

APPENDIX B

**Analysis of Alternative Decision Criteria for Determination of Sensitizers in the LLNA:
BrdU-ELISA**

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Appendix B-1 Substances Used to Evaluate Alternative Decision Criteria for the LLNA: BrdU-ELISA

No.	Substance Name	CASRN	Supplier	Lot Number	Purity	Traditional LLNA Result ¹
1	2,4-Dinitrochlorobenzene	97-007	Wako	TCR1735	99.4%	+
2	<i>p</i> -Benzoquinone	106-51-4	Kanto	012D2294	100%	+
3	Diphenylcyclopropenone	886-38-4	Wako	TCK7554	Not specified	+
4	Glutaraldehyde	111-30-8	Wako	PKM7225	Not specified	+
5	<i>p</i> -Phenylenediamine	106-50-3	SIGMA	59F0003	Not specified	+
6	<i>p</i> -Chloroaniline	106-47-8	Kanto	102D2015	Not specified	+
7	2-Mercaptobenzothiazole	149-30-4	Aldrich	10315HU	98%	+
8	Cinnamic aldehyde	104-55-2	Kanto	7687A	Not specified	+
9	Isoeugenol	97-54-1	Aldrich	06214HO	98%	+
10	<i>m</i> -Aminophenol	591-27-5	Kanto	009D2193	>97%	+
11	3-(4-Isopropylphenyl)iso-butyraldehyde (synonym: cyclamen aldehyde)	103-95-7	TCI	GL01	>90%	+
12	Citral	5392-40-5	Nacalai	M7K6160	97%	+
13	Eugenol	97-53-0	Kanebo	EG0704	>95w/v%	+
14	Hydroxycitronellal	107-75-5	Wako	KSK5525	>95%	+
15	α -Hexylcinnamaldehyde	101-86-0	Aldrich	01026AQ	85%	+
16	Isopropylmyristate	110-27-0	TCI	GH01	Not specified	+
17	Aniline	62-53-3	Kanto	009G1751	>99.0	+
18	Glycerol	56-81-5	Nacalai	V6M2408	>99%	-
19	Isopropanol	67-63-0	Nacalai	V0K7035	99.5%	-
20	2-Hydroxypropyl-methacrylate	923-26-2	Wako	SEM4085	95%	-
21	Diethyl phthalate	84-66-2	TCI	AY01	99%	-
22	Propyleneglycol	57-55-6	Wako	ELL5449	99.0%	-
23	Dimethylisophthalate	1459-93-4	TCI	810S4123	Not specified	-

Abbreviations: CASRN=Chemical Abstract Services Registry Number; LLNA: BrdU-ELISA= Murine local lymph node assay (LLNA) with enzyme-linked immunosorbent assay (ELISA) detection of bromodeoxyuridine (BrdU); TCI=Tokyo Chemical Industry; w/v=Weight to volume ration;

¹+ = Sensitizer; - = Non-sensitizer.

Appendix B-2 Positive/Negative Decisions from the LLNA: BrdU ELISA Based on Various Stimulation Index (SI) Cut-off Points

Substance	Alternative Stimulation Index ¹																		Trad. LLNA ¹	
	3.0	2.9	2.8	2.7	2.6	2.5	2.4	2.3	2.2	2.1	2.0	1.9	1.8	1.7	1.6	1.5	1.4	1.3		
Dimethylisophthalate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Propylene glycol ²	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Diethyl phthalate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Isopropanol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glycerol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2-Hydroxypropylmethacrylate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Aniline	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
<i>p</i> -Chloroaniline	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Isopropylmyristate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
α -Hexylcinnamaldehyde	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hydroxycitronellal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
Eugenol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Citral	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3-(4-Isopropylphenyl)iso-butylaldehyde (synonym: cyclamen aldehyde)	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
<i>m</i> -Aminophenol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Isoeugenol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cinnamic aldehyde	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2-Mercaptobenzothiazole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+
<i>p</i> -Phenylenediamine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glutaraldehyde	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Diphenylcyclopropenone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>p</i> -Benzoquinone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2,4-Dinitrochlorobenzene	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Abbreviations: LLNA: BrdU-ELISA = Murine local lymph node assay (LLNA) with enzyme-linked immunosorbent assay (ELISA) detection of bromodeoxyuridine (BrdU); SI=Stimulation Index; Trad.=Traditional

¹ + =Sensitizer; - = Non-sensitizer.

²Positive in the LLNA: BrdU-ELISA based on individual animal data submitted by Dr. Takeyoshi with SI =5.8 at 50% propylene glycol to support the graphical data in Takeyoshi et al. (2005).

Appendix B -3 Positive/Negative Decisions from the LLNA: BrdU-ELISA Based on Different Decision Criteria

Substance	Decision Criterion ¹								
	Statistics ²	≥95% CI ³	≥2 SD	≥3 SD	SI ≥3	SI ≥2.5	SI ≥2	SI ≥1.5	Trad. LLNA
Dimethylisophthalate	-	-	-	-	-	-	-	-	-
Propylene glycol ⁴	+	+	+	+	+	+	+	+	-
Diethyl phthalate	-	-	-	-	-	-	-	-	-
Isopropanol	-	-	-	-	-	-	-	-	-
Glycerol	-	-	-	-	-	-	-	-	-
2-Hydroxypropylmethacrylate	-	-	-	-	-	-	-	-	-
Aniline	-	+	+	-	-	-	-	+	+
<i>p</i> -Chloroaniline	+	+	+	+	-	+	+	+	+
Isopropylmyristate	+	+	+	+	+	+	+	+	+
Hexylcinnamaldehyde	+	+	+	+	+	+	+	+	+
Hydroxycitronellal	-	-	-	-	-	-	-	-	+
Eugenol	+	+	+	+	+	+	+	+	+
Citral	+	+	+	+	+	+	+	+	+
3-(4-Isopropylphenyl)isobutyraldehyde (synonym: cyclamen aldehyde)	+	+	+	+	-	-	-	+	+
<i>m</i> -Aminophenol	+	+	+	+	+	+	+	+	+
Isoeugenol	+	+	+	+	-	-	+	+	+
Cinnamic aldehyde	+	+	+	+	+	+	+	+	+
2-Mercaptobenzothiazole	+	+	+	+	-	-	-	+	+
<i>p</i> -Phenylenediamine	+	+	+	+	+	+	+	+	+
Glutaraldehyde	+	+	+	+	+	+	+	+	+
Diphenylcyclopropenone	+	+	+	+	+	+	+	+	+
<i>p</i> -Benzoquinone	+	+	+	+	+	+	+	+	+
2,4-Dinitrochlorobenzene	+	+	+	+	+	+	+	+	+

Abbreviations: CI = Confidence interval; LLNA: BrdU-ELISA = Murine local lymph node assay (LLNA) with enzyme-linked immunosorbent assay (ELISA) detection of bromodeoxyuridine (BrdU); SD = Standard deviation; SI = Stimulation Index; Trad. = Traditional

¹+ = Sensitizer; - = Non-sensitizer/

²Statistical test for difference of control group and treatment group means.

³Mean BrdU labeling index is outside the 95% confidence interval of the control group.

⁴Positive in the LLNA: BrdU-ELISA based on individual animal data submitted by Dr. Takeyoshi with SI =5.8 at 50% propylene glycol to support the graphical data in Takeyoshi et al. (2005).