyl adipate tetra-Aminopyrimidine sulfate Cetyl alcohol Oleyl alcohol Sodium stearate Sorbitan oleate PEG-40 stearate Sorbitan sesquioleate	12 Butylene glycol Propylene carbonate 2,4-Pentanediol Di-iso-butyl ketone Triethylene glycol Isopropyl Palmitate Ethylhexyl palmitate Diethylhexyl adipate Squalane Isocetyl stearate Safflower (Carthamus tinctorius) oil Sesame (Sesamum indicum) oil

Table 3 Predictive capacity of SIRC-CVS:TEA test in the applicability domain classified by log P using Shiseido's additional data

N=31	＋ (SIRC-CVS)	－ (SIRC-CVS)
＋ (in vivo) GHS 1,2B 2A	11 Chloroxylenol Benzophenone-1 Chlorophene Sodium naphthalenesulfonate Benzophenone-2 Benzalkonium chloride Lauramide DEA Cetrimonium chloride Phytantriol Stearalkonium chloride Dioctyl sodium sulfosuccinate	0
－ (in vivo) GHS NC	11 2,4-Difluoronitrobenzene iso-Octyl acrylate 2-(n-Dodecylthio)ethanol Diisopropyl adipate tetra-Aminopyrimidine sulfate Cetyl alcohol Oleyl alcohol Sodium stearate Sorbitan oleate PEG-40 stearate Sorbitan sesquioleateChloroxylenol	9 Di-iso-butyl ketone Triethylene glycol Isopropyl Palmitate Ethylhexyl palmitate Diethylhexyl adipate Squalane Isocetyl stearate Safflower (Carthamus tinctorius) oil Sesame (Sesamum indicum) oil