STUDY

Local Lymph Node Data for the Evaluation of Skin Sensitization Alternatives: A Second Compilation

Petra S. Kern, G. Frank Gerberick, Cindy A. Ryan, Ian Kimber, Aynur Aptula, and David A. Basketter

Background: **Development, evaluation and validation of alternatives to skin sensitisation testing require the availability of reliable databases with which comparative analyses can be conducted to establish performance characteristics. To facilitate this we have published previously a database comprising results from local lymph node assays (LLNAs) conducted with 211 chemicals. That database embraced a substantial range of chemistry, and of relative skin sensitising potency, and has found application in the assessment of new or refined methods.**

Objective: **In this paper we describe a second compilation to extend the LLNA database.**

Methods: **This second data compilation was derived from previously conducted LLNA studies involving an additional 108 chemicals. In addition, the first database contained a small number of inaccuracies, affecting results recorded with a few chemicals. In this paper these have been corrected.**

Results: **The inclusion of 108 new substances has served to extend and consolidate the areas of chemistry covered by the database. In addition, the entire dataset was evaluated for pre and prohaptens which will facilitate the choice of chemicals for alternative assay developments.**

Conclusions: **It is anticipated that the new revised and extended database totalling over 300 chemicals will now serve as the primary resource to support the development and evaluation of new approaches to hazard identification and potency assessment.**

THE EVALUATION of the potential for inducing allergic contact dermatitis is an important component of the overall safety assessment process for chemicals that may be encountered through the skin.[1] For many years, the guinea pig was the animal of choice for the identification of skin-sensitizing hazard (ie, the intrinsic property of a chemical to cause skin sensitization). More recently, however, the local lymph node assay (LLNA) has been accepted as an alternative approach in which activity is measured as a function of proliferative responses in draining lymph nodes induced by the topical exposure of mice to chemicals.[2–5] The Organization for Economic Cooperation and Development (OECD) has adopted the LLNA (as Guideline 429) as a stand-alone method for skin sensitization testing.[6] This adoption resulted from exhaustive independent validations of the LLNA in both the United States (Interagency Coordination Committee on the Validation of Alternative Methods [ICCVAM], 1999)[7] and Europe (European Centre for the Validation of Alternative Methods [ECVAM]).[8] It was subsequently also shown that, in addition to providing an accurate identification of hazard, the LLNA can provide a reliable measure of relative skin-sensitizing potency, information that is critical for effective risk assessment (ie, determination of whether exposure to a hazard is sufficient to cause a potential human health problem).[9] The relative potency of a skin-sensitizing chemical is measured by derivation of an EC3 value, which is the concentration of a test chemical necessary to produce a threefold increase in lymph node cell proliferation compared with concurrent vehicle controls (ie, a threshold positive response).[10] This approach has already been applied with some success, and the LLNA is now the preferred method for estimating the relative sensitizing potency of contact allergens.[11,12]

One critical requirement for the development of alternative test methods is the availability of robust in vivo data to calibrate, evaluate, and ultimately validate new approaches. We previously prepared and presented one substantial database of LLNA results.[13] In the present article, we describe the compilation of a further LLNA data set that extends the breadth and depth of coverage of a

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range of chemistries and skin-sensitizing activity. All chemicals have been evaluated in the LLNA; for some, it has been demonstrated that the LLNA EC3 value correlates closely with what is known of their relative ability to induce sensitization in humans.[14–20] In addition, a number of generally minor corrections to the original data set published in this journal in 2005 are presented in this article.

Materials and Methods

Chemicals

The chemicals identified in this article were evaluated for skin sensitization potential with the LLNA. Table 1 specifies the name, Chemical Abstracts Service (CAS) number, and two-dimensional structure of each chemical, together with its molecular weight, the concentrations tested, and the stimulation indices. The structures were drawn with *ChemDraw Ultra* version 7.03 (CambridgeSoft, Cambridge, MA).

Local Lymph Node Assay Protocol

The LLNA was conducted as described elsewhere.[2–6] Briefly, groups of CBA female mice (7–12 weeks of age) were exposed topically on the dorsum of both ears to 25 μL of test material or to an equal volume of the relevant vehicle alone. Treatment was performed daily for 3 consecutive days. Five days after the initiation of exposure, all mice were injected via the tail vein with 250 μL of phosphate buffered saline (PBS) containing 20 μCi of tritiated thymidine. Mice were sacrificed 5 hours later, and the draining auricular lymph nodes were excised and pooled for each experimental group or each individual animal. The incorporation of tritiated thymidine measured by beta scintillation counting was reported in disintegrations per minute (dpm). A stimulation index (SI) was calculated for each chemical-treated group as the ratio of the dpm of the treated group (or mean dpm when individual animals were assessed) to the dpm or mean dpm of the concurrent vehicle control group. LLNA methodology assesses skin sensitization, not photosensitization. A substance was classified as a skin sensitizer if it induced a threefold or greater increase in local lymph node proliferative activity at one or more test concentrations when compared with concurrent vehicle-treated controls ($SI \geq 3$). The compilation of data from numerous laboratories is reported in this article. The data are derived from previously conducted studies that were undertaken in accordance with Good Laboratory Practice principles and that were conducted and interpreted with the methods described in OECD Guideline 429.[7] References for the sources of LLNA data for each of the chemicals are provided in Table 1.

Potency Estimation in the LLNA

Dose response data were used to measure the relative skin sensitization potency of all of the chemicals that were positive. This approach, previously described in detail,[10] is centered on the EC3 value. When the LLNA dose-response curve included concentrations that induced at least one SI greater than 3 and one SI less than 3, EC3 values were calculated by linear interpolation. For chemicals that induced an SI greater than or equal to 3 at all concentrations tested, an EC3 value was extrapolated from the two lowest doses used.[21] For this extrapolation method to work, a dose response should be evident. The relative sensitizing potencies of the chemical allergens were categorized via a recently proposed arbitrary classification scheme.[11] This system, shown in Table 2, consists of four sensitization potency categories based on EC3 values. Compounds that did not induce a threefold increase at any concentration tested (generally $> 20\%$) have been categorized as nonsensitizing.

Results

Skin Sensitization Data Set

Chemical Information

Table 1 lists 108 chemicals along with their respective CAS numbers, two-dimensional chemical structures, molecular weights and concentrations tested, SIs, and calculated EC3 values. The LLNA results in this database were obtained from studies conducted with the standard LLNA protocol as described in OECD Guideline 429.[7] The specific reference for the source of the LLNA data for each chemical is indicated in Table 1. In the few cases in which there were multiple results, the data shown in Table 1 derive from a single representative experiment that we feel reflects accurately the results obtained with the chemical. When the chemical was tested in numerous vehicles, the data set for 4:1 volume per volume acetone and olive oil (AOO) was selected because it is the first-choice vehicle of the OECD test guideline. Furthermore, the results of a

Table 1. Chemical Structures, Molecular Weights, LLNA Data, Potency Categorizations, and Reaction Mechanistic Domains

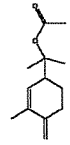
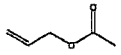
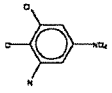
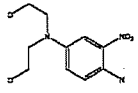
Chemical Structure	*CAS No.*	*MW*	*Vehicle*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA EC3 %*	*Potency Category*	*Reaction Mechanistic Domain*	*Reference**
8-Acetoxycarvotanacetone 	87578-93-6	208.30	Acetone	1.0	5.0	10.0	20.0	40.0	60.0	0.71	0.82	0.68	0.96	1.19	0.81	NC	Non-sensitizer	NR	P&G (unpublished)
Acetyl cedrene 	32388-55-9	246.39	1:3 E:D	2.5	5.0	10.0	25.0	50.0		1.6	1.7	2.4	4.7	5.0		13.9	Weak	NR	34
Allyl acetate 	591-87-7	100.1	AOO	2.5	5.0	10.0	25.0			1.4	1.8	1.2	0.8			NC	Non-sensitizer	S_N2	35
2-Amino-6-chloro-4-nitrophenol 	6358-09-4	188.57	DMSO	0.5	1.5	5.0	10.0			1.2	1.2	2	4.7			6.85	Moderate	Pro/Pre-MA	36
5-Amino-2-methylphenol 	2835-95-2	123.15	DMSO	0.5	1.5	3.0	5.0			2.6	2.4	2.8	3.9			3.4	Moderate	Pro/Pre-MA	37
4-Amino-3-methyl-phenol 	2835-99-6	123.15	DMSO	0.5	1.5	5	10			0.9	3.1	6.5	6.7			1.45	Moderate	Pro/Pre-MA	38
1-Amino-2-nitro-4-bis-(2-hydroxyethyl)-amino-benzol 	29705-39-3	241.24	DMSO	0.5	1.5	5.0	10.0			1.5	1.8	2.1	3.5			8.2	Moderate	Pro/Pre-MA	39

Table 1. Continued

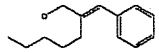
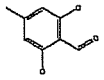
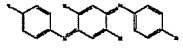
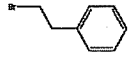
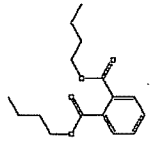
Chemical Structure	*CAS No.*	*MW*	*Vehicle*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA EC3 %*	*Potency Category*	*Reaction Mechanistic Domain*	*Reference**
4-Amino-3-nitrophenol 	610-81-1	154.12	AOO	0.05	0.1	0.5	1.0	2.5		1.7	1.8	6.9	8.9	27.7		0.2	Strong	Pro/Pre-MA	40
α-Amylcinnamyl alcohol 	101-85-9	204.31	1:3 E:D	1.0	2.5	5.0	10.0	25.0		1.2	0.8	1.4	1.7	2.9		~25	Non-sensitizer	NR	41
Anisyl alcohol 	105-13-5	138.17	1:3 E:D	2.5	5.0	10.0	25.0	50.0		1.8	2.8	3.9	5.1	5.3		5.9	Moderate	Pro/Pre-MA	42
Atranol 	526-37-4	152.15	1:3 E:D	0.5	1.0	2.5	5.0	10.0		2.4	5.5	6.9	8.2	11.4		0.6	Strong	Pro/Pre-MA or SB	43
Bandrowski's base 	20048-27-5	318.38	AOO	0.01	0.025	0.05	0.1	0.25		1.1	3.1	5.7	6.5	5.6		0.04	Extreme	Pro/Pre-MA	44
Benzene, (2-bromoethyl)- 	103-63-9	185.1	AOO	5.0	25.0	50.0				2.0	18.9	20.8				6.2	Moderate	S_N2	35
1,2-Benzenedicarboxylic acid, dibutyl ester 	84-74-2	278.3	AOO	10.0	25.0	50.0				1.4	1.8	2.2				NC	Non-sensitizer	NR	35

Table 1. Continued

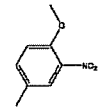
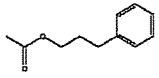
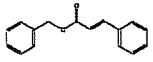
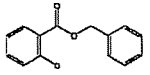
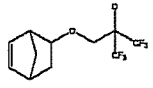
Chemical Structure	*CAS No.*	*MW*	*Vehicle*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA EC3 %*	*Potency Category*	*Reaction Mechanistic Domain*	*Reference**
Benzene, 1-methoxy-4-methyl-2-nitro- 	119-10-8	167.2	AOO	10.0	25.0	50.0				1.1	1.7	2.2				NC	Non-sensitizer	NR	35
Benzenepropanol, acetate 	122-72-5	178.2	AOO	10.0	25.0	50.0				0.8	1.2	2.7				NC	Non-sensitizer	NR	35
Benzyl alcohol 	100-51-6	108.14	1:3 E:D	2.5	5.0	10.0	25.0	50.0		1.0	0.9	0.5	0.6	1.2		NC	Non-sensitizer	NR	45
Benzyl cinnamate 	103-41-3	238.29	1:3 E:D	2.5	5.0	10.0	25.0	50.0		0.8	1.9	2.1	3.7	5.9		18.4	Weak	MA	45
Benzyl salicylate 	118-58-1	228.25	1:3 E:D	2.5	5.0	10.0	25.0	50.0		2.6	5.5	6.0	18.9	26.2		2.9	Moderate	S_N2	45
2[(bicyclo[2.2.1]hept-5-ene-2-yloxy)methyl]-1,1,1,3,3,3-hexafluoro-2-propanol (norbornene fluoroalcohol) 	305815-63-8	290.2	AOO	5.0	10.0	25.0	50.0	100		0.7	0.8	1.9	3.2	3.7		46	Weak	NR	46

Table 1. Continued

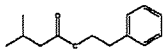
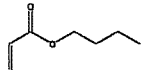
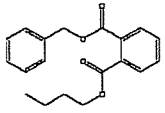
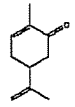
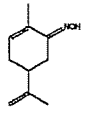
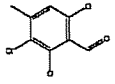
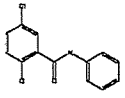
Chemical Structure	*CAS No.*	*MW*	*Vehicle*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA EC3 %*	*Potency Category*	*Reaction Mechanistic Domain*	*Reference**
Butanoic acid, 3-methyl-, 2-phenylethyl ester 	140-26-1	206.3	AOO	10.0	25.0	50.0				2.1	1.9	3.8				39.5	Weak	NR	35
Butyl acrylate 	141-32-2	128.17	AOO	1.0	2.5	5.0	10.0	25.0		0.7	1.3	1.4	2.5	8.7		11	Weak	MA	47
Butylbenzylphthalate 	85-68-7	312.4	AOO	0.5	5.0	50.0				1.2	1.5	2.5				NC	Non-sensitizer	S_N2	35
R-Carvone 	6485-40-1	150.22	AOO	6.0	12.0	20.0				1.3	2.6	6.2				12.9	Weak	MA	48
R-Carvoxime 	Not known	165.23	AOO	0.1	1.0	5.0				2.1	3.7	8.1				0.6	Strong	Pro/Pre-MA	48
Chloroatranol 	57074-21-2	186.59	1:3 E:D	0.25	0.5	1.0	2.5	5.0		2.0	3.3	5.1	7.8	5.0		0.4	Strong	Pro/Pre-MA or SB	49
5-Chlorosalicylanilide 	4638-48-6	247.68	DMSO	5.0	10.0	20.0	30.0			3.0	3.8	4.9	6.5			5	Moderate	Special case†	P&G (unpublished)

Table 1. Continued

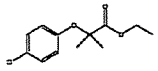
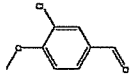
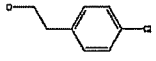
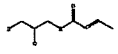
Chemical Structure	*CAS No.*	*MW*	*Vehicle*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA EC3 %*	*Potency Category*	*Reaction Mechanistic Domain*	*Reference**
Chlorothalonil 	1897-45-6	265.91	DMF	0.003	0.01	0.03	0.1	0.3		2.1	9.4	13.8	18.4	27.2		0.004	Extreme	S_NAr	50
d,l-Citronellol 	106-22-9	156.27	1:3 E:D	2.5	5.0	10.0	25.0	50.0		1.6	1.2	1.0	1.3	3.6		43.5	Weak	S_N1	51
Clofibrate 	637-07-0	242.70	Acetone	10.0	25.0	50.0				2.1	1.7	2.9				NC	Non-sensitizer	NR	P&G (unpublished)
3-Chloro-p-anisaldehyde 	4903-09-7	170.6	AOO	10.0	25.0	65.0				1.6	1.8	1.2				NC	Non-sensitizer	SB	35
1-Chloro-3-iodopropane 	6940-76-7	204.4	AOO	10.0	25.0	50.0				1.0	1.3	1.0				NC	Non-sensitizer	S_N2	35
2-Chloro-6-nitrotoluene 	83-42-1	171.6	AOO	1.0	10.0	25.0				1.2	0.3	1.5				NC	Non-sensitizer	NR	35
p-Chlorophenethylic alcohol 	1875-88-3	156.6	AOO	10.0	25.0	50.0				1.6	2.8	3.6				31.3	Weak	NR	35
Crotonyl thioglycerol 	Not known	176.23	70% EtOH	10	25	50				1.8	1	1.2				NC	Non-sensitizer	MA	P&G (unpublished)

Table 1. Continued

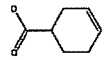
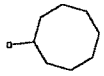
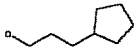
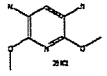
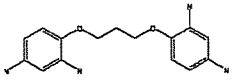
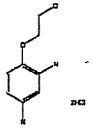
Chemical Structure	*CAS No.*	*MW*	*Vehicle*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA EC3 %*	*Potency Category*	*Reaction Mechanistic Domain*	*Reference**
Cyclohex-3-ene-1-carboxylic acid 	4771-80-6	126.1	AOO	5.0	10.0	25.0				1.8	3.2	17.3				9.3	Moderate	NR	35
Cyclooctanol 	696-71-9	128.2	AOO	10.0	25.0	50.0				1.2	1.4	1.6				NC	Non-sensitizer	NR	35
Cyclopentanepropanol 	767-05-5	128.2	AOO	10.0	25.0	50.0				1.4	2.1	2.1				NC	Non-sensitizer	NR	35
3,5-Diamino-2,6-dimethoxypyridine-dihydrochloride 	56216-28-5	242.11	AOO	0.5	1.5	5.0	10.0			1.2	1.3	2.7	3.5			6.9	Moderate	Pro/Pre-MA	52
1,3-Bis-(2,4-diaminophenoxy)-propane x 4HCl (HC Blue 16) 	74918-21-1	434.19	70% EtOH	5.0	25.0	50.0				1.2	4.9	4.3				14.7	Weak	Pro/Pre-MA	53
2,4-Diaminophenoxyethanol dihydrochloride 	66422-95-5	241.12	AOO	1	2.5	5.0	10.0	25.0		1.6	1.6	2.7	5.7	8.3		5.5	Moderate	Pro/Pre-MA	Unilever (unpublished)
2,5-Diaminotoluene sulfate 	615-50-9	220.25	AOO	0.5	1.5	2.8				4.4	10.4	19.4				0.4‡	Strong	Pro/Pre-MA	54

Table 1. Continued

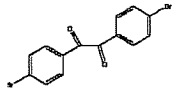
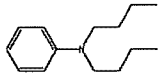
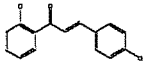
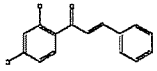
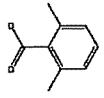
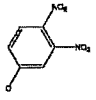
Chemical Structure	*CAS No.*	*MW*	*Vehicle*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA EC3 %*	*Potency Category*	*Reaction Mechanistic Domain*	*Reference**
4,4-Dibromobenzil 	35578-47-3	368.02	AOO	5.0	10.0	25.0	50.0			1.5	1.6	3.6	5.7			20.5	Weak	SB	Unilever (unpublished)
N,N-Dibutylaniline 	613-29-6	205.3	AOO	5.0	10.0	50.0				2.0	1.9	6.5				19.6	Weak	Pro/Pre-SB	35
N,N-Diethyl-m-toluamide 	134-62-3	191.27	Acetone	1.0	5.0	10.0	20.0	40.0	60.0	0.4	0.5	0.5	0.5	1.1	1.3	NC	Non-sensitizer	NR	P&G (unpublished)
2′-4-Dihydroxychalcone 	13323-66-5	240.25	DMSO	1.0	10.0	20.0				2.2	2.0	2.4				NC	Non-sensitizer	MA	P&G (unpublished)
2′,4′-Dihydroxylchalcone 	1776-30-3	240.26	DMSO	1.0	10.0	20.0				3.4	4.7	5.9				0.56‡	Strong	MA	P&G (unpublished)
2,6-Dimethylbenzoic acid 	632-46-2	149.2	AOO	5.0	10.0	35.0				1.2	1.0	1.5				NC	Non-sensitizer	NR	35
3,4-Dinitrophenol 	577-71-9	184.1	AOO	5.0	10.0	25.0				5.2	7.6	12.7				2.6‡	Moderate	Special case†	35

Table 1. Continued

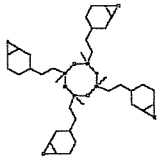
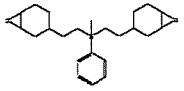
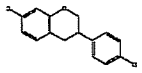
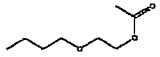
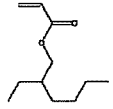
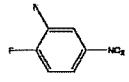
Chemical Structure	*CAS No.*	*MW*	*Vehicle*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA EC3 %*	*Potency Category*	*Reaction Mechanistic Domain*	*Reference**
3,4-Epoxyclohexylethyl-cyclopoly-methylsiloxane (Tet-sil) 	121225-98-7	753.29	AOO	50.0	100					1.2	1.2					NC	Non-sensitizer	S_N2	55
Bis-3,4-epoxycyclohexyl-ethyl-phenyl-methylsilane (Ph-Sil) 	154265-59-5	370.61	AOO	25.0	35.0	50.0				3.7	4.2	7.9				15.6‡	Weak	S_N2	55
Equol 	531-95-3	242.27	100% EtOH	0.5	5.0	15.0	40.0			1.1	1.4	1.7	2.0			NC	Non-sensitizer	NR	P&G (unpublished)
Ethanol, 2-butoxy-, acetate 	112-07-2	160.2	AOO	10.0	25.0	50.0				1.2	0.7	1.2				NC	Non-sensitizer	NR	35
2-Ethylhexyl acrylate 	103-11-7	184.28	AOO	0.5	1.0	2.5	5.0	10.0		1.1	1.2	0.9	1.2	3.1		10	Weak	MA	47
2-Fluoro-5-nitroaniline 	369-36-8	156.1	AOO	5.0	10.0	25.0	40.0			1.5	1.5	1.6	1.2			NC	Non-sensitizer	NR	35

Table 1. Continued

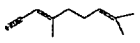
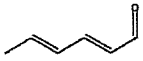
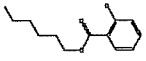
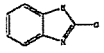
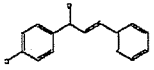
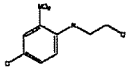
Chemical Structure	*CAS No.*	*MW*	*Vehicle*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA EC3 %*	*Potency Category*	*Reaction Mechanistic Domain*	*Reference**
Geranyl nitrile 	5146-66-7	149.24	1:3 E:D	2.5	5.0	10.0	2.5.0	50.0		2.0	1.5	1.5	1.8	2.8		NC	Non-sensitizer	NR	56
2-(Hexadecyloxy)ethanol 	2136-71-2	286.5	AOO	2.5	10.0	20.0				1.4	3.3	3.7				8.8	Moderate	NR	35
2,4-Hexadienal 	142-83-6	96.13	AOO	0.5	1.0	2.5	5.0	10.0		0.9	1.5	2.2	4.2	14.8		3.5	Moderate	MA	Unilever (unpublished)
Hexyl salicylate 	6259-76-3	222.28	1:3 E:D	0.05	0.25	0.5	1.0	2.5		1.9	3.6	5.6	10.8	11.8		0.18	Strong	Special case[†]	57
2-Hydroxybenzothiazole 	934-34-9	151.19	DMF	0.7	3.5	14.0	28.0			0.9	0.88	0.91	0.72			NC	Non-sensitizer	NR	P&G (unpublished)
4′-Hydroxychalcone 	2657-25-2	224.26	DMSO	1.0	10.0	20.0				8.6	10.6	10.8				0.002[‡]	Extreme	MA	P&G (unpublished)
4-((2-Hydroxyethyl)amino)-3-nitrophenol 	65235-31-6	198.18	DMF	0.03	0.09	0.3	0.8	2.5		2.2	3.5	6.4	7.6	11.2		0.07	Extreme	Pro/Pre-MA	58

Table 1. Continued

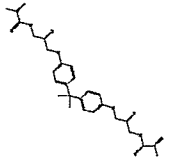
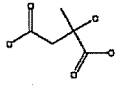
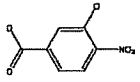
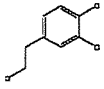
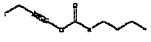
Chemical Structure	*CAS No.*	*MW*	*Vehicle*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA EC3 %*	*Potency Category*	*Reaction Mechanistic Domain*	*Reference**
2,2-Bis-[4-(2-hydroxy-3 methacryloxypropoxy)phenyl)]-propane (Bis-GMA) 	1565-94-2	512.65	AOO	35.0	75.0					2.0	5.9					45	Weak	MA	55
(s)-2-Hydroxy-2-methylsuccinic acid 	6236-09-5	148.1	AOO	10.0	25.0	45.0				1.8	1.4	1.2				NC	Non-sensitizer	NR	35
3-Hydroxy-4-nitrobenzoic acid 	619-14-7	183.1	DMSO	10.0	25.0	40.0				1.8	1.7	1.7				NC	Non-sensitizer	NR	35
3-Hydroxy-2-nitropyridine 	15128-82-2	140.10	DMF	0.5	5.0	10.0	26.0			1.0	1.7	1.2	1.3			NC	Non-sensitizer	NR	P&G (unpublished)
Hydroxytyrosol 	10597-60-1	154.16	100% EtOH	0.01	0.1	0.5	5.0			1.0	1.4	2.6	18.6			0.6	Strong	Pro/Pre-MA	P&G (unpublished)
Iodopropynyl butylcarbamate 	55406-53-6	281.09	AOO	0.1	1.0	5.0	10.0			0.7	3.4	4.2	12.0			0.9	Strong	S_N2	59

Table 1. Continued

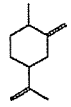
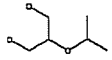
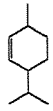
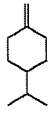
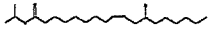
Chemical Structure	*CAS No.*	*MW*	*Vehicle*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA EC3 %*	*Potency Category*	*Reaction Mechanistic Domain*	*Reference**
(1R,4R)-4-Isopropenyl-1-methyl-2-methylenecyclohexane 	Not known	148.27	AOO	1.0	5.0	10.0	15.0	25.0		1.3	1.8	1.2	2.3	2.9		NC	Non-sensitizer	NR	60
(5R)-5-Isopropenyl-2-methyl-1-methylene-2-cyclohexene 	Not known	150.27	AOO	0.5	5.0	15.0				0.9	1.9	6.6				7.3	Moderate	NR	61
Isopropyl glycerol ether 	17226-43-6	134.17	dH2O	60.0	80.0	100				1.1	1.4	0.8				NC	Non-sensitizer	NR	P&G (unpublished)
(3S,6R)-3-Isopropyl-6-methylcyclohexene 	5113-93-9	138.25	AOO	1.0	10.0	25.0				0.8	1.0	2.9				NC	Non-sensitizer	NR	60
4-Isopropyl-1-methylenecyclohexane 	Not known	138.26	AOO	1.0	10.0	25.0				1.2	0.7	1.4				NC	Non-sensitizer	NR	60
Isopropyl Ricinoleamide 	Not known	339.56	Acetone	1.0	5.0	10.0	20.0			1.2	1.1	1.5	1.3			NC	Non-sensitizer	NR	P&G (unpublished)

Table 1. Continued

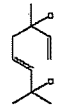
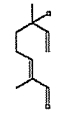
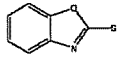
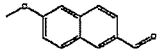
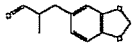
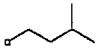
Chemical Structure	*CAS No.*	*MW*	*Vehicle*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA EC3 %*	*Potency Category*	*Reaction Mechanistic Domain*	*Reference**
Linalool alcohol 	Not known	170.25	AOO	1.0	10.0	30.0				1.0	1.3	1.3				NC	Non-sensitizer	NR	62
Linalool aldehyde 	Not known	168.24	AOO	1.0	5.0	15.0				1.2	2.0	4.2				9.5	Moderate	MA	62
2-Mercaptobenzoxazole 	2382-96-9	151.19	DMF	0.7	3.2	15.0	30.0			0.6	0.8	0.7	1.2			NC	Non-sensitizer	Pro/Pre-S_N2	P&G (unpublished)
6-Methoxynaphthalene-2-carbaldehyde 	3453-33-6	186.21	AOO	5.0	10.0	20.0				1.6	1.6	1.7				NC	Non-sensitizer	SB	35
Methyl acrylate 	96-33-3	86.09	AOO	1.0	2.5	5.0	10.0	25.0		0.8	0.8	1.3	1.6	3.8		20	Weak	MA	47
α-Methyl-1,3-benzodioxole-5-propionaldehyde 	1205-17-0	192.21	1:3 E:D	2.5	5.0	10.0	25.0	50.0		1.0	2.7	2.4	3.8	8.3		16.4	Weak	SB	63
3-Methyl-1-butanol 	123-51-3	88.15	AOO	10.0	25.0	50.0				0.9	0.8	1.0				NC	Non-sensitizer	NR	35

Table 1. Continued

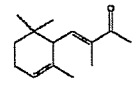
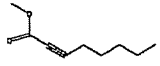
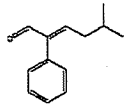
Chemical Structure	*CAS No.*	*MW*	*Vehicle*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA EC3 %*	*Potency Category*	*Reaction Mechanistic Domain*	*Reference**
α-iso-Methylionone 	127-51-5	206.33	1:3 E:D	2.5	5.0	10.0	25.0	50.0		0.6	0.6	1.5	3.4	4.6		21.8	Weak	MA	64
Methyl methacrylate 	80-62-6	100.12	AOO	10.0	30.0	50.0	75.0	100		1.4	1.5	1.5	2.1	3.6		90	Weak	MA	65
(4Z)-2-Methyl-6-methyleneoct-4-ene 	Not known	138.26	AOO	1.0	5.0	10.0	15.0	25.0		1.1	0.9	0.8	0.9	2.1		NC	Non-sensitizer	NR	60
Methyl 2-octynoate 	111-12-6	154.21	1:3 E:D	0.05	0.1	0.25	0.5	1.0		1.7	1.7	1.8	3.3	8.7		0.45	Strong	MA	66
5-Methyl-2-phenyl-2-hexenal 	21834-92-4	188.27	AOO	0.5	1.0	2.5	5.0	10.0		1.0	1.3	0.5	3.8	17.7		4.4	Moderate	SB or MA	Unilever (unpublished)
3-Methyl-1-phenylpyrazolone 	89-25-8	174.20	DMSO	0.03	0.3	3.0	30.0			1.0	1.0	2.2	6.3			8.5	Moderate	Special case†	P&G (unpublished)
Methyl pyruvate 	600-22-6	102.09	AOO	1.0	2.5	5.0	10.0			1.2	3.1	4.7	8.0			2.4	Moderate	SB	67

Table 1. Continued

Chemical Structure	*CAS No.*	*MW*	*Vehicle*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA EC3 %*	*Potency Category*	*Reaction Mechanistic Domain*	*Reference**
Dimethyl sulfone	67-71-0	94.13	DMSO	1.0	10.0	25.0				1.2	1.5	0.7				NC	Non-sensitizer	NR	P&G (unpublished)
4-Nitro-benzene-1,2-diamine	99-56-9	153.14	AOO	0.01	0.025	0.075	0.125	0.2		1.0	1.5	2.2	2.0	1.8		0.05‡	Extreme	Pro/Pre-MA	68
Nonanoyl amido caproylacid oxybenzenesulfonate	168151-92-6	449.54	dH2O	5.0	10.0	15.0	20.0			1.7	1.4	3.3	4.2			14.2	Weak	Acyl	69
Octanenitrile	124-12-9	125.2	AOO	10.0	25.0	50.0				1.0	1.1	1.4				NC	Non-sensitizer	NR	35
2,2′-Oxybisethanol	111-46-6	106.1	AOO	5.0	10.0	25.0				1.4	1.9	0.7				NC	Non-sensitizer	NR	35
α-Phellandrene	99-83-2	136.23	AOO	1.0	10.0	25.0				1.1	5.0	28.0				5.4	Moderate	Special case†	60
β-Phellandrene	555-10-2	136.23	AOO	1.0	10.0	20.0				1.1	4.8	23				5.6	Moderate	Special case†	60
Phenethyl alcohol	60-12-8	122.17	1:3 E:D	2.5	5.0	10.0	25.0	50.0		1.1	1.0	0.9	0.9	0.8		NC	Non-sensitizer	NR	45

Table 1. Continued

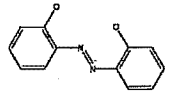
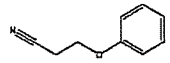
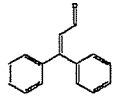
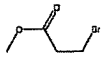
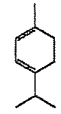
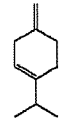
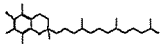
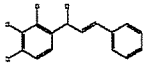
Chemical Structure	*CAS No.*	*MW*	*Vehicle*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA EC3 %*	*Potency Category*	*Reaction Mechanistic Domain*	*Reference**
Phenol, 2,2-azobis- 	2050-14-8	214.2	DMSO	10.0	25.0	50.0				2.7	2.7	5.3				27.9	Weak	NR	35
3-Phenoxypropiononitrile 	3055-86-5	147.2	AOO	10.0	25.0	49.0				1.0	0.9	0.8				NC	Non-sensitizer	NR	35
β-Phenylcinnamaldehyde 	1210-39-5	208.26	AOO	0.1	0.25	0.5	1.0	2.5		2.0	2.3	1.9	5.9	10.6		0.6	Strong	MA	Unilever (unpublished)
Propanoic acid, 3-bromo-, methyl ester 	3395-91-3	167.0	AOO	10.0	25.0	50.0				1.1	0.9	1.3				NC	Non-sensitizer	S_N2	35
α-Terpinene 	99-86-5	136.23	AOO	1.0	5.0	10.0	15.0	25.0		1.1	1.5	3.4	8.9	23		8.9	Moderate	Special case[†]	60
β-Terpinene 	Not known	136.24	AOO	1.0	10.0	25.0				1.4	1.3	2.1				NC	Non-sensitizer	Special case[†]	60

Table 1. Continued

Chemical Structure	*CAS No.*	*MW*	*Vehicle*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA EC3 %*	*Potency Category*	*Reaction Mechanistic Domain*	*Reference**
dl-α-Tocopherol 	10191-41-0	430.71	3:1 E:D	0.3	1.0	3.0	10.0	30.0		0.6	0.8	1.1	4.2	6.7		7.4	Moderate	NR	70
Trans-2-methyl-2-butenal 	497-03-0	84.12	AOO	10.0	25.0	50.0				1.5	1.0	2.8				NC	Non-sensitizer	MA	Unilever (unpublished)
Tridecane 	629-50-5	184.4	AOO	10.0	25.0	50.0				1.3	1.5	3.1				48.4	Weak	NR	35
2′,3′,4′-Trihydroxy chalcone 	1482-74-2	256.25	DMSO	1.0	10.0	20.0				4.4	5.9	5.4				0.11‡	Strong	MA	P&G (unpublished)
2,4,6-Trimethyl-3-cyclohexene-1-methanol 	68527-77-5	154.25	1:3 E:D	1.0	2.5	5.0	10.0	25.0		1.0	1.4	2.0	1.5	2.3		NC	Non-sensitizer	NR	71
Tropolone 	533-75-5	122.12	DMF	0.5	5.0	10.0	20.0			1.0	3.3	5.2	ND			4.3	Moderate	MA or S_N1	P&G (unpublished)

Acyl = acylating agents; AOO = acetone and olive oil (4:1); CAS = Chemical Abstracts Service; COLIPA = European Cosmetic, Toiletry and Perfumery Association; dH2O = distilled water; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EC3 = mathematically estimated concentration of test chemical necessary to induce a threefold stimulation index; E:D = ethanol:diethyl phthalate; EtOH = ethanol; HCl = hydrochloride; LLNA = local lymph node assay (LLNA % = weight per volume concentration); MA = Michael acceptor; MW = molecular weight; NC = not calculated; ND = not determined; NR = nonreactive; P&G = Procter & Gamble Co.; Pro/Pre = not direct-acting electrophiles but able to be converted to electrophiles by well-established transformations (abiotic or metabolic); RIFM=Research Institute for Fragrance Materials; SB = Schiff base formers; SCCP = Scientific Committee for Consumer Products; SI = stimulation index; S_N = nucleophilic substitution reaction; S_N1 = S_N1 electrophiles; S_N2 = S_N2 electrophiles; S_NAr = S_NAr electrophiles.

*Numbers denote numbered references in References section.

†Chemical does not fit any of the listed domains.

‡Value is estimated.

small number of cases in which the substance was not tested in a recommended OECD vehicle have been included. Nevertheless, 62 of the 108 chemicals were tested in AOO, and approximately 75% were tested in one of the OECD recommended vehicles. Many of the other chemicals were fragrance raw materials evaluated in a mixture of ethanol and diethyl phthalate and for which human test data will be published in due course. As previously reported for the original data set, a little over half of the present chemicals range in molecular weight from 100 to 200 D. Sensitizing and nonsensitizing chemicals in the list span a similar range of molecular weight from 84 to 753 D for the negatives and from 86 to 513 D for the positives. It is evident from surveying the structures themselves that the data set embraces the wide chemical diversity known to exist among skin allergens and enhances that presented in the earlier publication.[13]

Biologic Data

Of the 108 substances, 46 (43%) were not regarded as sensitizing whereas 62 (57%) gave positive results. The nonsensitizing materials included materials that did not give a positive response in the LLNA at up to the highest dose tested (generally > 20%). For positive chemicals in the LLNA database, EC3 values ranged from as low as 0.002% for 4′-hydroxychalcone to as high as 90% for methyl methacrylate. Table 1 includes a limited number of EC3 values estimated by the extrapolation method and indicated with a footnote. Caution should be used in interpreting and using extrapolated EC3 values because these have inevitably been estimated from less-than-ideal dose response data. However, we feel that these data can still provide important benchmark information to investigators developing alternative skin sensitization test methods. A good example of this is the identification of the extreme potency of 4′-hydroxychalcone by extrapolation of the dose response. It would not be helpful (and arguably would be quite misleading) simply to identify 4′-hydroxychalcone as a skin sensitizer and to fail to recognize that it is also a highly potent one. Overall, and using the same potency categorizations as in the previous publication (Table 2), the data set includes 21 weak, 24 moderate, 12 strong, and 5 extreme skin sensitizers, as well as 46 nonsensitizers.

Table 2. Classification of Relative Skin Sensitization Potency

EC3 Value (%)	*Potency Classification*
≥ 10 to ≤ 100	Weak
≥ 1 to < 10	Moderate
≥ 0.1 to < 1	Strong
< 0.1	Extreme

EC3 = mathematically estimated concentration of test chemical necessary to induce a threefold stimulation index.

An additional point to note is that there is now in this new data set a number of chemicals that have an EC3 value greater than 20%, indicating that they are at the weakest end of the sensitization classification spectrum; this was not the case with the original data set. The inclusion of these substances now provides a more realistic opportunity for a critical analysis of the sensitivity of in vitro alternatives in relation to the weakest sensitizers. Furthermore, the extra data ensure that there is a comprehensive selection of prohaptens and prehaptens. Table 1 lists the theoretical chemical reaction mechanism for each substance, following published criteria that include whether the chemical might be a prehapten or a prohapten and hence would require activation to be chemically reactive and act as a skin sensitizer.[22] We concluded that 19 of the 108 new substances presented in Table 1 can be regarded as falling into this category of sensitizers that are not directly reactive. This listing is not intended to be the ultimate analysis of all 108 substances in this respect but rather a useful guide that supplements the definitive analysis of the first data set, recently published separately.[22] However, for those who are particularly interested in the development of in vitro assays that can successfully identify this type of chemical, Table 3 lists the 60 substances (from the present and earlier lists) that are considered to be prehaptens or prohaptens.

As with the first data set, a number of chemicals may be regarded as false positive or false negative, a problem that may be expected with a predictive toxicology assay.[23] A classic false positive, sodium lauryl sulfate, was noted in that first article. In the present article, a good example of the same phenomenon would be tridecane, which delivered an SI of 3.1 at a concentration of 50%. No doubt, the list of chemicals in Table 1 and in the earlier data set could be inspected carefully and a small number of false positives and false negatives identified. However, these deliberations require not only detailed analysis but also expert judgment in the context of defined classification criteria; this is outside the scope of this simple presentation of additional LLNA results.[24,25]

Corrections to the Original Database

Table 4 lists nine substances in regard to which the original publication presented some minor errors, generally either

Table 3. Chemicals That Are Pro-electrophiles or Pre-electrophiles*

Chemical Name	*CAS No.*
Aniline	62-53-3
Anisyl alcohol	105-13-5
Atranol	526-37-4
trans-Anethole	104-46-1
Bandrowski's base	20048-27-5
(+/−) Linalool	78-70-6
1,2-Dibromo-2,4-dicyanobutane	35691-65-7
1,3-Phenylenediamine	108-45-2
1,3-Bis-(2,4-diaminophenoxy)-propane	74918-21-1
1,4-Phenylenediamine	106-50-3
1-Amino-2-nitro-4-bis-(2-hydroxyethyl)-amino-benzol	29705-39-3
1-Naphthol	90-15-3
2-Amino-6-chloro-4-nitrophenol	6358-09-4
2-Aminophenol	95-55-6
2-Mercaptobenzoxazole	2382-96-9
2-Methoxy-4-methylphenol	93-51-6
2-Methyl-5-hydroxyethylaminophenol	55302-96-0
2-Nitro-p-phenylenediamine	5307-14-2
2,4-Diaminophenoxyethanol dihydrochloride	66422-95-5
2,5-Diaminotoluene sulfate	615-50-9
2,5-Diaminotoluene	95-70-5
3,5-Diamino-2,6-dimethoxypyridine-dihydrochloride	56216-28-5
3-Aminophenol	591-27-5
3-Bromomethyl-5,5-dimethyl-dihydro-2(3H)-furanone	154750-20-6
3-(Dimethylamino)propylamine	109-55-7
3-Methylisoeugenol	186743-29-3
3-Methyleugenol	186743-26-0
4-Allylanisole	140-67-0
4-Amino-3-methyl phenol	2835-99-6
4-Amino-3-nitrophenol	610-81-1
5-Amino-2-methyl phenol	2835-95-2
5-Methyleugenol	186743-25-9
4-Nitro-benzene-1,2-diamine	99-56-9
4-([2-Hydroxyethyl]amino)-3-nitrophenol	65235-31-6
4-(N-ethyl-N-2-methan-sulfonamido-ethyl)-2-methyl-1,4-phenylenediamine	25646-71-3
6-Methylisoeugenol	13041-12-8
6-Methyleugenol	186743-24-8
7,12-Dimethylbenz(a)anthracene	57-97-6
Abietic acid	514-10-3
Benzo(a)pyrene	50-32-8
Cinnamyl alcohol	104-54-1
Chloroatranol	57074-21-2
Diethylenetriamine	111-40-0
Dihydroeugenol	2785-87-7
Ethylenediamine	107-15-3
Eugenol	97-53-0
Geraniol	106-24-1
HC Red No. 3	2871-01-4
Hydroquinone	123-31-9
Hydroxytyrosol	10597-60-1
Isoeugenol	97-54-1
Isopropyl isoeugenol	186743-30-6
Lauryl gallate	1166-52-5
Metol	55-55-0
N,N-Dibutylaniline	613-29-6
Pentachlorophenol	87-86-5
Resorcinol	108-46-3
R(+)-Limonene	5989-27-5
R-Carvoxime	(Not known)

CAS = Chemical Abstracts Service.
*Collated from both local lymph node assay data sets.

in the CAS number, the representation of the chemical structure, or (in one case) the LLNA EC3 value. One substance (cinnamic aldehyde [or 3-phenyl propenal or cinnamal]) was given a double entry in the original table and therefore does not appear in Table 4. Resorcinol has now been added because new data indicate in fact that it is positive in the LLNA.

Discussion

What was stated in the first article on the LLNA database remains true 4 years later: for the evaluation and eventual validation of nonanimal alternatives for skin sensitization testing (and for the LLNA in particular), there remains the need for an extensive good-quality database of in vivo results derived by an OECD standard method (or that, as put previously, "requires the selection of a robust chemical dataset to interrogate and calibrate the method"[13]). To ensure that this high standard is met, Table 4 records a modest number of corrections to the original publication, which listed 211 chemicals.[13]

It has been noted, however, that the original database did lack in certain areas or had only a limited number of examples of certain chemical classes. The present article provides the results of more than 100 additional chemicals, which we feel will address these issues to some extent. Nevertheless, the material presented here represents not a specific effort to address the gaps but rather (1) a collation

Table 4. Corrections to the Original Data Set*

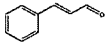
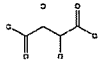
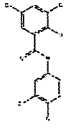
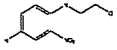
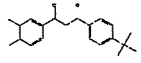
Chemical Structure†	*CAS No.*	*Log Kp*	*Log Ko/w*	*MW*	*Vehicle*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA EC3 %*	*Potency Category*	*Reference*‡
Cinnamic aldehyde (structure corrected) 	104-55-2	-1.90	2.29	132.16	AOO	0.5	1.0	2.5	5.0	10.0		1.4	0.9	1.9	7.1	15.8		3.0	Moderate	15
Tartaric acid (data changed; now nonsensitizer) 	87-69-4	-4.25	-0.87	150.09	DMF	5.0	10.0	25.0				1.0	0.9	1.5				NC	Non-sensitizer	Unilever (unpublished)
Undec-10-enal (structure corrected) 	112-45-8	-1.77	2.79	168.28	AOO	5.0	10.0	25.0	50.0	75.0		1.7	5.3	7.5	8.7	8.8		6.8	Moderate	72
3, 3′, 4′, 5-Tetrachlorosalicylanilide (structure corrected) 	1154-59-2	-2.38	3.49	351.02	Acetone	0.25	0.5	1.0				11.2	14.4	18.0				0.04	Extreme	73
Isopropyl eugenol (structure corrected) 	51474-90-9	-1.93	2.89	206.29	AOO	12.0	29.0	59.0				1.8	1.8	2.2				NC	Non-sensitizer	74

Table 4. Continued

Chemical Structure†	*CAS No.*	*Log Kp*	*Log Ko/w*	*MW*	*Vehicle*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA (%)*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA SI*	*LLNA EC3 %*	*Potency Category*	*Reference*‡
Isopropyl isoeugenol (structure and CAS number corrected) 	186743-30-6	-1.93	2.89	206.29	AOO	0.6	1.2	3.0				3.0	5.7	10.7				0.6	Strong	74
2-(4-Amino-2-nitro-phenylamino)-ethanol (HC Red No. 3) (CAS number corrected) 	2871-01-4	-3.84	0.12	197.19	AOO	0.1	0.25	0.5	1.0	2.5		0.5	1.2	1.9	1.8	3.3		2.2	Moderate	75
1-(2′,3′,4′,5′-Tetramethylphenyl)-3-(4′-tertbutylphenyl) propane-1,3-dione (CAS number corrected) 	Not known	-0.97	5.35	336.47	Ace-tone	10.0	20.0	40.0				1.6	1.2	1.6				NC	Non-sensitizer	P&G (unpublished)
Resorcinol (new LLNA data) 	108-46-3	-2.56	1.17	110.11	AOO	0.5	1.0	2.5	5.0	10.0	25.0	1.2	1.8	2.3	2.6	6.3	10.1	5.5	Moderate	76

AOO = acetone and olive oil (4:1); CAS = Chemical Abstracts Service; DMF = dimethylformamide; EC3 = mathematically estimated concentration of test chemical necessary to induce a threefold stimulation index; Ko/w = octanol-water partition coefficient; Kp = permeability coefficient; LLNA = local lymph node assay (LLNA % = weight per volume concentration); MW = molecular weight; NC = not calculated; P&G = Procter & Gamble Co.; SI = stimulation index.

*Original data set was published in Gerberick GF et al.[13]

†3-Phenyl propenal was deleted because it is an isomer of cinnamic aldehyde and so would constitute a double entry.

‡Numbers denote numbered references in References section.

of data published by us and others subsequent to the original database or (2) unpublished data from companies, presented here for the first time. These latter data represent approximately 18% of the total set but are an important addition, not least because these results would not normally be reported individually in the available peer-reviewed literature. The proportion of previously unpublished data was almost identical (17%) in the first data set.[13]

The additional information given here on the LLNA results for 108 substances further diversifies and extends the previous information. Chemical types represented include lactones, acrylates, chlorinated aromatics, organic silicones, nitriles, a range of thio-organics, and conjugated dienes, as well as further examples of preservative chemicals, a broad range of fragrance chemicals, hair dyes, and a substantial set of prohaptens. Furthermore, the data span not only a slightly wider range of molecular weights than in the first publication but also a fractionally wider range of potency values. However, these differences are marginal, and perhaps what is most important is that, when combined (including the corrected data on resorcinol), the data sets offer detailed results for 319 chemicals: 87 (27%) nonsensitizers in the LLNA plus 88 (28%) weak, 93 (29%) moderate, 33 (10%) strong, and 18 (6%) extreme sensitizers, according to the original potency classification. When considered in terms of the new globally harmonized scheme for skin sensitization classification, the combined database will deliver information on 87 (27%) nonclassified substances, 163 (51%) weaker sensitizers (ie, an EC3 value of $> 2\%$), and 69 (22%) stronger sensitizers.[26]

The LLNA EC3 values listed in Table 1 show an enormous range of potency that covers four orders of magnitude from the weakest to the most potent allergens. Since the publication of the first database, the importance of potency assessment has become more clear, as has the contribution that EC3 values can make to risk assessment.[12,14–20,27–33] It is vital to remember that these are assessments of intrinsic potency and that whether these are seen as clinically important skin sensitizers depends almost entirely on the extent to which there is human exposure; extreme sensitizers can be used quite safely if exposure is adequately low.

The list of chemicals in this second data set complements that in the first, providing an extended list of substances that represents both the chemical and biologic diversity of chemical allergens and nonallergens. We hope that this additional LLNA data set will provide investigators with a complete package of information needed to accelerate the development and subsequent evaluation of alternative test methods and ultimately reduce the reliance on animals for assessing the skin sensitization potential of new chemicals.

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