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APPENDIX D

**Comparative LLNA: BrdU-ELISA, Traditional LLNA, Guinea Pig Skin Sensitization,
and Human Data**

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37 **Appendix D-1 Comparative Performance of the LLNA: BrdU-ELISA, Traditional LLNA, Guinea Pig, and Human**
 38 **Tests (Alphanumeric Order)**
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Substance Name	CASRN	BrdU-ELISA LLNA Result ^{1,16}	Overall BrdU-ELISA LLNA Result ¹	BrdU-ELISA Reference	Traditional LLNA Result ^{1,2}	GPMT/BT Outcome ^{1,2}	Human Outcome ^{1,2}
2,4-Dinitrochlorobenzene	97-00-7	+	+	Takeyoshi 2005	+	+	+
		+		Takeyoshi 2006			
2,2'-Dihydroxyl-3,3'-dimethoxy-5,5'-diallyl-biphenyl		-	-	Takeyoshi 2004a	NA	-	NA
2-Hydroxypropylmethacrylate	923-26-2	-	-	Takeyoshi 2007b	-	-	+ ³
2-Mercaptobenzothiazole	149-30-4	-	-	Takeyoshi 2007b	+	+	+
2-Methoxy-4-(7-methoxy-3-methyl-5-propenyl-2,3-dihydro-benzofuran-2yl)-phenol (Synonym: Dehydroiisoeugenol)		+	+	Takeyoshi 2007a	NA	+	NA
3-(4-Isopropylphenyl)isobutyraldehyde (Synonym: cyclamen aldehyde)	103-95-7	-	-	Takeyoshi 2007b	+ ¹⁵	NA	+ ¹⁵
4-[1-Hydroxy-2-(2-methoxy-4-propenyl-phenyloxy)-propyl]-2-methoxy-phenol (Synonym: β-O-4-Dilignol)		-	-	Takeyoshi 2007a	NA	- ⁴	NA
4,5'-Diallyl-2'-hydroxy-2,3'-dimethoxyphenyl ether		+	+	Takeyoshi 2004a	NA	+	NA
4-Chloroaniline	106-47-8	-	-	Takeyoshi 2007b	+	+	NA
4-Phenylenediamine	106-50-3	+	+	Takeyoshi 2005	+	+	+
m-Aminophenol	591-27-5	+	+	Takeyoshi 2007b	+	+ ⁵	+
Aniline	62-53-3	-	-	Takeyoshi 2007b	+ ¹²	+	+
p-Benzoquinone	106-51-4	+	+	Takeyoshi 2004b	+	+	NA

Substance Name	CASRN	BrdU-ELISA LLNA Result ^{1,16}	Overall BrdU-ELISA LLNA Result ¹	BrdU-ELISA Reference	Traditional LLNA Result ^{1,2}	GPMT/BT Outcome ^{1,2}	Human Outcome ^{1,2}
trans-Cinnamaldehyde	14371-10-9	+	+	Takeyoshi 2005	NA	NA	NA
Cinnamic aldehyde	104-55-2	+	+	Takeyoshi 2007b	+	+	+
Citral	5392-40-5	+	+	Takeyoshi 2005	+	+	+
Diethyl phthalate	84-66-2	-	-	Takeyoshi 2007b	-	NA	+
Dimethylisophthalate	1459-93-4	-	-	Takeyoshi 2007b	-	-	NA
Diphenylcyclopropanone	886-38-4	+	+	Takeyoshi 2005	+	NA	+ ⁶
Eugenol	97-53-0	+	+	Takeyoshi 2004a	+	+	+
		+		Takeyoshi 2007b			
		+		Takeyoshi 2005			
		+		Takeyoshi 2006			
Glutaraldehyde	111-30-8	+	+	Takeyoshi 2005	+ ⁷	NA	+ ⁸
Glycerol	56-81-5	-	-	Takeyoshi 2007b	-	-	- ¹⁰
Hexane	110-54-3	-	-	Takeyoshi 2005	-	NA	-
Hexyl cinnamic aldehyde	101-86-0	+	+	Takeyoshi 2003	+	+	+
		+		Takeyoshi 2005			
		+		Takeyoshi 2006			

Substance Name	CASRN	BrdU-ELISA LLNA Result ^{1,16}	Overall BrdU-ELISA LLNA Result ¹	BrdU-ELISA Reference	Traditional LLNA Result ^{1,2}	GPMT/BT Outcome ^{1,2}	Human Outcome ^{1,2}
Hydroxycitronellal	107-73-5	-	-	Takeyoshi 2007b	+	+	+
Isoeugenol	97-54-1	+	+ ¹³	Takeyoshi 2005	+	+	+
		+		Takeyoshi 2005			
		-		Takeyoshi 2006			
		+		Takeyoshi 2007b			
Isopropanol	67-63-0	-	-	Takeyoshi 2007b	-	-	+ ⁹
Isopropyl myristate	110-27-0	+	+	Takeyoshi 2005	+ ¹⁰	NA	- ¹¹
Propylene glycol	57-55-6	+	+ ¹⁴	Takeyoshi 2005	-	-	+
		-		Takeyoshi 2006			

40 Abbreviations: BrdU-ELISA LLNA = Murine local lymph node assay with enzyme-linked immunosorbent assay detection of bromodeoxyuridine; CASRN = Chemical Abstract Services Registry
 41 Number; LLNA = Murine local lymph node assay; NA = Not available; SI = Stimulation index.

42 ¹+ = Sensitizer; - = Non-sensitizer

43 ²Data from ICCVAM (1999) unless otherwise noted.

44 ³Bjorkner (1984).

45 ⁴Takeyoshi et al. (2007a).

46 ⁵Data from nonstandard assay (ICCVAM 1999). Not used in accuracy analyses.

47 ⁶Data from Basketter et al. (2000).

48 ⁷Hilton et al. (1998)

49 ⁸Marzulli et al. (1974).

50 ⁹Kwon et al. (2003).

51 ¹⁰Ryan et al. (2000).

52 ¹¹Opdyke (1976).

53 ¹²Gerberick et al. (2005).

54 ¹³Since three tests produced a positive result and one test produced a negative result, the overall result was deemed to be positive (i.e., a weight of evidence approach was used). The maximum
 55 concentration tested by Takeyoshi et al. (2005; 2006) was 10% while the maximum concentration tested by Takeyoshi et al. (2007b) was 30%.

56 ¹⁴Because one test produced a positive result and one test produced a negative result, the overall result was deemed to be positive (i.e., a conservative approach was used). Both tests used a
 57 maximum concentration of 50%. The 2005 paper by Takeyoshi et al. indicated (in bar graphs) that the SI<3, however, individual animal data submitted by Dr. Takeyoshi showed that SI=5.8 (i.e.,
 58 positive for skin sensitization potential). The results for same concentration from the 2006 paper indicated that propylene glycol was negative (confirmed by SI=0.7 from individual animal data
 59 submitted by Dr. Takeyoshi).

60 ¹⁵Data from Basketter et al. (2005).

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¹⁶All tests used acetone:olive oil (4:1) as the vehicle except 2-mercaptobenzothiazole, which used dimethylformamide, and glycerol, which was tested neat.

64 **Appendix D-2 Substances Tested in the LLNA: BrdU-ELISA (Ordered by Traditional LLNA EC3 Value)**

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Chemical Name	Traditional LLNA				BrdU-ELISA LLNA		
	LLNA Result	EC3 (%) ¹	N ²	SI (Conc. Tested [%]) ³	LLNA Result ⁴	EC3 (%) ⁵	Takeyoshi et al. Reference
Diphenylcyclopropenone	+	0.005	1	NA	+	<2 ^{&}	2005
Benzoquinone	+	0.01	1	NA	+	0.22 [#]	2004b
2,4-Dinitrochlorobenzene	+	0.049	15	NA	+	0.2 [*] , 0.1	2005, 2006
4-Phenylenediamine	+	0.11	6	NA	+	<2 ^{&}	2005
Glutaraldehyde	+	0.14	2	NA	+	<2 ^{&}	2005
Isoeugenol	+	1.5	31	NA	+	5.6 [*] , 9.6, 12.7	2005, 2006, 2007a
Cinnamic aldehyde	+	2.4		NA	+	ND	2007b
3-Aminophenol	+	3.2	1	NA	+	ND	2007b
4-Chloroaniline	+	6.5	1	5	-	NC	2007b
Citral	+	9.8	2	NA	+	13.9 [*]	2005
2-Mercaptobenzothiazole	+	9.8	2	NA	-	NC	2007b
Hexyl cinnamic aldehyde	+	9.9	15	NA	+	40.8 [*] , 45.5, 37.2	2005, 2006, 2003
Eugenol	+	10	22	NA	+	9.64 [#] , 25.1, 40.6, 22.8	2005, 2004a, 2006, 2007a
3-(4-Isopropylphenyl)isobutyraldehyde (Synonym: cyclamen aldehyde)	+	22.3	1	NA	-	ND	2007b
Hydroxycitronellal	+	23.8	6	NA	-	NC	2007b
Isopropyl myristate	+	44	1	NA	+	36.7 [*]	2005
Aniline	+ ⁶	63	2	NA	-	NC	2007b
2-Hydroxypropyl methacrylate	-	NC	NA	1.3 (50)	-	NC	2007b
Diethylphthalate	-	NC	NA	1.5 (100)	-	NC	2007b

Chemical Name	Traditional LLNA				BrdU-ELISA LLNA		
	LLNA Result	EC3 (%) ¹	N ²	SI (Conc. Tested [%]) ³	LLNA Result ⁴	EC3 (%) ⁵	Takeyoshi et al. Reference
Dimethyl isophthalate	-	NC	NA	1.0 (25)	-	NC	2007b
Glycerol	-	NC	NA	1.1 (25)	-	NC	2007b
Hexane	-	NC	NA	2.2 (100)	-	NC	2005
Isopropanol	-	NC	NA	1.7 (10)	-	NC	2007b
Propylene glycol	-	NC	NA	1.6 (100)	+	NC	2005, 2006
2,2'-Dihydroxyl-3,3'-dimethoxy-5,5'-diallyl-biphenyl	NK	NK	NA	NA	-	>30	2004a
2-Methoxy-4-(7-methoxy-3-methyl-5-propenyl-2,3-dihydro-benzofuran-2yl)-phenol	NK	NK	NA	NA	+	9.4	2007
4,5'-Diallyl-2'-hydroxy-2,3'-dimethoxyphenyl ether	NK	NK	NA	NA	+	2.3	2004a
4-[1-Hydroxy-2-(2-methoxy-4-propenyl-phenoxy)-propyl]-2-methoxy-phenol	NK	NK	NA	NA	-	>30	2007
trans-Cinnamaldehyde	NK	NK	NA	NA	+	<10 ^{&}	2005

- 66 Abbreviations: BrdU-ELISA LLNA = Murine local lymph node assay with enzyme-linked immunosorbent assay detection of bromodeoxyuridine; CASRN = Chemical
67 Abstracts Registry Service Number; Conc. = Concentration; EC3 = Concentration at SI=3; LLNA = Murine local lymph node assay; NA = Not applicable; NC = Not
68 calculated; ND = No data available to calculate EC3; NK = Not known; SI = Stimulation index.
69 *Value interpolated by NICEATM from SI and concentration values provided in the reference using $EC3 = c + [(3-d)/(b-d)] * (a-c)$, where a is the concentration of the point with
70 SI > 3, b is the associated SI, c is the concentration of the point with SI < 3, and d is the associated SI.
71 # Value extrapolated by NICEATM from SI and concentration values provided in the reference using $EC3 = 2^{\log_2 c + [(3-d)/(b-d)] * (\log_2 a - \log_2 c)}$, where a is the
72 concentration of the point with the higher SI, b is the higher SI, c is the concentration of the point with the lower SI, and d is the lower SI.
73 &Data inadequate for the calculation of EC3 values (i.e., only one concentration tested).
74 + = Sensitizer.
75 - = Non-sensitizer.
76 ¹EC3 values from the NICEATM LLNA database. All tests use acetone:olive oil (4:1) as the vehicle.
77 ²Number of values used to calculate mean.
78 ³Highest SI value and the test concentration at which it was observed reported for substances for which an SI ≥ 3 was not observed at any concentration tested. All tests used
79 acetone:olive oil (4:1) as the vehicle, except glycerol, which used dimethylformamide and isopropanol, which used water.
80 ⁴All tests used acetone:olive oil (4:1) as the vehicle except 2-mercaptobenzothiazole, which used dimethylformamide, and glycerol, which was tested neat.
81 ⁵EC3 values reported by Takeyoshi et al. or calculated using data from Takeyoshi et al.
82 ⁶Gerberick et al. (2005).