Plan B

Appendix 8.15 Physicochemical explanation of applicability domain

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

1. Chemicals with an acid dissociation constant (pKa) of 4 or less
2. Chemicals with a distribution coefficient (log D) of greater than -1.5 and less than 2

We think that there are some acids that yield false negative results, because the test chemicals are diluted using a culture medium with buffering capacity. On the other hand, a distribution coefficient of $-1.5 < \log D < 2$ indicates that a chemical is amphiphilic. For example, many amphiphilic chemicals, such as ethanol, are known to exhibit ocular irritation potential.[1] But since amphiphilic ocular irritants (undiluted ingredients) generally do not exhibit cytotoxicity at the levels of concentration (0.5% or less) used in the SIRC-CVS test method, they yield in false negative results. Chemicals with a distribution coefficient other than $-1.5 < \log D < 2$ as well as macromolecules, surfactants, organic salts, or other chemicals for which a log D value cannot be obtained fall within the applicability domain, provided they do not have an acid dissociation constant (pKa) of 4 or less. Chemicals that fall into the SIRC-CVS applicability domain and yield negative results during testing can be considered ocular non-irritants.

In determining the applicability domain, we looked at 116 chemicals. Of the 120 chemicals tested in the validation study, there was one that was a duplicate, one that could not be tested using the SIRC-CVS test method, and two for which no in vivo data was available, leaving 116 chemicals.

Next, we will present a physiochemical rationale for exclusion from the applicability domain.

The reason for excluding chemicals with an acid dissociation constant (pKa) of 4 or less is as follows. Conditions of the SIRC-CVS test differ from in vivo in that the test chemical is immersed in a buffer solution, which we think inhibits effects from hydrogen ions. Discrepancies in strong acids are obvious, and the threshold was set to a pKa value of 4. The acid disassociation constant is a quantitative measure of the strength of an acid in solution, and the smaller the pKa value, the stronger the acid. Table 1 shows the test chemicals with a pKa value of 4 or less that were used in this validation.

Table 1: Test chemicals excluded from the applicability domain due to a pKa value of 4 or less

Code No.	Chemical Name	pKa value (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

P3-094	glycolic acid	3.74 ±0.11
P3-100	lactic acid	3.91 ±0.11

The rationale for excluding chemicals with a distribution coefficient of $1.5 < \log D < 2$ is as follows. We looked at 111 test chemicals, which is to say, the 116 test chemicals used in the validation study but minus the five test chemicals with a pKa of 4 or less. The distribution coefficient has been reported to be a factor in the kinetics of percutaneous absorption and other types of transmission through biomembranes.[2] As an expression of the octanol/water partition coefficient, log D is an indicator of solubility in either water or oil.

A chemical with a low log D value will have excellent solubility in water but poor cellular membrane permeability. The reason for this is that cellular membranes are made of bilayer lipid membranes. Ordinarily, a chemical's effect on the cellular membrane decreases when the log D value is sufficiently low, and the effect on the cornea derived from cells also decreases. The relationship between log D and ocular irritation potential for some typical test chemicals is shown in Table 2. Glycerol is a typical example from Table 2 of a chemical with a low log D value.

Conversely, a chemical with a high log D 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. Isopropyl myristate is a typical example from Table 2 of a chemical with a high log D value.

Chemicals with intermediate log D 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. (See Table 1.) It should be noted, however, that triethanolamine, which is used as a relative control, is not excluded from the applicability domain, even though its log D value is -1.89.

Excluding from the SIRC-CRV applicability domain any amphiphilic test chemical with a log D value between -1.5 and 2.0 improves sensitivity to the point that any test chemical yielding a negative result can confidently be predicted to be an ocular non-sensitizer.

We consider false positives to result of effects that are modified by halogen, aldehyde, or other functional group.

Table 2: Relationship of log D and ocular irritation potential

Test substance	log D (pH7)	In vitro IC50 (μg/mL)	In vivo GHS or MAS	Evaluation	Citation
Triethanolamine	-1.89	2090	GHS:NC	True negative	Tani et al (1999)
Glycerol	-1.85	11600	GHS:NC	True negative	Tani et al (1999)
Triethylene glycol	-1.65	> 5000	Non irritant	True negative	Hagino CIR
Ethylene glycol	-1.36	25524	GHS:2B	False negative	Kitagaki et al (2006) CICAD 45 (2002)
Methanol	-0.69	7566	GHS:2	False negative	Kitagaki et al (2006)

					EHC 196 (1997)
Ethanol	-0.18	10000 <	MAS: 32.7	False negative	Tani et al (1999)
Acetone	-0.04	9353	MMAS: 65.8	False negative	Kitagaki et al (2006) Bagley et al (1999)
Isopropanol	0.17	8672	MMAS: 30.5	False negative	Kitagaki et al (2006) Bagley et al (1999)
Methoxyisopropyl acetate	0.48	2482.4,4172.9	Irritant	False negative	Hagino CIR
Isobutanol	0.68	3480	MMAS: 60.3	False negative	Kitagaki et al (2006) Bagley et al (1999)
Propasol Solvent P	0.68	3889.9, 3816.8	GHS: 2B	False negative	Hagino ICCVAM
Ethyl-2-methyl acetoacetate	0.72	2978.4, 3410.9	GHS: 2B	False negative	Hagino CIR
Cyclopentanol	0.75	2684.1, 2366.4	GHS: 2B	False negative	Hagino ICCVAM
Butyl Dipropasol Solvent	0.8	2729.9, 3646.0	GHS: 2B	False negative	Hagino ICCVAM
Butoxy ethanol	0.83	2099.4, 2275.0	MMAS: 68.7	False negative	Hagino Bagley
n-Butanol	0.84	4400 <	MMAS: 60.8	False negative	Tani et al (1999)
Benzyl alcohol	1.06	1190	MAS: 31.0	True positive	Tani et al (1999)
Phenethyl alcohol	1.5	621.0, 753.3	Irritant	True positive	Hagino CIR
2-Methyl-1-pentanol	1.7	1665.9, 1558.9	GHS: 2B	False negative	Hagino ICCVAM
n-Hexanol	1.86	1374.4 (Negative)	2B	False negative	This validation ICCVAM
2,2-Dimethyl-3-pentanol	1.96	1399.8, 976.2	GHS: NC	True positive	Hagino ICCVAM
2,6-Dimetyl-4-heptanone	2.70	>5000	MMAS: 0.7	True negative	Hagino Bagley et al (1999)
Octanol	2.88	397	MAS: 41.0	True positive	Kitagaki et al (2006) Bagley et al
Isopropyl Myristate	7.25	>5000, 3606	GHS: NC	True negative	Hagino CIR

Cronin et al (1994) contains historical data establishing a distribution coefficient of $-1.5 < \log D < 2$ as a physical characteristic of amphiphilic chemicals that exhibit ocular irritation potential. 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, equivalent to ignoring dissociation per pH in log D), and found that some amphiphilic chemicals are ocular irritants. (See Fig. 1) Conversely, this tendency was not found in non-irritants. (See Fig. 2)

Fig. 1: Log P of some ocular irritants

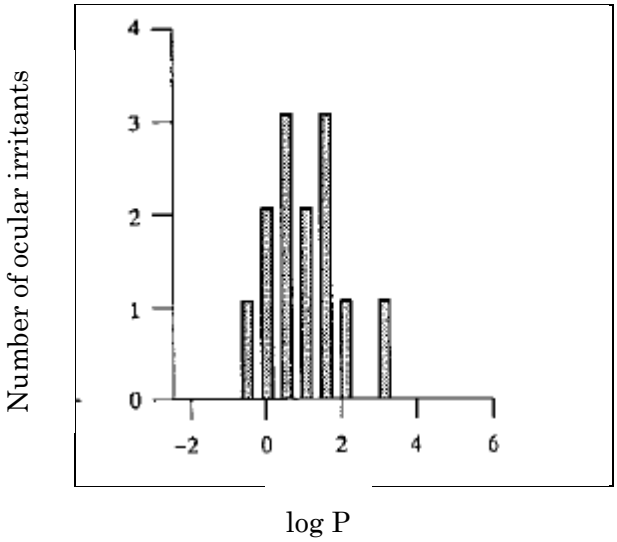

Fig 2: Log P of some ocular non-irritants

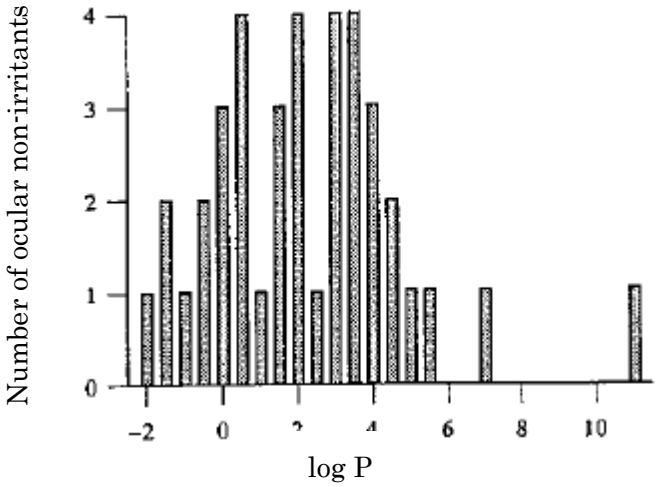

Table 3 shows chemicals with a log D value of greater than -1.5 and less than 2, while Table 4 shows chemicals with a log D value outside that range. Although there were some instances of small discrepancies in stated values or lack of data depending upon source, all chemicals in either Scifinder or ADME Predictor that met the $-1.5 < \log D < 2$ criteria have been excluded from the SIRC-CVS applicability domain. The only example of a chemical with a discrepancy between these two sources was 2,2-Dimethyl-3-pentanol (P3-037), the log D value of which was listed as 1.96 in Scifinder but as 2.148 in ADME Predictor, either of which is close to the upper limit of 2.

Table 5 shows predictive capacity of the SIRC-CVS test method when test chemicals with either an acid dissociation constant (pKa) of 4 or less or a distribution coefficient (log D) of greater than -1.5 and less than 2 have been excluded from the applicability domain.

As described above, we have shown that excluding test chemicals with either an acid dissociation constant (pKa) of 4 or less or a distribution coefficient (log D) of greater than -1.5 and less than 2 will reduce false negatives when using the SIRV-CVS test method to screen for ocular non-irritants.

Bibliography

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) Atobe, T., Mori, M., Yamashita, F., Hashida, M. and Kouzuki, H (2015) Artificial neural network analysis for predicting human percutaneous absorption taking account of vehicle properties, J. Toxicol. Sci., Vol.40, No.2, 277-294.

Table 3: Chemicals with a distribution constant of $-1.5 < \log D < 2$

Code	Chemical Name	Molecular weight	Log D (by Scifinder)	Log D (by ADME predictor)	GHS	In vitro
P2-002	2,5-Dimethylhexaediol	146.23	0.76	1.141	2B	0
P2-003	1-(2-Propoxy-1-methylethoxy)-2-propanol	176.25	0.8	1.276	2A	0
P2-009	Propylene glycol propyl ether	118.17	0.68	0.742	No	1
P2-010	Ethyl thioglycolate	120.17	1.1	0.654	2B	1
P2-015	Isobutyraldehyde	72.11	0.76	0.711	2B	0
P2-016	1-Naphthaleneacetic acid	186.21	-0.14	-0.174	1	0
P2-020	Cyclopentanol	86.13	0.75	0.941	2A	1
P3-001	2-Ethoxyethyl methacrylate	158.19	1.44	1.624	No	1
P3-005	2-(2-Ethoxyethoxy)ethanol	134.17	-0.42	-0.244	No	0
P3-008	Glycidyl methacrylate	142.15	0.34	0.742	No	1
P3-015	3,4-Dimethoxy benzaldehyde	166.17	1.37	1.619	No	1
P3-016	3-Chloropropionitrile	89.52	0.29	0.471	2B	1
P3-017	2-Methyl-1-pentanol	102.17	1.70	1.727	2B	0
P3-018	Ethyl-2-methylacetoacetate	144.17	0.72	0.869	2B	0
P3-024	2-Amino-3-hydroxy pyridine	110.11	-0.44	-0.097	2A	0
P3-025	Sodium benzoate	144.1	-	-0.991	2A	0
P3-026	Methylthioglycolate	106.14	0.59	0.146	1	1
P3-028	Tetraethylene glycol	302.32	0.53	0.762	1	1
P3-030	1,2-Benzisothiazol-3(2H)-one	151.18	1.95	1.555	1	1
P3-031	2-Hydroxy-1,4-naphthoquinone	174.15	-0.74	-0.445	2B	1
P3-032	Disodium 4,4'-bis(2-sulfonatostyryl)biphenyl	562.56	-	0.713	1	1
P3-033	Gamma-Butyrolactone	86.09	-0.63	-0.057	No	0
P3-037	2,2-Dimethyl-3-pentanol	116.2	1.96	2.148	No	0
P3-044	Isopropyl acetoacetate	144.17	0.72	0.79	2B	0
P3-047	2-Benzyloxyethanol	152.19	1.11	1.26	2A	0
P3-048	Butanol	74.12	0.84	0.733	1	0
P3-049	Isobutyl alcohol	74.12	0.68	0.66	1	1
P3-050	Isopropyl alcohol	60.1	0.17	0.091	2A	0
P3-053	n-Butanal	72.11	0.91	0.666	2B	1
P3-055	m-Phenylenediamine	108.14	-0.19	-0.022	1	1
P3-056	Ethyl acetate	88.11	0.79	0.527	No	0
P3-058	Methoxyethyl acrylate	130.14	0.51	0.611	1	1
P3-059	Methyl acetate	74.08	0.28	-0.024	2A	0
P3-061	Imidazole	68.08	-0.7	-0.487	1	1

P3-062	Pyridine	79.1	0.83	0.629	1	0
P3-064	Cyclohexanone	98.14	0.82	0.95	No	0
P3-065	2-Methylbutyric acid	102.13	-1.14	-0.797	1	0
P3-069	Sodium salicylate	160.1	-	-1.217	1	0
P3-071	n-Lauroylsarcosine sodium salt	293.38	-	1.36	2B	1
P3-072	Sodium lauryl sulfate	288.38	-	1.175	2A?	1
P3-077	3-Methoxy-1.2-propanediol	106.12	-0.94	-1.186	No	0
P3-078	Cyclohexanol	100.16	1.28	1.317	1	0
P3-079	Ethanol	46.068	-0.18	-0.305	2A	0
P3-080	n-Hexanol	102.17	1.86	1.879	2A	0
P3-084	Acetone	58.08	-0.04	-0.061	2A	0
P3-087	Methyl ethyl ketone (2-butanone)	72.11	0.47	0.449	2A	0
P3-088	Methyl isobutyl ketone(4-methyl 2-pentanol)	100.16	1.33	1.44	No	0
P3-097	Methyl para-Hydroxybenzoate	152.15	1.86	1.736	2?	1
P3-98	Silic acid	78.1	-	0.535	NC	1
P3-099	Benzyl alcohol	108.14	1.06	1.091	1	0

Table 4: Chemicals with a distribution constant other than -1.5 < log D < 2

Code	Chemical Name	Molecular weight	Log D (by Scifinder)	Log D (by ADME predictor)	GHS	In vitro
P2-001	Piperonylbutoxide	338.44	4.75	3.902	No	1
P2-004	Ammonium nitrate	80.04	-	-	2B	1
P2-005	Potassium tetrafluoroborate	125.9	-	6.027	No	0
P2-006	3,4,4'-Trichlorocarbanilide	315.58	6.07	4.856	No	1
P2-007	1-Bromohexane	165.07	3.85	3.798	No	1
P2-008	4,4'-Methylenebis(2,6-di-tert-butylphenol)	424.66	8.97	9.862	No	0
P2-011	Sodium oxalate	134	-	-2.879	1	1
P2-012	2-Phospho-L-ascorbic acid trisodium salt	322.05	-	-2.407	No	0
P2-013	1-Bromo-4-chlorobutane	171.46	2.75	2.786	No	1
P2-014	Sodium hydrogensulfite	104.06	-	-2.279	No	1
P2-017	Propyl 4-hydroxybenzoate	180.2	2.88	2.693	No	1
P2-018	Ethyl 2,6-dichloro-5-fluoro-beta-oxo-3-pyridinepropionate	280.08	1.84	2.501	2B	1
P2-019	Camphene	136.23	4.24	4.131	2B	1
P3-002	Iso-octylthioglycolate	204.33	-	3.747	No	0
P3-003	Dipropyl disulfide	150.31	4.19	3.441	No	1
P3-004	1-Bromo-octane	193.12	4.87	4.899	No	1
P3-006	Dioctyl ether	242.44	7.15	7.334	No	1
P3-007	3-Phenoxybenzyl alcohol	200.23	3.39	3.022	No	1
P3-009	2-Ethylhexylthioglycolate	204.33	3.99	3.715	No	0
P3-010	n,n-Dimethylguanidine sulfate	272.33	-	-2.054	No	0
P3-011	6-Hydroxy-2,4,5-triaminopyrimidine Sulfate	239.21	-4.86	-2.103	No	1
P3-012	Polyethylene hydrogenated caster oil (40E.O.)	About 400		12	No	0
P3-013	2,2'-Methylene-bis-(6-(2Hbenzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol)	658.87	14.32		No	0
P3-014	Cellulose 2-(2-hydroxy-3-(trimethylammonio)propoxy) ethyl ether chloride	>257	-		No	0

P3-019	Diethyl toluamide	191.27	2.42	2.141	2B	1
P3-021	Sodium chloroacetate	116.48	-	-1.927	2B	1
P3-022	2,4,11,13-Tetraazatetra (Chlorohexidine glucocinate)	897.76	-		2A	1
P3-027	3-(2-Aminoethylamino)propyl]trimethoxysilane	222.36	-2.33	-1.582	1	1
P3-029	Dodecanoic acid	200.32	2.56	2.91	1	1
P3-034	1-Methylpropyl benzene	134.22	4.09	3.902	No	0
P3-035	4-(Methylmercapto)benzaldehyde	152.21	2.21	2.253	No	1
P3-036	1,9-Decaine	138.25	4.99	4.859	No	1
P3-038	1-Ethyl-3-methylimidazolium ethylsulfate	236.29	-		No	0
P3-039	1,2,4-Triazole,sodium salt	91.05	-		1	1
P3-040	4,4'-(4,5,6,7-Tetrabromo-1,1-dioxido-3H-2,1-benzoxathiole-3,3-diyl)bis[2,6-dibromophenol]	985.54	9.72	3.959	1	1
P3-041	Benzenamine,4,4'-(4-aimino-3-methylphenyl)(4-imino-3-methyl-2,5-cyclohexadien-1-ylidene)methyl-2-methy HCL	365.9	-		1	1
P3-042	1-(9H-Carbozol-4-yloxy)-3-[[2-(2-methoxy phenoxy)ethyl] amino]-2-propanol	406.47	2.69	2.893	No	1
P3-043	3-Methyl-1,5-di(2,4-xylyl)-1,3,5-Triazapenta-1,4-dien	293.41	5.59	4.237	No	1
P3-045	(3R,4R)-4-Acetoxy-3-[(R)-(tert-butyldimethylsilyloxy)ethyl]-2-azetidinone	287.43	2.37		2A	1
P3-046	1-Octanol	130.23	2.88	3.032	2A	1
P3-051	Myristyl alcohol	214.39	5.93	6.192	2A	1
P3-052	Hexyl cinnamic aldehyde	216.32	4.87	4.637	2B	1
P3-054	Monoethanolamine	61.08	-4.08	-2.929	2B	1
P3-057	Isopropyl myristate	270.45	7.25	7.568	No	0
P3-063	Isopropyl bromide	122.99	2.16	2.231	No	0
P3-070	Distearyldimethylammonium chloride	586.5	-	11.271	1	1

P3-073	Triton X-100 (5%)	324.41	-	4.645	2B	1
P3-074	2-Ethylhexyl p-dimethyl-amino benzoate	277.4	5.41	5.709	No	1
P3-075	Promethazine hydrochloride	320.88	-		1	1
P3-076	2-Ethyl-1-hexanol	130.23	2.72	2.788	2A	1
P3-081	3,3-Dimethylpentane	100.2	4.02	4.162	No	1
P3-082	Methyl cyclopentane	84.16	3.17	3.372	No	1
P3-083	Toluene	92.14	2.72	2.512	2B?	0
P3-085	Gluconolactone	178.14	-3.47	-2.32	No	0
P3-086	Methyl amyl ketone (2-heptanol)	114.19	2	2.032	No	0
P3-089	Glycerol	92.09	-1.85	-1.883	No	0
P3-090	Cetylpyridinium bromide	384.44	-	3.228	1	1
P3-091	Triton X-100	324.41	-	4.645	1	1
P3-092	Tween20	346.46	-	5.237	No	1
P3-093	Sodium hydroxide	40	-	-3.842	1	1
P3-096	Sucrose fatty acid ester	>342.3	-		2A?	1

Table 5: Predictive capacity of the SIRC-CVS test method when excluding test chemicals with either an acid dissociation constant (pKa) of 4 or less or a distribution coefficient (log D) of greater than -1.5 and less than 2 are excluded from the applicability domain

N=61	+ (SIRC-CVS)	− (SIRC-CVS)
+ (in vivo) GHS 1,2A, 2B	25 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 P3-022 2,4,11,13-Tetraazatetra (Chlorohexidine glucocinate) P3-027 3-(2-Aminoethylamino)propyl]trimethoxysilane P3-029 Dodecanoic acid P3-039 1,2,4-Triazole,sodium salt P3-040 4,4'-(4,5,6,7-Tetrabromo-1,1-dioxido-3H-2,1 -benzoxathiole-3,3-diyl)bis[2,6-dibromophenol] P3-041 Benzenamine,4,4'-(4-aimino-3-methylphenyl) (4-imino-3-methyl-2,5-cyclohexadien-1-ylidene)methyl-2-methy HCL P3-045 (3R,4R)-4-Acetoxy-3-[(R)-(tert -butyldimethylsilyloxy)ethyl]-2-azetidinone P3-046 1-Octanol P3-051 Myristyl alcohol P3-052 Hexyl cinnamic aldehyde P3-054 Monoethanolamine P3-070 Distearyldimethylammonium chloride P3-073 Triton X-100 (5%) P3-075 Promethazine hydrochloride P3-076 2-Ethyl-1-hexanol P3-090 Cetylpyridinium bromide P3-091 Triton X-100 P3-093 Sodium hydroxide P3-096 Sucrose fatty acid ester	1 P3-083 Toluene

– (in vivo)	19	16
GHS NC	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	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 P3-012 Polyethylene hydrogenated caster oil (40E.O.) P3-013 2,2'-Methylene-bis-(6-(2H benzotriazol-2-yl) -4- (1,1,3,3-tetramethylbutyl) phenol) P3-014 Cellulose 2-(2-hydroxy-3-(trimethylammo nio)propoxy) ethyl ether chloride P3-034 1-Methylpropyl benzene P3-038 1-Ethyl-3-methylimidazolium ethylsulfate P3-057 Isopropyl myristate P3-063 Isopropyl bromide P3-085 Gluconolactone P3-086 Methyl amyl ketone (2-heptanol) P3-089 Glycerol

1) According to in vivo data, toluene is either Category 2B or No Category under UN GHS.