

Appendix F

ICCVAM LLNA Performance Standards: Recommended Reference Substances

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Appendix F1

Recommended Reference Substances – Alphabetically Sorted

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Substance Name	CASRN	MW (g/mol)	Physical Form	LLNA	Veh ¹	EC3 ²	N ³	0.5x - 2.0x EC3	Max SI (Conc)	GPMT/BT ⁴	HMT	HPTA	Additional Human Skin Sensitization Data/Information ⁵	Peptide Reactivity ⁶
5-Chloro-2 methyl-4-isothiazolin-3-one	26172-55-4	149.599	Liquid	+	DMF	0.009	1	0.0045-0.018	22.7 (0.1%)	+		+	DSA05HRIPT=4.3 (Cardin et al. 1986) Human data is for mixture (Kathon CG) – no human data for single substance	High
Chlorobenzene	108-90-7	112.557	Liquid	-	AOO	NC	1	NA	1.7 (10%)	-			No human data located ⁷	Minimal
Cinnamic alcohol	104-54-1	134.18	Solid	+	AOO	21	1	10.5-42	5.7 (90%)	+	+		DSA05HRIPT=3704; DSA05HMT=625 (Jordan and King 1977)	
Citral	5392-40-5	152.233	Liquid	+	AOO	9.2	6	4.6-18.3	20.5 (20%)	+	+		DSA05HRIPT=840; DSA05HMT=862 (Steltenkamp et al. 1980)	
Cobalt chloride	7646-79-9	129.84	Solid	+	DMSO ⁸	0.6	2	0.3-1.2	7.2 (5%)	+	+	+	DSA05HMT=172 (Kligman 1966b) human data is for cobalt sulfate	
2,4-Dinitrochlorobenzene	97-00-7	202.552	Solid	+	AOO	0.049	15	0.025-0.099	43.9 (0.025%)	+			DSA05HRIPT=5.5 (Friedmann et al. 1983)	High
Ethylene glycol dimethacrylate	97-90-5	198.216	Liquid	+	MEK	28	1	14-56	7 (50%)	-		+	+ human from Basketter et al. 1999a	High
Eugenol	97-53-0	164.201	Liquid	+	AOO	10.1	11	5.05-20.2	17 (50%)	+		+	DSA05HRIPT=5926 (Marzulli and Maibach 1980)	
Hexyl cinnamic aldehyde ⁹	101-86-0	216.319	Liquid	+	AOO ¹⁰	9.7	21	4.8-19.5	20 (50%)	+			DSA(NOEL)HRIPT=23622 (RIFM submission and Basketter et al. 2005b)	Minimal
Lactic acid	50-21-5	90.078	Liquid	-	DMSO	NC	1	NA	2.2 (25%)	-			No human data located ⁷	Minimal
Imidazolidinyl urea	39236-46-9	388.294	Solid	+	DMF	24	1	12-48	5.5 (50%)	+		+	DSA05HRIPT=3846 (Jordan and King 1977)	Moderate
Isoeugenol	97-54-1	164.201	Liquid	+	AOO ¹¹	1.5	47	0.77-3.1	31 (5%)	+		+	DSA(LOEL) HRIPT=69 (Griem et al. 2003); DSA(LOEL)HMT=5217	
Isopropanol	67-63-0	60.095	Liquid	-	AOO	NC	1	NA	1.7 (10%)	-			Studies indicate substance produces skin sensitization ¹²	Minimal
2-Mercaptobenzothiazole	149-30-4	167.253	Solid	+	DMF	1.7	1	0.85-3.4	8.6 (10%)	+	+	+	DSA05HMT=1642 (Kligman 1966a)	High
Methyl methacrylate	80-62-6	100.12	Liquid	+	AOO ¹³	90 ¹³	1	45-100	3.6 (100%)	+			Information derived from clinical experience ¹³	
Methyl salicylate	119-36-8	152.147	Liquid	-	AOO	NC	9	NA	2.7 (20%)	-	-	-	DSA05HMT=5517 (Schneider and Akkan 2004)	Minimal

ICCVAM LLNA Performance Standards – Appendix F1

Substance Name	CASRN	MW (g/mol)	Physical Form	LLNA	Veh ¹	EC3 ²	N ³	0.5x - 2.0x EC3	Max SI (Conc)	GPMT/BT ⁴	HMT	HPTA	Additional Human Skin Sensitization Data/Information ⁵	Peptide Reactivity ⁶
Nickel chloride	7718-54-9	129.599	Solid	-	DMSO ¹⁴	NC	2	NA	2.4 (5%)	+			DSA05HMT=28 (Kligman 1966a for nickel sulfate – but data expressed as nickel)	
Phenyl benzoate ⁹	93-99-2	198.217	Solid	+	AOO	13.6	3	6.8-27.2	11.1 (25%)	+			DSA05HRIPT=52489 (Basketter et al. 2005b)	
4-Phenylenediamine	106-50-3	108.14	Solid	+	AOO	0.11	6	0.055-0.22	26.4 (1%)	+	+	+	DSA05HMT=16.4 (Kligman 1966a); DSA05HRIPT=6.9 (Marzulli and Maibach 1974)	
Salicylic acid	69-72-7	138.121	Solid	-	AOO	NC	1	NA	2.5 (25%)	-	-	-	DSA(NOEL) HMT=13793 (Kligman 1966b)	
Sodium lauryl sulfate	151-21-3	288.38	Solid	+	DMF	8.1	5	4.05-16.2	8.9 (10%)	-	-	-	DSA(NOEL) HMT=6897 (Kligman 1966b)	
Xylene	1330-20-7	106.17	Liquid	+	AOO	95.8 ¹⁵	1	47.9-100	3.1 (100%)	NA ¹⁶	-		DSA (NOEL)=68966 (Kligman 1966b)	

Abbreviations: AOO = acetone: olive oil (4:1); BT = Buehler Test; CASRN = Chemical Abstracts Service Registry Number; Conc. = concentration tested; DMF = *N,N*-dimethylformamide; DMSO = dimethyl sulfoxide; DSA = dose per skin area; DSA05 = dose per skin area leading to a sensitization incidence of 5%; EC3 = estimated concentration needed to produce a stimulation index of 3; GPMT = Guinea Pig Maximization Test; HMT = Human Maximization Test; HPTA = Human Patch Test Allergen; HRIPT = Human Repeat Insult Patch Test; LLNA = murine local lymph node assay; LOEL = lowest observed effect level; Max = maximum; MEK = methyl ethyl ketone; MW = molecular weight; NA = not available; NC = not calculated; NOEL = no observed effect level; RIFM = Research Institute for Fragrance Materials; SI = stimulation index; Veh = vehicle

¹ Unless noted otherwise, vehicle information obtained from Gerberick et al. 2005.

² Unless noted otherwise, where the number of LLNA studies equals one, EC3 values obtained from Gerberick et al. 2005.

³ Number of LLNA studies from which data were obtained.

⁴ Results obtained from Guinea Pig Maximization Test and/or Buehler Test.

⁵ Human Quantitative Data obtained from literature where human data was compared to LLNA. All data are expressed as DSA ($\mu\text{g}/\text{cm}^2$). DSA05HMT and DSA05HRIPT were obtained by linear interpolation from the lowest observed effect level to a dose corresponding to the estimated sensitization incidence of 5% (Schneider and Akkan 2004). DSA (NOEL) refers to the maximum no observed effect level. In absence of negative data, the lowest observed effect level was used, provided that the percentage of people sensitized was less than 8% (Basketter et al. 2005b).

⁶ Peptide reactivity data obtained from Gerberick et al. 2007.

⁷ Presumed to be a non-sensitizer in humans based on the fact that no clinical patch test results were located, it is not included as a patch test kit allergen, and no case reports of human sensitization were located.

⁸ Basketter and Scholes 1992; Ikarashi et al. 1992

⁸ Human data based on following studies: (1) Rees et al. 1989 (2) Zina et al. 1987.

⁹ Presumed to be a strong human allergen (search for human data ongoing)

¹⁰ Dearman et al. 2001

¹¹ Basketter and Cadby 2004

¹² Human data based on Kwon et al. 2003

¹³ Betts et al. 2006

¹⁴ Basketter and Scholes 1992

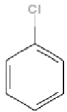
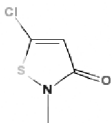
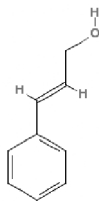
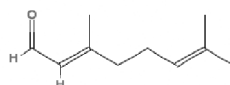
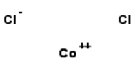
¹⁵ Estrada et al. 2003

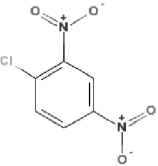
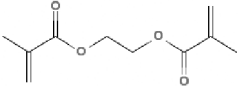
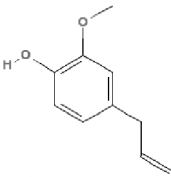
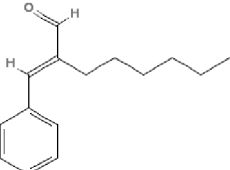
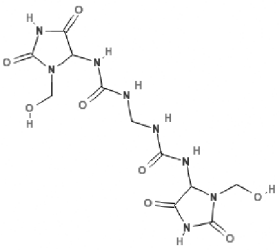
¹⁶ Personal Communication (D. Basketter 2008)

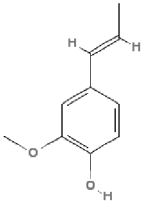

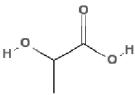
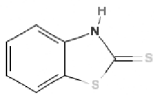
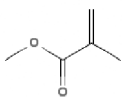
Appendix F2

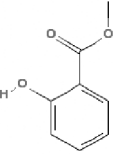

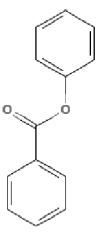
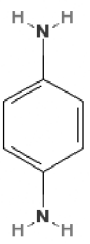
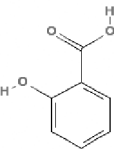
Recommended Reference Substances – Structures and Product Uses

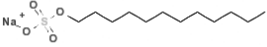
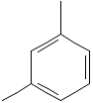
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Substance Name	CASRN	Structure	Product Uses
Chlorobenzene	108-90-7		Phenol manufacture Aniline manufacture DDT manufacture Solvent for paints
5-Chloro-2-methyl-4-isothiazolin-3-one	26172-55-4		Disinfectant
Cinnamic alcohol	104-54-1		Perfume manufacture
Citral	5392-40-5		Flavor additive Perfume manufacture
Cobalt chloride	7646-79-9		Humidity and water indicator Preparation of catalysts Fertilizer and feed additive Vitamin B12 manufacture

Substance Name	CASRN	Structure	Product Uses
2,4-Dinitrochlorobenzene	97-00-7		<p>Color photo processing Explosives manufacture</p>
Ethylene glycol dimethacrylate	97-90-5		<p>Polymerization agent</p>
Eugenol	97-53-0		<p>Fragrance and flavoring agent Insect attractant</p>
Hexyl cinnamic aldehyde	101-86-0		<p>Perfume manufacture</p>
Imidazolidinyl urea	39236-46-9		<p>Cosmetic preservative Antimicrobial</p>

Substance Name	CASRN	Structure	Product Uses
Isoeugenol	97-54-1		<p>Perfume manufacture Flavoring additive Topical pharmaceutical</p>
Isopropanol	67-63-0		<p>Topical pharmaceutical Gasoline additive Cleaning agent</p>
Lactic Acid	50-21-5		<p>Manufacture of lactates which are used in food products, in medicine, and as solvents</p>
2-Mercaptobenzothiazole	149-30-4		<p>Rubber manufacture Anticorrosive</p>
Methyl methacrylate	80-62-6		<p>Used in the production of polymers such as surface coating resins, plastics (Plexiglas and Lucite), ion exchange resins and plastic dentures.</p>

Substance Name	CASRN	Structure	Product Uses
Methyl salicylate	119-36-8		Topical pharmaceutical Flavor additive
Nickel chloride	7718-54-9		Electroplating agent Battery manufacture
Phenyl benzoate	93-99-2		Production of industrial chemicals
4-Phenylenediamine	106-50-3		Hair dye Textile dye
Salicylic acid	69-72-7		Pharmaceutical Food preservative

Substance Name	CASRN	Structure	Product Uses
Sodium lauryl sulfate	151-21-3		<p>Detergent Cosmetic</p>
Xylene	1330-20-7		<p>Solvent Production of industrial chemicals</p>

Abbreviations: CASRN = Chemical Abstracts Service Registry Number

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Appendix F3

Recommended Reference Substances – Murine Local Lymph Node Assay Data

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Substance Name	CASRN	Veh	Dose 1 Conc. (%)	Dose 1 SI	Dose 2 Conc. (%)	Dose 2 SI	Dose 3 Conc. (%)	Dose 3 SI	Dose 4 Conc. (%)	Dose 4 SI	Dose 5 Conc. (%)	Dose 5 SI	EC3 (%)	Rationale for High Dose ¹	LLNA Result	Reference
Chlorobenzene	108-90-7	AOO	5	1.1	10	1.7	25	1.6	ND	ND	ND	ND	NC	A, C	-	Gerberick et al. 2005
5-Chloro-2-methyl-4-isothiazolin-3-one	26172-55-4	DMF	0.01	3.5	0.03	12.3	0.10	22.7	ND	ND	ND	ND	0.009	A, C	+	Gerberick et al. 2005
Cinnamic alcohol	104-54-1	AOO	10	1.8	25	3.5	50	3.9	90	5.7	ND	ND	21	C	+	Gerberick et al. 2005
Citral	5392-40-5	AOO	5	1.2	10	2.1	25	6.3	ND	ND	ND	ND	13	C	+	Gerberick et al. 2005
Citral	5392-40-5	AOO	5	2.1	10	5.0	20	9.3	ND	ND	ND	ND	6.6	B	+	Basketter et al. 1991
Citral	5392-40-5	AOO	5	0.9	10	2.2	20	6.2	ND	ND	ND	ND	12.0	B	+	Basketter et al. 1991
Citral	5392-40-5	AOO	5	2.2	10	8.1	20	20.5	ND	ND	ND	ND	5.7	B	+	Basketter et al. 1991
Citral	5392-40-5	AOO	5	0.9	10	2.4	20	4.7	ND	ND	ND	ND	12.6	B	+	Basketter et al. 1991
Citral PQ extra	5392-40-5	AOO	5	2.9	10	6.4	25	12.9	ND	ND	ND	ND	5.1	A, C	+	Ashby et al. 1995
Cobalt chloride	7646-79-9	DMSO	0.5	3.2	1	3.7	2.5	2.8	ND	ND	ND	ND	0.4	B	+	Basketter and Scholes 1992
Cobalt chloride	7646-79-9	DMSO	0.5	2.1	1	3.5	2.5	3.8	5	7.2	ND	ND	0.8	A	+	Ikarashi et al. 1992
DNCB	97-00-7	AOO	0.010	1.5	0.025	1.8	0.050	2.4	0.100	8.9	0.250	38.0	0.048	E	+	Gerberick et al. 2005
DNCB	97-00-7	AOO	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.08	N	+	Basketter et al. 2005a
DNCB	97-00-7	AOO	0.010	1.4	0.025	2.2	0.050	4.0	0.100	9.8	0.250	16.2	0.036	C	+	Betts et al. 2006
DNCB	97-00-7	AOO	0.010	2.0	0.025	2.3	0.050	5.3	0.100	10.5	0.250	35.5	0.027	C	+	Kimber et al. 1995
DNCB	97-00-7	AOO	0.010	0.8	0.025	1.8	0.050	3.3	0.100	8.7	0.250	40.9	0.046	C	+	Kimber et al. 1995
DNCB	97-00-7	AOO	0.010	1.1	0.025	1.4	0.050	2.5	0.100	4.6	0.250	11.5	0.062	C	+	Kimber et al. 1995
DNCB	97-00-7	AOO	0.010	0.8	0.025	1.2	0.050	1.7	0.100	3.1	0.250	22.5	0.094	C	+	Kimber et al. 1995
DNCB	97-00-7	AOO	0.010	1.3	0.025	1.5	0.050	2.1	0.100	7.7	0.250	43.9	0.057	C	+	Kimber et al. 1995
DNCB	97-00-7	AOO	0.010	1.5	0.025	1.9	0.050	3.1	0.100	6.5	0.250	25.0	0.05	E	+	Loveless et al. 1996
DNCB	97-00-7	AOO	0.010	1.2	0.025	0.9	0.050	2.9	0.100	4.5	0.250	13.0	0.06	E	+	Loveless et al. 1996
DNCB	97-00-7	AOO	0.010	2.5	0.025	2.9	0.050	3.2	0.100	7.1	0.250	25.0	0.033	E	+	Loveless et al. 1996
DNCB	97-00-7	AOO	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.02	A, C, D	+	Basketter et al. 2007
DNCB	97-00-7	AOO	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.05	A, C, D	+	Basketter et al. 2007
DNCB	97-00-7	AOO	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.03	A, C, D	+	Basketter et al. 2007
DNCB	97-00-7	AOO	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.05	A, C, D	+	Basketter et al. 2007

ICCVAM LLNA Performance Standards – Appendix F3

Substance Name	CASRN	Veh	Dose 1 Conc. (%)	Dose 1 SI	Dose 2 Conc. (%)	Dose 2 SI	Dose 3 Conc. (%)	Dose 3 SI	Dose 4 Conc. (%)	Dose 4 SI	Dose 5 Conc. (%)	Dose 5 SI	EC3 (%)	Rationale for High Dose ¹	LLNA Result	Reference
Ethylene glycol dimethacrylate	97-90-5	MEK	10	1.2	25	2.4	50	7.0	ND	ND	ND	ND	28	N	+	Gerberick et al. 2005
Eugenol	97-53-0	AOO	2.5	1.6	5	1.5	10	2.4	25	5.5	50	16.1	11.9	E	+	Gerberick et al. 2005
Eugenol	97-53-0	AOO	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	9.80	E	+	Loveless et al. 1996
Eugenol	97-53-0	AOO	2.5	2.0	5	2.8	10	3.2	25	13.0	50	17.0	5.80	E	+	Loveless et al. 1996
Eugenol	97-53-0	AOO	2.5	1.6	5	1.5	10	2.4	25	5.5	50	16.0	14.50	E	+	Loveless et al. 1996
Eugenol	97-53-0	AOO	2.5	1.1	5	1.7	10	1.8	25	9.1	50	12.4	8.90	E	+	Loveless et al. 1996
Eugenol	97-53-0	AOO	2.5	2.4	5	2.1	10	1.2	25	5.3	50	9.6	13.80	E	+	Loveless et al. 1996
Eugenol	97-53-0	AOO	2.5	1.5	5	4.3	10	4.6	25	14.0	50	6.1	6.00	E	+	Loveless et al. 1996
Eugenol	97-53-0	AOO	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	15	E	+	Basketter et al. 2007
Eugenol	97-53-0	AOO	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	12.9	E	+	Basketter et al. 2007
Eugenol	97-53-0	AOO	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	4.9	E	+	Basketter et al. 2007
Eugenol	97-53-0	AOO	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	7.5	E	+	Basketter et al. 2007
HCA	101-86-0	AOO	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	8	N	+	Basketter and Kimber 2001
HCA	101-86-0	AOO	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	11	C	+	Dearman et al. 2001
HCA	101-86-0	AOO	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	12.02	C	+	Patelwicz et al. 2001
HCA	101-86-0	AOO	10	3.2	25	6.0	50	10.0	ND	ND	ND	ND	9.40	A, C	+	Ashby et al. 1995
HCA	101-86-0	AOO	2.5	1.3	5	1.1	10	2.5	25	10.0	50	17.0	11	E	+	Loveless et al. 1996
HCA	101-86-0	AOO	2.5	1.3	5	1.1	10	2.5	25	10.0	50	17.0	8.40	E	+	Loveless et al. 1996
HCA	101-86-0	AOO	2.5	1.3	5	2.1	10	2.7	25	7.8	50	13.4	10.6	C	+	Dearman et al. 2001
HCA	101-86-0	AOO	2.5	1.7	5	2.1	10	4.4	25	8.1	50	14.5	6.6	C	+	Dearman et al. 2001
HCA	101-86-0	AOO	2.5	1.7	5	2.1	10	2.4	25	7.2	50	14.1	11.3	C	+	Dearman et al. 2001
HCA	101-86-0	AOO	2.5	2.2	5	3.2	10	7.1	25	13.9	50	17.6	4.4	C	+	Basketter et al. 1999c
HCA	101-86-0	AOO	2.5	1.0	5	1.4	10	2.0	25	8.7	50	11.6	11.5	C	+	Basketter et al. 1999c
HCA	101-86-0	AOO	2.5	1.3	5	1.5	10	4.4	25	8.8	50	16.0	7.60	E	+	Loveless et al. 1996
HCA	101-86-0	AOO	2.5	1.4	5	2.1	10	3.3	25	8.3	50	14.0	7.90	E	+	Loveless et al. 1996
HCA	101-86-0	AOO	2.5	1.1	5	2.2	10	4.4	25	9.8	50	20.0	7.00	E	+	Loveless et al. 1996
HCA	101-86-0	AOO	5	1.6	10	2.5	25	6.8	ND	ND	ND	ND	11.70	C	+	Dearman et al. 2001

Substance Name	CASRN	Veh	Dose 1 Conc. (%)	Dose 1 SI	Dose 2 Conc. (%)	Dose 2 SI	Dose 3 Conc. (%)	Dose 3 SI	Dose 4 Conc. (%)	Dose 4 SI	Dose 5 Conc. (%)	Dose 5 SI	EC3 (%)	Rationale for High Dose ¹	LLNA Result	Reference
HCA	101-86-0	AOO	5	1.4	10	2.7	25	5.3	ND	ND	ND	ND	11.70	C	+	Dearman et al. 2001
HCA	101-86-0	AOO	2.5	1.7	5	2.2	10	2.8	25	8.2	ND	ND	10.60	C	+	Dearman et al. 2001
HCA	101-86-0	AOO	2.5	1.4	5	2.1	10	3.3	25	8.4	50	14.0	8.8	C	+	Dearman et al. 2001
HCA	101-86-0	AOO	2.5	1.0	5	1.4	10	2.0	25	8.7	50	11.6	12.20	C	+	Dearman et al. 2001
HCA	101-86-0	AOO	2.5	1.3	5	1.3	10	4.2	25	8.8	50	17.0	8.10	E	+	Loveless et al. 1996
HCA	101-86-0	AOO	1	1.0	2.5	1.0	5	1.5	10	1.8	25	5.7	14.7	N	+	Basketter et al. 2001
Imidazolidinyl urea	39236-46-9	DMF	10	1.7	25	3.1	50	5.5	ND	ND	ND	ND	24	B	+	Gerberick et al. 2005
Isoeugenol	97-54-1	AOO	0.5	0.7	1	2.3	5	13.8	ND	ND	ND	ND	1	G	+	Basketter and Cadby 2004
Isoeugenol	97-54-1	AOO	0.5	0.8	1	1.6	5	14.1	ND	ND	ND	ND	1.1	G	+	Basketter and Cadby 2004
Isoeugenol	97-54-1	AOO	0.5	0.8	1	2.8	5	5.6	ND	ND	ND	ND	2.1	G	+	Basketter and Cadby 2004
Isoeugenol	97-54-1	AOO	0.5	0.9	1	6.3	5	31.0	ND	ND	ND	ND	0.5	G	+	Basketter and Cadby 2004
Isoeugenol	97-54-1	AOO	0.5	0.9	1	1.0	5	7.2	ND	ND	ND	ND	1.9	G	+	Basketter and Cadby 2004
Isoeugenol	97-54-1	AOO	0.5	1.0	1	1.1	5	12.4	ND	ND	ND	ND	1.2	G	+	Gerberick et al. 2005
Isoeugenol	97-54-1	AOO	0.5	1.0	1	1.3	5	7.5	ND	ND	ND	ND	1.8	G	+	Basketter and Cadby 2004
Isoeugenol	97-54-1	AOO	0.5	1.1	1	1.8	5	23.2	ND	ND	ND	ND	0.8	G	+	Basketter and Cadby 2004
Isoeugenol	97-54-1	AOO	0.5	1.2	1	4.2	5	18.4	ND	ND	ND	ND	0.7	G	+	Basketter and Cadby 2004
Isoeugenol	97-54-1	AOO	0.5	1.2	1	1.4	5	19.3	ND	ND	ND	ND	1.8	G	+	Basketter and Cadby 2004
Isoeugenol	97-54-1	AOO	0.5	1.3	1	2.2	5	13.1	ND	ND	ND	ND	1	G	+	Basketter and Cadby 2004
Isoeugenol	97-54-1	AOO	0.5	1.3	1	3.3	5	14.7	ND	ND	ND	ND	1.5	G	+	Basketter and Cadby 2004
Isoeugenol	97-54-1	AOO	0.5	1.4	1	1.5	5	4.9	ND	ND	ND	ND	2.6	G	+	Basketter and Cadby 2004

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Substance Name	CASRN	Veh	Dose 1 Conc. (%)	Dose 1 SI	Dose 2 Conc. (%)	Dose 2 SI	Dose 3 Conc. (%)	Dose 3 SI	Dose 4 Conc. (%)	Dose 4 SI	Dose 5 Conc. (%)	Dose 5 SI	EC3 (%)	Rationale for High Dose ¹	LLNA Result	Reference
Isoeugenol	97-54-1	AOO	0.5	1.4	1	1.2	5	6.7	ND	ND	ND	ND	2	G	+	Basketter and Cadby 2004
Isoeugenol	97-54-1	AOO	0.5	1.5	1	2.6	5	19.2	ND	ND	ND	ND	0.8	G	+	Basketter and Cadby 2004
Isoeugenol	97-54-1	AOO	0.5	1.5	1	2.5	5	29.8	ND	ND	ND	ND	0.6	G	+	Basketter and Cadby 2004
Isoeugenol	97-54-1	AOO	0.5	1.6	1	2.2	5	7.5	ND	ND	ND	ND	1.6	G	+	Basketter and Cadby 2004
Isoeugenol	97-54-1	AOO	0.5	1.6	1	2.2	5	19.0	ND	ND	ND	ND	0.8	G	+	Basketter and Cadby 2004
Isoeugenol	97-54-1	AOO	0.5	1.6	1	4.3	5	24.4	ND	ND	ND	ND	0.6	G	+	Basketter and Cadby 2004
Isoeugenol	97-54-1	AOO	0.5	1.7	1	1.2	5	5.0	ND	ND	ND	ND	2.6	G	+	Basketter and Cadby 2004
Isoeugenol	97-54-1	AOO	0.5	1.8	1	2.9	5	23.2	ND	ND	ND	ND	0.6	G	+	Basketter and Cadby 2004
Isoeugenol	97-54-1	AOO	0.5	2.0	1	1.4	5	7.6	ND	ND	ND	ND	1.6	G	+	Basketter and Cadby 2004
Isoeugenol	97-54-1	AOO	0.5	2.3	1	1.6	5	23.6	ND	ND	ND	ND	0.6	G	+	Basketter and Cadby 2004
Isoeugenol	97-54-1	AOO	2.5	7.8	5	13.1	10	14.6	ND	ND	ND	ND	1.3	A, B	+	Kimber et al. 1991
Isoeugenol	97-54-1	AOO	2.5	9.9	5	17.0	10	29.5	ND	ND	ND	ND	1.3	A, B	+	Kimber et al. 1991
Isoeugenol	97-54-1	AOO	2.5	4.2	5	11.8	10	21.3	ND	ND	ND	ND	2.2	A	+	Kimber et al. 1991
Isoeugenol	97-54-1	AOO	2.5	7.5	5	13.1	10	25.3	ND	ND	ND	ND	1.4	A, B	+	Kimber et al. 1991
Isoeugenol	97-54-1	AOO	0.25	1.5	0.50	2.2	1	2.5	2.5	4.9	5	10.0	1.3	E	+	Loveless et al. 1996
Isoeugenol	97-54-1	AOO	0.25	1.0	0.50	1.3	1	2.1	2.5	2.3	5	4.1	3.3	E	+	Loveless et al. 1996
Isoeugenol	97-54-1	AOO	0.25	2.9	0.50	1.7	1	2.3	2.5	3.8	5	6.8	1.8	E	+	Loveless et al. 1996
Isoeugenol	97-54-1	AOO	0.25	0.7	0.50	0.7	1	0.9	2.5	2.1	5	7.2	3.1	E	+	Loveless et al. 1996
Isoeugenol	97-54-1	AOO	0.25	1.2	0.50	1.7	1	2.6	2.5	4.3	5	11.0	1.6	E	+	Loveless et al. 1996
Isoeugenol	97-54-1	AOO	0.5	1.8	1	2.9	2.5	7.7	5	11.1	10	11.7	1		+	No reference
Isoeugenol	97-54-1	AOO	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.9	C, E	+	Basketter et al. 2007
Isoeugenol	97-54-1	AOO	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.3	C, E	+	Basketter et al. 2007
Isoeugenol	97-54-1	AOO	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.8	C, E	+	Basketter et al. 2007

Substance Name	CASRN	Veh	Dose 1 Conc. (%)	Dose 1 SI	Dose 2 Conc. (%)	Dose 2 SI	Dose 3 Conc. (%)	Dose 3 SI	Dose 4 Conc. (%)	Dose 4 SI	Dose 5 Conc. (%)	Dose 5 SI	EC3 (%)	Rationale for High Dose ¹	LLNA Result	Reference
Isoeugenol	97-54-1	AOO	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.9	C, E	+	Basketter et al. 2007
Isoeugenol	97-54-1	AOO	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.7	C, E	+	Basketter et al. 2007
Isoeugenol	97-54-1	AOO	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.2	C, E	+	Basketter et al. 2007
Isoeugenol	97-54-1	AOO	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.4	C, E	+	Basketter et al. 2007
Isoeugenol	97-54-1	AOO	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.3	C, E	+	Basketter et al. 2007
Isoeugenol	97-54-1	AOO	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.2	C, E	+	Basketter et al. 2007
Isoeugenol	97-54-1	AOO	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.7	C, E	+	Basketter et al. 2007
Isoeugenol	97-54-1	AOO	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.3	C, E	+	Basketter et al. 2007
Isoeugenol	97-54-1	AOO	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.3	C, E	+	Basketter et al. 2007
Isoeugenol	97-54-1	AOO	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.7	C, E	+	Basketter et al. 2007
Isoeugenol	97-54-1	AOO	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.7	C, E	+	Basketter et al. 2007
Isopropanol	67-63-0	AOO	10	1.7	25	1.1	50	1.0	ND	ND	ND	ND	NC	C	-	Gerberick et al. 2005
Lactic acid	598-82-3	DMSO	5	1.0	10	1.4	25	2.2	ND	ND	ND	ND	NC	C	-	Gerberick et al. 2005
2-Mercapto-benzothiazole	149-30-4	DMF	1	2.3	3	4.4	10	8.6	ND	ND	ND	ND	1.7	A, C	+	Gerberick et al. 2005
Methyl methacrylate	80-62-6	AOO	10	1.4	30	1.5	50	1.5	75	2.1	100	3.6	90	C	+	Betts et al. 2006
Methyl salicylate	119-36-8	AOO	1	1.0	2.5	1.1	5	1.6	10	1.4	20	0.9	NC	C	-	Gerberick et al. 2005
Methyl salicylate	119-36-8	AOO	1	1.1	2.5	1.0	5	1.1	10	1.6	20	1.9	NC	C	-	Kimber et al. 1995
Methyl salicylate	119-36-8	AOO	1	1.8	2.5	2.7	5	2.6	ND	ND	ND	ND	NC	A, B	-	Kimber et al. 1991
Methyl salicylate	119-36-8	AOO	1	1.0	2.5	0.7	5	1.2	ND	ND	ND	ND	NC	C	-	Kimber et al. 1991
Methyl salicylate	119-36-8	AOO	1	1.2	2.5	1.5	5	1.2	10	1.8	20	2.9	NC	C	-	Kimber et al. 1995
Methyl salicylate	119-36-8	AOO	1	2.1	2.5	1.4	5	1.5	10	1.9	20	2.1	NC	C	-	Kimber et al. 1995
Methyl salicylate	119-36-8	AOO	1	0.7	2.5	0.9	5	0.8	10	0.5	20	1.1	NC	C	-	Kimber et al. 1995
Methyl salicylate	119-36-8	AOO	1	1.3	2.5	1.0	5	0.8	ND	ND	ND	ND	NC	C	-	Kimber et al. 1991

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Substance Name	CASRN	Veh	Dose 1 Conc. (%)	Dose 1 SI	Dose 2 Conc. (%)	Dose 2 SI	Dose 3 Conc. (%)	Dose 3 SI	Dose 4 Conc. (%)	Dose 4 SI	Dose 5 Conc. (%)	Dose 5 SI	EC3 (%)	Rationale for High Dose ¹	LLNA Result	Reference
Methyl salicylate	119-36-8	AOO	1	0.9	2.5	1.2	5	1.8	10	1.6	20	2.3	NC	C	-	Kimber et al. 1995
Nickel chloride	7718-54-9	DMSO	0.5	1.0	1	1.7	2.5	2.2	ND	ND	ND	ND	NC	C	-	Basketter et al. 1999b
Nickel chloride	7718-54-9	DMSO	1	1.5	2.5	2.2	5	2.4	ND	ND	ND	ND	NC	B	-	Basketter and Scholes 1992
Phenyl benzoate	93-99-2	AOO	5	2.3	10	2.1	25	3.5	ND	ND	ND	ND	20	N	+	Gerberick et al. 2005
Phenyl benzoate	93-99-2	AOO	1	2.0	2.5	6.4	5	9.3	10	8.7	25	11.1	1.2	C	+	Basketter et al. 1999c
Phenyl benzoate	93-99-2	AOO	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	19.60	F	+	Basketter et al. 2005b
4-Phenylene-diamine	106-50-3	AOO	0.05	2.0	0.10	3.3	0.25	10.2	0.50	20.5	1	26.4	0.10	B, C	+	Gerberick et al. 2004
4-Phenylene-diamine	106-50-3	AOO	0.05	1.9	0.10	2.3	0.25	4.0	0.50	5.7	1	6.6	0.16	C	+	Gerberick et al. 2005
4-Phenylene-diamine	106-50-3	AOO	0.05	2.2	0.10	4.2	0.25	13.7	0.50	20.8	1	25.3	0.07	C	+	Warbrick et al. 1999
4-Phenylene-diamine	106-50-3	AOO	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.09	F	+	Basketter et al. 2005b
4-Phenylene-diamine	106-50-3	AOO	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.07	C	+	Warbrick et al. 1999
4-Phenylene-diamine	106-50-3	AOO	0.05	1.6	0.10	2.6	0.25	5.6	0.50	9.5	1	9.4	0.15	C	+	Warbrick et al. 1999
Salicylic acid	69-72-7	AOO	5	0.8	10	1.5	25	2.5	ND	ND	ND	ND	NC	C	-	Gerberick et al. 2005
Sodium lauryl sulfate	151-21-3	DMF	1	0.9	2.5	1.1	5	1.7	10	2.6	20	3.5	13.40	E	+	Loveless et al. 1996
Sodium lauryl sulfate	151-21-3	DMF	1	1.5	2.5	2.3	5	3.8	10	4.1	20	5.3	4.40	E	+	Loveless et al. 1996
Sodium lauryl sulfate	151-21-3	DMF	1	2.7	2.5	4.2	5	4.6	10	8.9	20	8.6	1.50	E	+	Loveless et al. 1996
Sodium lauryl sulfate	151-21-3	DMF	1	1.6	2.5	2.1	5	2.8	10	1.6	20	3.6	17.10	E	+	Loveless et al. 1996
Sodium lauryl sulfate	151-21-3	DMF	1	1.2	2.5	1.7	5	4.3	10	5.4	20	8.0	4.00	E	+	Loveless et al. 1996

Substance Name	CASRN	Veh	Dose 1 Conc. (%)	Dose 1 SI	Dose 2 Conc. (%)	Dose 2 SI	Dose 3 Conc. (%)	Dose 3 SI	Dose 4 Conc. (%)	Dose 4 SI	Dose 5 Conc. (%)	Dose 5 SI	EC3 (%)	Rationale for High Dose ¹	LLNA Result	Reference
Xylene	1330-20-7	AOO	25	1.3	50	3.0	100	3.1	ND	ND	ND	ND	95.8	C	+	Basketter et al. 1996; Personal Communication (Basketter 2008); Estrada et al. 2003

Abbreviations: AOO = acetone: olive oil (4:1); CASRN = Chemical Abstracts Service Registry Number; Conc. = concentration; DMF = *N,N*-dimethylformamide; DMSO = dimethyl sulfoxide; DNCB = 2,4-dinitrochlorobenzene; EC3 = estimated concentration needed to produce a stimulation index of 3; HCA = hexyl cinnamic aldehyde; LLNA = murine local lymph node assay; MEK = methyl ethyl ketone; NC = not calculated; ND = no data; SI = stimulation index

¹ Rationale for High Dose Key:

A: Kimber and Weisenberger 1989. Test concentrations were determined from data available in the literature and previous experience in this laboratory.

B: Basketter et al. 1991. The choice of test concentrations was based primarily upon previous experience in guinea pig tests and the physical properties of the test material (e.g., solubility and viscosity).

C: Kimber and Basketter 1992. Selected three consecutive concentrations from the following range: 50, 25, 10, 5, 2.5, 1, 0.5, 0.25 and 0.1% (w/v). The selection was made to provide the highest possible test concentration, while avoiding unacceptable dermal trauma or systemic toxicity.

D: Kimber et al. 1995. Test concentrations were selected on the basis of previous experience in these and in other laboratories.

E: Loveless et al. 1996. Test concentrations were selected on the basis of previous experience in these or in other laboratories.

F: Ryan et al. 2000. Test concentrations were chosen to provide the highest possible concentration based on either solubility in the selected vehicle or lack of systemic toxicity of the chemical. For those chemicals that were deemed to be non-sensitizing in man, the highest possible dose, up to 100% in some cases, was used.

G: OECD TG 429 (OECD 2002). Doses were selected from the concentration series 100%, 50%, 25%, 10%, 5%, 2.5%, 1%, 0.5% etc. Three consecutive concentrations were selected so that the highest concentration maximizes exposure whilst avoiding systemic toxicity and excessive local skin irritation.

N: No information; reference did not provide specific information for test chemical preparations.