

**APPENDIX C**

**Comparative LLNA: DA, Traditional LLNA, Guinea Pig Skin Sensitization, and  
Human Skin Sensitization**

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1 **Appendix C-1. Comparative Performance of the LLNA: DA, Traditional LLNA, Guinea Pig Skin Sensitization, and**  
 2 **Human Skin Sensitization Test Data for the Substances Tested in the LLNA: DA (Alphabetic Order)**

Substance Name	CASRN	LLNA: DA Result <sup>1</sup>	Trad. LLNA Result <sup>2</sup>	GPMT/BT Outcome <sup>3</sup>	Human Outcome <sup>4</sup>
Abietic acid <sup>a</sup>	514-10-3	+	+	+	+
3-Aminophenol <sup>c</sup>	591-27-5	-	+	+(nonstd)	+
Benzalkonium chloride	8001-54-5	+	-	-	+
Benzocaine	94-09-7	+	+/- <sup>5</sup>	+	+/- <sup>5</sup>
1-Bromobutane	109-65-9	-	-		
Chlorobenzene	108-90-7	-	-	-	
Cinnamic aldehyde	104-55-2	+	+	+	+
Citral	5392-40-5	+	+	+	+
Cobalt chloride <sup>a, b</sup>	7646-79-9	+	+	+	+
Diethyl phthalate	84-66-2	-	- <sup>6</sup>		- <sup>7</sup>
Dimethyl isophthalate <sup>c</sup>	1459-93-4	-	-	-	
2,4-Dinitrochlorobenzene <sup>a</sup>	97-00-7	+	+	+	+ <sup>7</sup>
Eugenol	97-53-0	+	+	+	+
Formaldehyde <sup>a</sup>	50-00-0	+	+	+	+
Glutaraldehyde <sup>a</sup>	111-30-8	+	+ <sup>6</sup>		+ <sup>7</sup>
Hexane	110-54-3	-	-		-
Hexyl cinnamic aldehyde <sup>a, b</sup>	101-86-0	+	+	+	+ <sup>8</sup>
Hydroxycitronellal	107-75-5	+	+	+	+
Imidazolidinyl urea	39236-46-9	+	+	+	+

Substance Name	CASRN	LLNA: DA Result <sup>1</sup>	Trad. LLNA Result <sup>2</sup>	GPMT/BT Outcome <sup>3</sup>	Human Outcome <sup>4</sup>
Isoeugenol <sup>a</sup>	97-54-1	+	+	+	+
Isopropanol <sup>a</sup>	67-63-0	-	-	-	- <sup>9</sup>
Lactic acid <sup>b</sup>	50-21-5	-	-	-	
2-Mercaptobenzothiazole	149-30-4	-	+	+	+
Methyl salicylate <sup>a</sup>	119-36-8	-	-	-	-
Nickel (II) sulfate hexahydrate <sup>a, b</sup>	10101-97-0	-	- <sup>10</sup>	+	+
p-Phenylenediamine	106-50-3	+	+	+	+
Phthalic anhydride	85-44-9	+	+	+	+ <sup>9</sup>
Potassium dichromate <sup>b</sup>	7778-50-9	+	+	+	+
Propyl paraben	94-13-3	-	-	-	+
Resorcinol	108-46-3	+	+ <sup>11</sup>	-	+
Sodium lauryl sulfate	151-21-3	+	+	-	-
Toluene 2,4-diisocyanate	584-84-9	+	+ <sup>12</sup>		+ <sup>9</sup>
Trimellitic anhydride	552-30-7	+	+ <sup>13</sup>	+ <sup>14</sup>	+ <sup>9</sup>

3 Abbreviations: CASRN=Chemical Abstracts Registry Service Number; GPMT/BT=Guinea Pig Maximization Test/Buehler Test; LLNA=Local Lymph Node  
4 Assay; LLNA: DA=Local Lymph Node Assay Modified by Daicel Chemical Industries, Ltd. Based on ATP Content; Trad.=Traditional; + nonstd=Non-standard  
5 guinea pig maximization tests.

6 <sup>1</sup>Data from Daicel Chemical Industries, Ltd. presented at 6<sup>th</sup> World Congress on Alternatives and Animal Use in the Life Sciences (2007).

7 <sup>2</sup>Data from ICCVAM (1999) unless otherwise noted.

8 <sup>3</sup>Data from ICCVAM (1999), unless otherwise noted, and based on studies using either the Guinea Pig Maximization Test or the Buehler Test.

9 <sup>4</sup>Data from ICCVAM (1999), unless otherwise noted, and based on studies using either the Human Maximization Test or the inclusion of the test chemical in a  
10 Human Patch Test Allergen Kit.

11 <sup>5</sup>Equivocal data (ICCVAM 1999). Substance not included in accuracy analyses.

12 <sup>6</sup>Gerberick et al. 2005.

13 <sup>7</sup>Schneider and Akkan 2004.

- 14 <sup>8</sup>Basketter et al. 2005.
- 15 <sup>9</sup>Basketter et al. 2001.
- 16 <sup>10</sup>ICCVAM 1999 data represents Nickel(II) sulfate heptahydrate (CASRN 10101-98-1).
- 17 <sup>11</sup>Basketter et al. 2007.
- 18 <sup>12</sup>van Och et al. 2001. Modified version of LLNA protocol not in accordance with ICCVAM 1999 and Dean et al. 2001; Substance not included in accuracy
- 19 analysis.
- 20 <sup>13</sup>Basketter and Scholes 1992.
- 21 <sup>14</sup>Gad et al. 1986.
- 22 <sup>a</sup>Tested among the 31 substances used to assess the performance of the LLNA: DA (Daicel Chemical Industries, Ltd. 2007) and in the first interlaboratory
- 23 validation study on LLNA: DA (Ikarashi et al. 2007).
- 24 <sup>b</sup>Tested among the 31 substances used to assess the performance of the LLNA: DA (Daicel Chemical Industries, Ltd. 2007) and in the second
- 25 interlaboratory validation study on LLNA: DA (Kanazawa et al. 2007).
- 26 <sup>c</sup>Not tested among the 31 substances used to assess the performance of the LLNA: DA (Daicel Chemical Industries, Ltd. 2007) but in the first
- 27 interlaboratory validation study on LLNA: DA (Ikarashi et al. 2007).
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53 **Appendix C-2. Performance of the LLNA: DA versus Traditional LLNA (Substances Sorted Based on Traditional**  
 54 **LLNA EC3)**

Substance Name <sup>1</sup>	Traditional LLNA				LLNA: DA	
	LLNA Result	EC3 (%)	Number <sup>2</sup>	SI (Conc. Tested %)	LLNA Result	EC3 (%) <sup>3</sup>
2,4-Dinitrochlorobenzene <sup>a</sup>	+	0.049	15	NA	+	0.05
Glutaraldehyde <sup>a</sup>	+	0.083	3	NA	+	0.10
p-Phenylenediamine	+	0.11	6	NA	+	0.07
Potassium dichromate <sup>b</sup>	+	0.11	6	NA	+	0.14
Toluene 2,4-diisocyanate	+	0.11	1	NA	+	0.05
Trimellitic anhydride	+	0.22	1	NA	+	0.17
Phthalic anhydride	+	0.36	1	NA	+	<0.1
Formaldehyde <sup>a</sup>	+	0.50	4	NA	+	1.16
Isoeugenol <sup>a</sup>	+	1.53	49	NA	+	2.35
Cinnamic aldehyde	+	2.38	5	NA	+	2.98
<b>2-Mercaptobenzothiazole</b>	+	<b>2.5</b>	<b>2</b>	<b>NA</b>	-	<b>NC (SI=2 at 10%)</b>
3-Aminophenol <sup>c</sup>	+	3.2	1	NA	+	NP
Cobalt chloride <sup>a, b</sup>	+	4.8	1	NA	+	3.27
Resorcinol	+	6.7	1	NA	+	6.44
Sodium lauryl sulfate	+	8.08	5	NA	+	8.28
Citral	+	9.8	2	NA	+	15.63
Hexyl cinnamic aldehyde <sup>a, b</sup>	+	9.93	22	NA	+	11.62
Eugenol	+	10.09	11	NA	+	4.50
Abietic acid <sup>a</sup>	+	11.92	5	NA	+	7.90
Benzocaine	+/-	22 <sup>4</sup>	1	NA	+	6.57
Hydroxycitronellal	+	23.75	6	NA	+	13.74
Imidazolidinyl urea	+	24	1	NA	+	18.77
<b>Benzalkonium chloride</b>	-	<b>NA</b>	<b>NA</b>	<b>NA</b>	+	<b>0.58</b>
1-Bromobutane	-	NA	NA	NA	-	NC (SI=1.65 at 25%)
Chlorobenzene	-	NA	NA	NA	-	NC (SI=2.44 at 25%)
Diethyl phthalate	-	NA	NA	NA	-	NC (SI=1.09 at 25%)
Dimethyl isophthalate <sup>c</sup>	-	NA	NA	NA	-	NP
Hexane	-	NA	NA	NA	-	NC (SI=2.31 at 100%)

Substance Name <sup>1</sup>	Traditional LLNA				LLNA: DA	
	LLNA Result	EC3 (%)	Number <sup>2</sup>	SI (Conc. Tested %)	LLNA Result	EC3 (%) <sup>3</sup>
Isopropanol <sup>a</sup>	-	NA	NA	NA	-	NC (SI=1.08 at 25%)
Lactic acid <sup>b</sup>	-	NA	NA	NA	-	NC (SI=1.06 at 10%)
Methyl salicylate <sup>a</sup>	-	NA	NA	NA	-	NC (SI=1.2 at 25%)
Nickel (II) sulfate hexahydrate <sup>a, b</sup>	-	NA	NA	NA	-	NC (SI=2.17 at 2.5%)
Propylparaben	-	NA	NA	NA	-	NC (SI=1.28 at 25%)

55 Bolded and italicized text denotes the discordant substances in the LLNA: DA compared to the traditional LLNA.

56 Abbreviations: Conc.=Concentration; LLNA=Local Lymph Node Assay; LLNA: DA=Local Lymph Node Assay Modified by Daicel Chemical Industries,  
57 Ltd. Based on ATP Content; NA=Not applicable; NC=Not calculated; NP=Not provided; SI=Stimulation Index

58 <sup>1</sup>Total of 33 substances: includes the 31 substances from the original validation study performed in one laboratory and the two interlaboratory validation  
59 studies (14 substances; two substances not among the 31 original substances assessed).

60 <sup>2</sup>Refers to the number of LLNA studies from which the EC3 data were obtained.

61 <sup>3</sup>Based on EC3 values calculated by Daicel Chemical Industries, Ltd. (2007); For substances predicted as non-sensitizers by the LLNA: DA, the mean SI for the  
62 highest dose tested is provided in parenthesis.

63 <sup>4</sup>An EC3 value is reported here for benzocaine, but variable and equivocal responses in the traditional LLNA were reported for this substance in the ICCVAM  
64 1999 report.

65 <sup>a</sup>Tested among the 31 substances used to assess the performance of the LLNA: DA (Daicel Chemical Industries, Ltd. 2007) and in the first interlaboratory  
66 validation study on LLNA: DA (Ikarashi et al. 2007).

67 <sup>b</sup>Tested among the 31 substances used to assess the performance of the LLNA: DA (Daicel Chemical Industries, Ltd. 2007) and in the second  
68 interlaboratory validation study on LLNA: DA (Kanazawa et al. 2007).

69 <sup>c</sup>Not tested among the 31 substances used to assess the performance of the LLNA: DA (Daicel Chemical Industries, Ltd. 2007) but in the first  
70 interlaboratory validation study on LLNA: DA (Ikarashi et al. 2007).

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