

## Appendix 8.5

### Report on the selection of test substances for SIRC-CVS:TEA test validation study

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This report describes the selection process for test substances used in the SIRC-CVS:TEA test validation study.

The objective of this study was to evaluate the within- and between-laboratory reproducibility and predictive capacity of the SIRC-CVS:TEA test on eye irritation (consistency with the two categories, Irritant and Non-irritant) as the initial step in a bottom-up approach.

In a complementary study, the validation management team (VMT) evaluated predictive capacity for the Category 1, Category 2, and Non-irritant classifications of the United Nations Globally Harmonized System of Classification and Labeling of Chemicals (UN GHS) as well as four classifications used by the United States Environmental Protection Agency (EPA).

To this end, phase II-A, phase II-B and phase III studies were conducted by three laboratories using the test substances as shown in Table 1. These test substances were selected by the VMT without any participation by delegates from the three laboratories.

In addition, the list of these test substances included chemical categories or physical and chemical properties (molecular weight, solubility in the medium, etc.) to facilitate study of an optimal applicable domain.

Table 1: Breakdown of the SIRC-CVS:TEA test validation study

Phase	No. of test substances	No. of repetitions	Subject
II-A	5	3	Within- and between- laboratory reproducibility
II-B	15	3	
III	100 (Including common test substances)	1	Between- laboratory reproducibility and predictability

#### 1. Phase II study

In the Phase II study, the twenty test substances shown in Table 1 were selected by the VMT for use in assessing within- and between-laboratory reproducibility. Selections were made from the following lists with an eye toward maintaining a balance between UN GHS or EPA labeling and solid or liquid.

- Extant individual animal data for test substances were available for classifying the eye irritating hazard under UN GHS.
- Test substances had already been evaluated in other *in vitro* eye irritation tests.

Twenty test substances comprising 10 irritants and 10 non-irritants are listed in Table 2. To assess within- and between-laboratory reproducibility, the VMT distributed 3 sets of each coded test substance to each laboratory. The three sets were tested separately, but the order in which they were tested was considered immaterial. The VMT distributed 15 coded test substances (5 different test substances) in the phase II-A study and 45 coded test substances (15 different test substances) in the phase II-B study to each laboratory.

Table 2-1 : List of the 20 substances selected for phase II in SIRC-CSV:TEA test validation study

## Phase II-A study

No.	Test substance	CAS No.	Solid/ Liquid	Supplier	Storage	Lab. Code			GHS	EPA
						SA	SB	SC		
						Nihon Kolmar	Bozo	Biototech		
1	piperonylbutoxide	51-03-6	Liquid	Sigma Aldrich	rt	SA008	SB010	SC011	No	III
						SA013	SB001	SC004		
						SA002	SB009	SC006		
2	2,5-dimethylhexaediol	110-03-2	Solid	Sigma Aldrich	rt	SA001	SB005	SC010	I	I
						SA010	SB013	SC002		
						SA015	SB003	SC015		
3	1-(2-propoxy-1-methylet hoxo)-2-propanol	29911-27-1	Liquid	Sigma Aldrich	rt	SA005	SB015	SC008	2B	III
						SA012	SB007	SC003		
						SA007	SB012	SC014		
4	ammonium nitrate	6484-52-2	Solid	Sigma Aldrich	rt	SA004	SB002	SC007	2B	III
						SA011	SB006	SC013		
						SA009	SB014	SC005		
5	potassium tetrafluoroborate	14075-53-7	Solid	Sigma Aldrich	rt	SA014	SB011	SC009	No	IV
						SA003	SB004	SC012		
						SA006	SB008	SC001		

rt: room temp.

Set1

Set 2

Set 3

Table 2-2: List of the 20 substances selected for phase II in SIRC-CSV:TEA test validation study

## Phase II-B study

No.	Test substance	CAS No.	Solid/ Liquid	Supplier	Storage	Lab. Code			GHS	EPA
						SA	SB	SC		
						Nihon Kolmar	Bozo	Biotoxtech		
6	3,4,4'-trichlorocarbaniide	101-20-2	Solid	Sigma Aldrich	rt	SA049	SB017	SC031	No	IV
						SA029	SB054	SC021		
						SA057	SB060	SC043		
7	1-bromohexane	111-25-1	Liquid	Sigma Aldrich	rt	SA016	SB029	SC042	No	IV
						SA034	SB043	SC055		
						SA039	SB053	SC047		
8	4,4'-methylenebis (2,6-di-tert-butylphenol)	118-82-1	Solid	Sigma Aldrich	rt	SA022	SB057	SC016	No	IV
						SA048	SB037	SC030		
						SA028	SB044	SC053		
9	propylene glycol propyl ether	1569-01-3	Liquid	Sigma Aldrich	rt	SA038	SB028	SC033	2A	II
						SA040	SB042	SC054		
						SA017	SB031	SC041		
10	ethyl thioglycolate	623-51-8	Liquid	Sigma Aldrich	rt	SA035	SB055	SC017	No	III
						SA052	SB018	SC032		
						SA027	SB027	SC046		
11	sodium oxalate	62-76-0	Solid	Sigma Aldrich	rt	SA030	SB038	SC023	1	I
						SA050	SB020	SC029		
						SA026	SB045	SC040		
12	2-phospho-L-ascorbic acid trisodium salt	66170-10-3	Solid	Sigma Aldrich	rt	SA018	SB034	SC022	No	III
						SA045	SB052	SC034		
						SA031	SB030	SC044		
13	1-bromo-4-chlorobutane	6940-78-9	Liquid	Sigma Aldrich	rt	SA046	SB016	SC039	No	IV
						SA025	SB032	SC020		
						SA044	SB023	SC058		
14	sodium hydrogensulfite	7631-90-5	Solid	Sigma Aldrich	rt	SA019	SB041	SC024	No	III
						SA037	SB046	SC045		
						SA032	SB019	SC048		
15	isobutyraldehyde	78-84-2	Liquid	Sigma Aldrich	4°C	SA036	SB056	SC025	2B	III
						SA033	SB022	SC035		
						SA021	SB050	SC038		
16	1-naphthaleneacetic acid	86-87-3	Solid	Wako Pure Chemicals	rt	SA041	SB024	SC056	1	I
						SA053	SB021	SC026		
						SA024	SB047	SC049		
17	propyl 4-hydroxybenzoate	94-13-3	Solid	Sigma Aldrich	rt	SA054	SB039	SC057	No	III
						SA020	SB026	SC060		
						SA059	SB051	SC018		
18	ethyl 2,6-dichloro- 5-fluoro-beta-oxo-3- pyridinepropionate	96568-04-6	Solid	Sigma Aldrich	rt	SA051	SB035	SC036	2B	III
						SA023	SB059	SC050		
						SA056	SB048	SC059		
19	camphene	79-92-5	Solid	Sigma Aldrich	rt	SA060	SB040	SC027	2B	III
						SA042	SB033	SC052		
						SA058	SB049	SC051		
20	cyclopentanol	96-41-3	Liquid	Sigma Aldrich	rt	SA047	SB058	SC028	2B	II
						SA055	SB025	SC037		
						SA043	SB036	SC019		

rt: room temp.

Set 1

Set 2

Set 3

## 2. Phase III study

According to the objective in the study plan, the 120 coded test substances (100 different test substances) were prepared to evaluate the predictability and to confirm between-laboratory reproducibility of SIRC-CVS:TEA validation studies. The 40 coded test substances (forty different test substances) were distributed to each laboratory for the Phase III validation study. Of the 40 test substances, the ten substances in Table 3 were used as common test substances. Therefore, a total of 100 test substances were tested to evaluate the predictive capacity in the Phase III study.

Of these 100 test substances, nearly 60 had been used in validation studies of a three-dimensional corneal model (such as EpiOcular) by the European Union Reference Laboratory for Alternatives to Animal Testing (EURL-ECVAM)<sup>1)</sup> and nearly 60 had been used in the Short Time Exposure (STE) test validation study by the Japanese Center for the Validation of Alternative Methods (JaCVAM) and Independent peer review by Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)<sup>2,3,4)</sup>.

In the Phase III study, all test substances were selected from the following lists with an eye toward maintaining a balance between UN GHS or EPA labeling and solid or liquid.

- Extant individual animal data for test substances were available for classifying the eye irritating hazard under UN GHS and EPA.
- A uniform balance between solids and liquids.
- Test substances had already been evaluated in other *in vitro* eye irritation tests.
- Test substances represented a variety of categories such as alcohol, ester, ketone, surfactant and so on.

Table 3 :List of 100 test substances selected for phase III in SIRC-CVS:TEA test validation study

No.	Test substance	CAS No.	Solid/ Liquid	Supplier	Lab. Code	GHS	EPA	Source
1#	2-ethoxyethyl methacrylate	2370-63-0	Liquid	Sigma Aldrich	SB062	No	IV	ECETOC
2	iso-octylthioglycolate	25103-09-7	Liquid	Wako Pure	SC072	No	IV	ECETOX
3#	dipropyl disulfide	629-19-6	Liquid	Sigma Aldrich	SA082 SB079 SC061	No	IV	STE review
4	1-bromo-octane	111-83-1	Liquid	Sigma Aldrich	No	IV	STE review	Halogen compound
5#	2-(2-ethoxyethoxy)ethanol	111-90-0	Liquid	Sigma Aldrich	SA089, SB072 SC062	No	III	Cosing
6	dioctyl ether	629-82-3	Liquid	Sigma Aldrich	SC077	No	IV	Cognis
7	3-phenoxybenzyl alcohol	13826-35-2	Liquid	Sigma Aldrich	SC079	No	III	NICEATM
8	glycidyl methacrylate	106-91-2	Liquid	Sigma Aldrich	SB063	No	III	STE review
9	2-ethylhexylthioglycolate	7659-86-1	Liquid	Sigma Aldrich	SC080	No	IV	ECETOC
10#	n,n-dimethylguanidine sulfate	598-65-2	Solid	Sigma Aldrich	SA090, SB071, SC063	No	III	STE review
11	6-hydroxy-2,4,5-triaminopyrimidine sulfate	1603-02-7	Solid	Wako Pure	SC081	No	IV	Cosing
12#	polyethylene hydrogenated castor oil (40E.O.)	61788-85-0	Solid	Sigma Aldrich	SA084 SB077 SC064	No	IV	STE review
13	2,2'-methylenebis-(6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol)	103597-45-1	Solid	Sigma Aldrich	SC082	No	IV	Ciba
14	cellulose, 2-(2-hydroxy-3-(trimethylammonio)propoxy) ethyl ether chloride	68610-92-4	Solid	Sigma Aldrich	SC083	No	III	J&J
15	3,4-dimethoxy benzaldehyde	120-14-9	Solid	Sigma Aldrich	SC084	No	III	NICEATM
16	3-chloropropionitrile	542-76-7	Liquid	Wako Pure	SC085	2B	III	ECETOC
17	2-methyl-1-pentanol	105-30-6	Liquid	Sigma Aldrich	SC087	2B	III	STE review
18	ethyl-2-methylacetoacetate	609-14-3	Liquid	Sigma Aldrich	SC088	2B	III	STE review
19#	diethyl toluamide	134-62-3	Liquid	Sigma Aldrich	SA088 SB073, SC065	2B	III	US-EPA
20#	4-nitrobenzoic acid	62-23-7	Solid	Sigma Aldrich	SA083 SB078, SC066	2B	III	NICEATM
21	sodium chloroacetate	3926-62-3	Solid	Sigma Aldrich	SC090	2B	III	STE review
22	2,4,11,13-tetraazatetra (chlorohexidine glucocinate)	18472-51-0	Liquid	Wako Pure	SA061	2A	II	NICEATM
23	-	-	-	-	-	-	-	-

24#	2-amino-3-hydroxy pyridine	16867-03-1	Solid	Sigma Aldrich	SA086, SB075, SC068	2A	III	Cosing
25	sodium benzoate	532-32-1	Solid	Sigma Aldrich	SC092	2A	II	Cosing
26	methylthioglycolate	2365-48-2	Liquid	Sigma Aldrich	SC093	1	II	ECETOC
27	3-(2-aminoethylamino)propyl]trimethoxysilane	1760-24-3	Liquid	Chemos	SA096	1	I	Evonik
28#	tetraethylene glycol	17831-71-9	Liquid	Sigma Aldrich	SA085 SB076 SC069	1	I	TSCA
29#	dodecanoic acid	143-07-7	Solid	Sigma Aldrich	SA087, SB074, SC070	1	I	ECETOC
30	1,2-benzisothiazol-3(2H)-one	2634-33-5	Solid	Wako Pure	SC097	1	I	Cosing
31	2-hydroxy-1,4-naphthoquinone	83-72-7	Solid	Sigma Aldrich	SC089	2B	III	Cosing
32	disodium 4,4'-bis(2-sulfonatostyryl)biphenyl	27344-41-8	Solid	Wako Pure	SC098	1	II	Ciba
33#	gamma-butyrolactone	96-48-0	Liquid	Sigma Aldrich	SA081, SB080, SC067	2A	II	STE review
34	1-methylpropyl benzene	135-98-8	Liquid	Wako Pure	SC071	No	IV	STE review
35	4-(methylmercapto)benzaldehyde	3446-89-7	Liquid	Sigma Aldrich	SC073	No	IV	ECETOX
36	1,9-decaine	1647-16-1	Liquid	Sigma Aldrich	SC075	No	IV	STE review
37	2,4-dimethyl-3-pentanol	3970-62-5	Liquid	Sigma Aldrich	SC076	No	III	STE review
38	1-ethyl-3-methylimidazolium ethylsulfate	342573-75-5	Liquid	AlfaAesar	SC078	No	III	Evonik
39	1,2,4-triazole,sodium salt	41253-21-8	Solid	Sigma Aldrich	SC095	1	I	ECETOC
40	4,4'-(4,5,6,7-tetrabromo-1,1-dioxido-3H-2,1-benzoxathiole-3,3-diyl)bis[2,6-dibromophenol]	4430-25-5	Solid	Sigma Aldrich	SC096	1	I	Coising
41	benzenamine,4,4'-(4-aimino-3-methylphenyl)(4-imino-3-methyl-2,5-cyclohexadien-1-ylidene)methyl-2-methyl HCl	3248-91-7	Solid	Sigma Aldrich	SC099	1	I	Cosing
42	1-(9H-carbozol-4-yloxy)-3-[[2-(2-methoxy phenoxy)ethyl] amino]-2-propanol	72956-09-3	Solid	LKT.Labs,Inc	SA062	No	IV	Glaxo
43	3-methyl-1,5-di(2,4-xylyl)-1,3,5-triazapenta-1,4-dien	33089-61-1	Solid	LKT.Labs,Inc	SB061	No	IV	US-EPA
44	isopropyl acetoacetate	542-08-5	Liquid	Wako Pure	SC086	2B	III	NICEATM
45	(3R,4R)-4-acetoxy-3-[(R)-(tert-butyldimethylsilyloxy)ethyl]-2-azetidinone	76855-69-1	Solid	Sigma Aldrich	SA063	2A	II	Glaxo
46	1-octanol	111-87-5	Liquid	Wako Pure	SB064	2A	II	STE review
47	2-benzyloxyethanol	622-08-2	Liquid	Wako Pure	SB065	2A	II	STE review
48	butanol	71-36-3	Liquid	Wako Pure	SB066	1	I	STE review
49	isobutyl alcohol	78-83-1	Liquid	Sigma Aldrich	SB067	1	I	STE review
50	isopropyl alcohol	67-63-0	Liquid	Wako Pure	SB068	2A	III	STE review
51	myristyl alcohol	112-72-1	Solid	Wako Pure	SB069	2A	III	STE review
52	hexyl cinnamic aldehyde	101-86-0	Liquid	Wako Pure	SB070	2B	II	STE review

53	n-butanal	123-72-8	Liquid	Wako Pure	SB081	2B	III	STE review
54	monoethanolamine	141-43-5	Liquid	Sigma Aldrich	SB082	2B	III	NICEATM
55	m-phenylenediamine	108-45-2	Solid	TCI	SB083	1	I	STE review
56	ethyl acetate	141-78-6	Liquid	Sigma Aldrich	SB084	No	III	STE review
57	isopropyl myristate	110-27-0	Liquid	Wako Pure	SB085	No	IV	STE review
58	methoxyethyl acrylate	3121-61-7	Liquid	Wako Pure	SB086	1	>II	STE review
59	methyl acetate	79-20-9	Liquid	Sigma Aldrich	SB087	2A	II	STE review
60	methyl cyanoacetate	105-34-0	Liquid	Sigma Aldrich	SB088	2A	II	STE review
61	imidazole	288-32-4	Solid	Sigma Aldrich	SB089	1	I	STE review
62	pyridine	110-86-1	Liquid	Sigma Aldrich	SB090	1	I	STE review
63	isopropyl bromide	75-26-3	Liquid	Wako Pure	SB091	No	IV	STE review
64	cyclohexanone	108-94-1	Liquid	Sigma Aldrich	SB092	No	III	STE review
65	2-methylbutyric acid	116-53-0	Liquid	Sigma Aldrich	SB093	1	I	STE review
66	calcium thioglycollate trihydrate	5793-98-6	Solid	TCI	SB094	1	I	Ohno(1999)
67	citric acid	77-92-9	Solid	Sigma Aldrich	SB095	2A?	II?	Kojima (2013)
68	potassium sorbate	24634-61-5	Solid	Sigma Aldrich	SB096	2A?	II?	Kojima (2013)
69	sodium salicylate	54-21-7	Solid	Wako Pure	SB097	1	I	STE review
70	distearyldimethylammonium chloride	107-64-2	Solid	TCI	SB098	1	I	STE review
71	n-lauroylsarcosine sodium salt	137-16-6	Solid	Wako Pure	SB099	2B	III	NICEATM
72	sodium lauryl sulfate	151-21-3	Solid	Wako Pure	SB100	2A?	III	STE review
73	triton X-100 (5%)	9002-93-1	Liquid	Sigma Aldrich	SA065	2B	III	NICEATM
74	2-ethylhexyl p-dimethyl-amino benzoate	21245-02-3	Liquid	Wako Pure	SA076	No	IV	STE review
75	promethazine hydrochloride	58-33-3	Solid	Sigma Aldrich	SA064	1	I	STE review
76	2-ethyl-1-hexanol	104-76-7	Liquid	Wako Pure	SA067	2A	II	STE review
77	3-methoxy-1,2-propanediol	623-39-2	Liquid	TCI	SA080	No	IV	STE review
78	cyclohexanol	108-93-0	Liquid	Sigma Aldrich	SA070	1	I	STE review
79	ethanol	64-17-5	Liquid	Wako Pure	SA091	2A	I	STE review
80	n-hexanol	111-27-3	Liquid	Sigma Aldrich	SA072	2A	II	STE review
81	3,3-dimethylpentane	562-49-2	Liquid	Sigma Aldrich	SA078	No	IV	STE review
82	methyl cyclopentane	96-37-7	Liquid	TCI	SA098	No	III	STE review
83	toluene	108-88-3	Liquid	Wako Pure	SA069	2B?	III	STE review
84	acetone	67-64-1	Liquid	Sigma Aldrich	SA092	2A	II	STE review
85	gluconolactone	90-80-2	Solid	Wako Pure	SA097	No	IV	NICEATM
86	methyl amyl ketone (2-heptanol)	110-43-0	Liquid	Wako Pure	SA071	No	III	STE review
87	methyl ethyl ketone (2-butanone)	78-93-3	Liquid	TCI	SA094	2A	III	STE review
88	methyl isobutyl ketone(4-methyl 2-pentanol)	108-10-1	Liquid	Sigma Aldrich	SA068	No	III	STE review
89	glycerol	56-81-5	Liquid	Wako Pure	SA079	No	IV	STE review
90	cetylpyridinium bromide	140-72-7	Solid	Sigma Aldrich	SA075	1	I	STE review



91	triton X-100	9002-93-1	Liquid	Sigma Aldrich	SC094	1	I	STE review
92	tween20	9005-64-5	Liquid	Sigma Aldrich	SC100	No	III	STE review
93	sodium hydroxide	1310-73-2	Solid	Wako Pure	SA074	1	I	STE review
94	glycolic acid	79-14-1	Solid	Sigma Aldrich	SA095	2B	III	NICEATM
95	3,3-dithiodipropionic acid	1119-62-6	Solid	Wako Pure	SC091 SA073	2B	II	NICEATM
96	sucrose fatty acid ester	Non	Solid	TCI	SA100	2A?	II?	STE review
97	methyl para-Hydroxybenzoate	99-76-3	Solid	Wako Pure	SA099	2 ?	II?	Ohno(1999)
98	silic acid, dehydrogate	7699-41-4	Solid	Wako Pure	SA093	No	IV	Ohno(1999)
99	benzyl alcohol	100-51-6	Liquid	Sigma Aldrich	SA066	1	I	STE review
100	lactic acid	50-21-5	Liquid	Wako Pure	SA077	1	I	STE review

#: Ten test substances (No.1-No.10) distributed to three participated laboratories as common test substances.

\$: Tow test substances (No.35 and No.38) could not confirmed individual animal data

&: One test substance (No.60) duplicated.

### 3. Information on test substances selected for SIRC-CVS:TEA test validation study

The 116 test substances listed in Table 4 were used to analyze the predictive capacity of the SIRC-CVA:TEA test. These include the 20 test substances used in the Phase II study plus the 100 test substances used in the Phase III study. Of these 120 test substances, 3,3-dithiodipropionic acid was included twice by JaCVAM, so the duplication was excluded from the analysis. Citric acid and potassium sorbate did not have a clear source for individual animal data and were excluded from the analysis. A clear source for individual animal data was identified for the remaining 117 test substances in association of the NICEATM.

The UN GHS or EPA classifications of the 117 test substances selected for the validation study are shown in Table 5. The VMT considered this a reasonable balance of test substances. The ratio of solids to liquids for the 117 test substances selected for the validation study are shown in Table 6. This information may be useful in determining an optimal applicability domain for this assay.

Table 4: List of test substances used in SIRC-CVS:TEA test validation study

NO.	Code No.	Test substance	CAS	Solid: Liquid	Supplier	GHS	EPA	Source	Final chemical class
001	P2-016	1-naphthaleneacetic acid	86-87-3	Solid	Wako Pure	1	I	ECETOC	Carboxylic acid, Polycyclic compound
002	P3-030	1,2-benzisothiazol-3(2H)-one	2634-33-5	Solid	Wako Pure	1	I	Cosing	Heterocyclic compound, Thio compound, Amide
003	P3-039	1,2,4-triazole, sodium salt	41253-21-8	Solid	Sigma Aldrich	1	I	ECETOC	Heterocyclic compound
005	P3-065	2-methylbutyric acid	116-53-0	Liquid	Sigma Aldrich	1	I	STE review	Carboxylic acid
004	P2-002	2,5-dimethylhexaediol	110-03-2	Solid	Sigma Aldrich	1	I	STE review	Alcohol
006	P3-027	3-(2-aminoethylamino)propyl]trimethoxysilane	1760-24-3	Liquid	Chemos	1	I	Evonik	Silicon compound
007	P3-040	4,4'-(4,5,6,7-tetrabromo-1,1-dioxido-3H-2,1-benzoxathiole-3,3-diyl)bis[2,6-dibromophenol]	4430-25-5	Solid	Sigma Aldrich	1	I	Coising	Halogen compound, Phenol, Sulfonic acid
008	P3-041	benzenamine, 4,4'-(4-amino-3-methylphenyl)(4-imino-3-methyl-2,5-cyclohexadien-1-ylidene)methyl-2-methyl HCl	3248-91-7	Solid	Sigma Aldrich	1	I	Cosing	Organic salt
009	P3-099	benzyl alcohol	100-51-6	Liquid	Sigma Aldrich	1	I	STE review	Alcohol
010	P3-048	butanol	71-36-3	Liquid	Wako Pure	1	I	STE review	Alcohol
011	P3-066	calcium thioglycollate trihydrate	5793-98-6	Solid	TCI	1	I	STE review	Thio compound, Organic salt
012	P3-090	cetylpyridinium bromide	140-72-7	Solid	Sigma Aldrich	1	I	STE review	Surfactant (cationic)
013	P3-078	cyclohexanol	108-93-0	Liquid	Sigma Aldrich	1	I	STE review	Alcohol
014	P3-032	disodium 4,4'-bis(2-sulfonatostyryl)biphenyl	27344-41-8	Solid	Wako Pure	1	II	Ciba	Sulfonic acid
015	P3-070	distearyldimethylammonium chloride	107-64-2	Solid	TCI	1	I	STE review	Quaternary ammonium compound, Surfactant
016	P3-029	dodecanoic acid	143-07-7	Solid	Sigma Aldrich	1	I	ECETOC	Fatty acid
017	P3-061	imidazole	288-32-4	Solid	Sigma Aldrich	1	I	STE review	Heterocyclic compound, Amine
018	P3-049	isobutyl alcohol	78-83-1	Liquid	Sigma Aldrich	1	I	STE review	Alcohol
019	P3-100	lactic acid	50-21-5	Liquid	Wako Pure	1	I	STE review	Carboxylic acid
020	P3-058	methoxyethyl acrylate	3121-61-7	Liquid	Wako Pure	1	>II	STE review	Acrylate, Ether, Ester
021	P3-026	methylthioglycolate	2365-48-2	Liquid	Sigma Aldrich	1	II	ECETOC	Thio compound, Ester
022	P3-055	m-phenylenediamine	108-45-2	Solid	TCI	1	I	STE review	Amine
023	P3-075	promethazine hydrochloride	58-33-3	Solid	Sigma Aldrich	1	I	STE review	Heterocyclic compound, Organic salt
024	P3-062	pyridine	110-86-1	Liquid	Sigma Aldrich	1	I	STE review	Heterocyclic compound
025	P3-093	sodium hydroxide	1310-73-2	Solid	Wako Pure	1	I	STE review	Alkali
026	P2-011	sodium oxalate	62-76-0	Solid	Sigma Aldrich	1	I	ECETOC	Organic salt
027	P3-069	sodium salicylate	54-21-7	Solid	Wako Pure	1	I	STE review	Organic salt, Phenol
028	P3-028	tetraethylene glycol	17831-71-9	Liquid	Sigma Aldrich	1	I	TSCA	Acrylate, Ether, Ester
029	P3-091	triton X-100	9002-93-1	Liquid	Sigma Aldrich	1	I	STE review	Surfactant (nonionic)
030	P3-046	1-octanol	111-87-5	Liquid	Wako Pure	2A	II	STE review	Fatty alcohol

031	P3-024	2-amino-3-hydroxy pyridine	16867-03-1	Solid	Sigma Aldrich	2A	III	Cosing	Heterocyclic compound, Amine
032	P3-047	2-benzyloxyethanol	622-08-2	Liquid	Wako Pure	2A	II	STE review	Alcohol, Ether
033	P3-076	2-ethyl-1-hexanol	104-76-7	Liquid	Wako Pure	2A	II	STE review	Fatty alcohol
034	P3-022	2,4,11,13-tetraazatetra (chlorohexidine glucocinate)	18472-51-0	Liquid	Wako Pure	2A	II	NICEATM	Organic salt, Halogen Compound
035	P3-045	(3R,4R)-4-acetoxy-3-[(R)-(tert-butylidimethylsilyloxy)ethyl]-2-azetidinone	76855-69-1	Solid	Sigma Aldrich	2A	II	Glaxo	Silicon compound
036	P3-084	acetone	67-64-1	Liquid	Sigma Aldrich	2A	II	STE review	Ketone
037	P3-079	ethanol	64-17-5	Liquid	Wako Pure	2A	I	STE review	Alcohol
038	P3-033	gamma-butyrolactone	96-48-0	Liquid	Sigma Aldrich	2A	II	STE review	Heterocyclic compound, Ketone
039	P3-050	isopropyl alcohol	67-63-0	Liquid	Wako Pure	2A	III	STE review	Alcohol
040	P3-059	methyl acetate	79-20-9	Liquid	Sigma Aldrich	2A	II	STE review	Ester
041	P3-060	methyl cyanoacetate	105-34-0	Liquid	Sigma Aldrich	2A	II	STE review	Ester, Nitrile compound
042	P3-087	methyl ethyl ketone (2-butanone)	78-93-3	Liquid	TCI	2A	III	STE review	Ketone
043	P3-097	methyl para-Hydroxybenzoate	99-76-3	Solid	Wako Pure	2?	II?	Ohno(1999)	Ester, Phenol
044	P3-051	myristyl alcohol	112-72-1	Solid	Wako Pure	2A	III	STE review	Fatty alcohol
045	P3-080	n-hexanol	111-27-3	Liquid	Sigma Aldrich	2A	II	STE review	Alcohol
046	P2-009	propylene glycol propyl ether	1569-01-3	Liquid	Sigma Aldrich	2A	II	NICEATM	Alcohol, Ether
047	P3-025	sodium benzoate	532-32-1	Solid	Sigma Aldrich	2A	II	Cosing	Organic salt
048	P3-072	sodium lauryl sulfate	151-21-3	Solid	Wako Pure	2A?	III	STE review	Surfactant (anionic)
049	P3-096	sucrose fatty acid ester	Non	Solid	TCI	2A?	II?	STE review	Polyol, Ester
050	P2-003	1-(2-propoxy-1-methylethoxy)-2-propanol	29911-27-1	Liquid	Sigma Aldrich	2B	III	STE review	Alcohol, Ether
051	P3-031	2-hydroxy-1,4-naphthoquinone	83-72-7	Solid	Sigma Aldrich	2B	III	Cosing	Phenol compound
052	P3-017	2-methyl-1-pentanol	105-30-6	Liquid	Sigma Aldrich	2B	III	STE review	Fatty alcohol
053	P3-016	3-chloropropionitrile	542-76-7	Liquid	Wako Pure	2B	III	ECETOC	Halogen compound, Nitrile compound
054	P3-023, P3-095	3,3-dithiodipropionic acid	1119-62-6	Solid	Wako Pure	2B	II	NICEATM	Carboxylic acid, Thio compound
055	P3-020	4-nitrobenzoic acid	62-23-7	Solid	Sigma Aldrich	2B	III	NICEATM	Carboxylic acid
056	P2-004	ammonium nitrate	6484-52-2	Solid	Sigma Aldrich	2B	III	NICEATM	Inorganic salt
057	P2-019	camphene	79-92-5	Solid	Sigma Aldrich	2B	III	STE review	Hydrocarbon
058	P2-020	cyclopentanol	96-41-3	Liquid	Sigma Aldrich	2B	II	ECETOC	Alcohol
059	P3-019	diethyl toluamide	134-62-3	Liquid	Sigma Aldrich	2B	III	US-EPA	Amide
060	P3-018	ethyl-2-methylacetoacetate	609-14-3	Liquid	Sigma Aldrich	2B	III	STE review	Ester, Ketone
061	P2-018	ethyl 2,6-dichloro-5-fluoro-beta-oxo-3-pyridinepropionate	96568-04-6	Solid	Sigma Aldrich	2B	III	NICEATM	Halogen compound, Heterocyclic compound, Ester, Ketone
062	P3-094	glycolic acid	79-14-1	Solid	Sigma Aldrich	2B	III	NIEATM	Carboxylic acid
063	P3-052	hexyl cinnamic aldehyde	101-86-0	Liquid	Wako Pure	2B	II	STE review	Aldehyde

064	P2-015	isobutyraldehyde	78-84-2	Liquid	Sigma Aldrich	2B	III	STE review	Aldehyde
065	P3-044	isopropyl acetoacetate	542-08-5	Liquid	Wako Pure	2B	III	NICEATM	Ester, Ketone
066	P3-054	monoethanolamine	141-43-5	Liquid	Sigma Aldrich	2B	III	NICEATM	Alkanolamine
067	P3-053	n-butanal	123-72-8	Liquid	Wako Pure	2B	III	STE review	Aldehyde
068	P3-071	n-lauroylsarcosine sodium salt	137-16-6	Solid	Wako Pure	2B	III	NICEATM	Surfactant (anionic)
069	P3-021	sodium chloroacetate	3926-62-3	Solid	Sigma Aldrich	2B	III	STE review	Organic salt, Halogen compound
070	P3-073	triton X-100 (5%)	9002-93-1	Liquid	Sigma Aldrich	2B	III	NICEATM	Surfactant (nonionic)
071	P3-083	toluene	108-88-3	Liquid	Wako Pure	2B?	III	STE review	Hydrocarbon (aromatic)
072	P3-042	1-(9H-carbozol-4-yloxy)-3-[[2-(2-methoxy phenoxy)ethyl] amino]-2-propanol	72956-09-3	Solid	LKT.Labs, Inc	No	IV	Glaxo	Polycyclic compound, Alcohol
073	P2-013	1-bromo-4-chlorobutane	6940-78-9	Liquid	Sigma Aldrich	No	IV	STE review	Halogen compound
074	P2-007	1-bromohexane	111-25-1	Liquid	Sigma Aldrich	No	IV	STE review	Halogen compound
075	P3-004	1-bromo-octane	111-83-1	Liquid	Sigma Aldrich	No	IV	STE review	Halogen compound
076	P3-038	1-ethyl-3-methylimidazolium ethylsulfate	342573-75-5	Liquid	AlfaAesar	No	III	Evonik	Heterocyclic compound, Inorganic salt
077	P3-034	1-methylpropyl benzene	135-98-8	Liquid	Wako Pure	No	IV	STE review	Hydrocarbon(aromatic)
078	P3-036	1,9-decaine	1647-16-1	Liquid	Sigma Aldrich	No	IV	STE review	Alkene
079	P3-001	2-ethoxyethyl methacrylate	2370-63-0	Liquid	Sigma Aldrich	No	IV	ECETOC	Methacrylate, Ester, Ether
080	P3-074	2-ethylhexyl p-dimethyl-amino benzoate	21245-02-3	Liquid	Wako Pure	No	IV	STE review	PABA derivative
081	P3-009	2-ethylhexylthioglycolate	7659-86-1	Liquid	Sigma Aldrich	No	IV	ECETOC	Thiol compound, Ester
082	P2-012	2-phospho-L-ascorbic acid trisodium salt	66170-10-3	Solid	Sigma Aldrich	No	III	BASF	Heterocyclic compound, Organic salt, Phosphorus compound
083	P3-005	2-(2-ethoxyethoxy)ethanol	111-90-0	Liquid	Sigma Aldrich	No	III	Cosing	Alcohol, Ether
084	P3-013	2,2'-methylenebis-(6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol)	103597-45-1	Solid	Sigma Aldrich	No	IV	Ciba	Phenol compound, Heterocyclic compound
085	P3-037	2,4-dimethyl-3-pentanol	3970-62-5	Liquid	Sigma Aldrich	No	III	STE review	Fatty alcohol
086	P3-077	3-methoxy-1,2-propanediol	623-39-2	Liquid	TCI	No	IV	STE review	Alcohol, Ether
087	P3-043	3-methyl-1,5-di(2,4-xylyl)-1,3,5-triazapenta-1,4-dien	33089-61-1	Solid	LKT.Labs, Inc	No	IV	US-EPA	Triazapentadien compound
088	P3-007	3-phenoxybenzyl alcohol	13826-35-2	Liquid	Sigma Aldrich	No	III	NICEATM	Alcohol
089	P3-081	3,3-dimethylpentane	562-49-2	Liquid	Sigma Aldrich	No	IV	STE review	Hydrocarbon
090	P3-015	3,4-dimethoxy benzaldehyde	120-14-9	Solid	Sigma Aldrich	No	III	NICEATM	Aldehyde
091	P2-006	3,4,4'-trichlorocarbanilide	101-20-2	Solid	Sigma Aldrich	No	IV	Cosing	Halogen compound, Amide
092	P3-035	4-(methylmercapto)benzaldehyde	3446-89-7	Liquid	Sigma Aldrich	No	IV	ECETOX	Thio compound, Aldehyde
093	P2-008	4,4'-methylenebis(2,6-di-tert-butylphenol)	118-82-1	Solid	Sigma Aldrich	No	IV	ECETOC	Phenol compound
094	P3-011	6-hydroxy-2,4,5-triaminopyrimidine sulfate	1603-02-7	Solid	Wako Pure	No	IV	Cosing	Heterocyclic compound(salt)
095	P3-014	cellulose, 2-(2-hydroxy-3-(trimethylammonio)propoxy) ethyl ether chloride	68610-92-4	Solid	Sigma Aldrich	No	III	J&J	Quaternary ammonium compound, Synthetic polymer

096	P3-064	cyclohexanone	108-94-1	Liquid	Sigma Aldrich	No	III	STE review	Ketone, Hydrocarbon(cyclic)
097	P3-006	dioctyl ether	629-82-3	Liquid	Sigma Aldrich	No	IV	Cognis	Ether
098	P3-003	dipropyl disulfide	629-19-6	Liquid	Sigma Aldrich	No	IV	STE review	Disulfide compound
099	P3-056	ethyl acetate	141-78-6	Liquid	Sigma Aldrich	No	III	STE review	Ester
100	P2-010	ethyl thioglycolate	623-51-8	Liquid	Sigma Aldrich	No	III	NICEATM	Thiol compound, Ester
101	P3-085	gluconolactone	90-80-2	Solid	Wako Pure	No	IV	NICEATM	Polyol
102	P3-089	glycerol	56-81-5	Liquid	Wako Pure	No	IV	STE review	Polyol
103	P3-008	glycidyl methacrylate	106-91-2	Liquid	Sigma Aldrich	No	III	STE review	Methacrylate, Ester
104	P3-002	iso-octylthioglycolate	25103-09-7	Liquid	Wako Pure	No	IV	ECETOX	Thio compound, Ester
105	P3-063	isopropyl bromide	75-26-3	Liquid	Wako Pure	No	IV	STE review	Halogen compound
106	P3-057	isopropyl myristate	110-27-0	Liquid	Wako Pure	No	IV	STE review	Ester
107	P3-086	methyl amyl ketone (2-heptanol)	110-43-0	Liquid	Wako Pure	No	III	STE review	Ketone
108	P3-082	methyl cyclopentane	96-37-7	Liquid	TCI	No	III	STE review	Hydrocarbon
109	P3-088	methyl isobutyl ketone(4-methyl 2-pentanol)	108-10-1	Liquid	Sigma Aldrich	No	III	STE review	Ketone
110	P3-010	n,n-dimethylguanidine sulfate	598-65-2	Solid	Sigma Aldrich	No	III	STE review	Organic salt
111	P2-001	piperonylbutoxide	51- 03- 6	Liquid	Sigma Aldrich	No	III	US-EPA	Ether
112	P3-012	polyethylene hydrogenated castor oil (40E.O.)	61788-85-0	Solid	Sigma Aldrich	No	IV	STE review	Surfactant (nonionic)
113	P2-005	potassium tetrafluoroborate	14075-53-7	Solid	Sigma Aldrich	No	IV	ECETOC	Inorganic salt, Halogen compound
114	P2-017	propyl 4-hydroxybenzoate	94-13-3	Solid	Sigma Aldrich	No	III	LNS	Ester, Phenol
115	P3-098	silic acid, dehydrogenate	7699-41-4	Solid	Wako Pure	No	IV	Ohno(1999)	Silicon compound
116	P2-014	sodium hydrogensulfite	7631-90-5	Solid	Sigma Aldrich	No	III	NICEATM	Inorganic salt
117	P3-092	tween20	9005-64-5	Liquid	Sigma Aldrich	No	III	STE review	Surfactant (nonionic)

Table 5. Distribution of test substances (lank of *in vivo*) selected for SIRC-CVS:TEA test validation study

GHS				Total
Category 1	Category 2A	Category 2B	No	
29	20	22	46	117
EPA				Total
I	II	III	IV	
27	19	44	27	117

Table 6. Distribution of test substances (chemical properties) selected for SIRC-CVS:TEA test validation study

Solid	Liquid	Total
49	68	117

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