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CONTACT DERMATITIS

A chemical dataset for evaluation of alternative approaches to skin-sensitization testing

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Allergic contact dermatitis resulting from skin sensitization is a common occupational and environmental health problem. In recent years, the local lymph node assay (LLNA) has emerged as a practical option for assessing the skin-sensitization potential of chemicals. In addition to accurate identification of skin sensitizers, the LLNA can also provide a reliable measure of relative sensitization potency, information that is pivotal in successful management of human health risks. However, even with the significant animal welfare benefits provided by the LLNA, there is interest still in the development of non-animal test methods for skin sensitization. Here, we provide a dataset of chemicals that have been tested in the LLNA and the activity of which correspond with what is known of their potential to cause skin sensitization in humans. It is anticipated that this will be of value to other investigators in the evaluation and calibration of novel approaches to skin-sensitization testing. The materials that comprise this dataset encompass both the chemical and biological diversity of known chemical allergens and provide also examples of negative controls. It is hoped that this dataset will accelerate the development, evaluation and eventual validation of new approaches to skin-sensitization testing.

Key words: allergen replacement; allergens; risk assessment; skin sensitization.

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Skin sensitization resulting in allergic contact dermatitis is a common occupational and environmental health problem and the most common manifestation of immunotoxicity in humans. The acquisition of skin sensitization and the subsequent elicitation of an allergic hypersensitivity reaction in the skin are processes dependent upon the induction of specific T-lymphocyte responses (1). Chemical allergens encountered in the skin are recognized by Langerhans' cells (LCs) resident in the epidermis. LCs are part of a wider family of dendritic cells that collectively are responsible for initiating primary immune responses. Following topical sensitization, epidermal LCs transport antigen from the skin to draining lymph nodes. They are induced to migrate from the epidermis, via afferent lymphatics, to draining lymph nodes where they present antigen to responsive T lymphocytes (2). Antigen-specific T lymphocytes are activated and are stimulated to divide and differentiate. Cell division results in the clonal expansion of allergen-responsive cells, such that if the now-sensitized subject is exposed subsequently to the inducing allergen then an accelerated and more aggressive secondary response will be provoked causing allergic contact dermatitis.

For many years, guinea pigs were the species of choice for the hazard identification of skin-sensitizing chemicals. More recently, however, the local lymph node assay (LLNA) has been developed as an alternative approach based upon characterization of induced proliferative responses in draining lymph nodes following topical exposure of mice to chemicals (3–7). The LLNA has been adopted recently, as Guideline 429, by the Organization for Economic Cooperation and Development (OECD) (8) as a stand-alone test method for skin-sensitization testing. This adoption was predicated on an exhaustive and independent

validation of the LLNA in both the USA (9) and Europe (10). Comprehensive details of the dataset on which these validations were based are available elsewhere (4). It is clear that the LLNA offers significant scientific advantages, in addition to important animal welfare benefits (in terms of both reduction and refinement), compared with conventional guinea-pig tests. More recently, it has been shown also that, in addition to the accurate identification of skin-sensitization hazard, the LLNA can provide a reliable measure of relative sensitization potency as the first step in a risk assessment process. This is achieved by consideration of the vigour with which chemical allergens provoke proliferative responses by draining lymph node cells (LNCs). The approach has already been applied with some success such that the LLNA is the preferred method for estimation of the potency of an allergen (11). Here, potency is measured by derivation of a mathematically estimated concentration of chemical required to induce a 3-fold stimulation index (SI) value (EC3), the concentration of a test chemical necessary to produce a 3-fold stimulation of proliferation in draining lymph nodes compared to concurrent vehicle controls (12). Thus, the intrinsic sensitizing potency of a chemical is defined as a function of the concentration required to elicit a LLNA response of the magnitude which in practice is necessary for classification as a contact allergen. It is important to emphasize that the EC3 value denotes the amount of chemical that is required to induce a SI of 3 in the LLNA. For this reason, the lower the EC3 value, the greater the relative skin-sensitizing potency of the chemical.

Here, we describe an extensive chemical dataset that embraces a range of chemistry and skin-sensitizing activity. All materials have been evaluated in the LLNA, and for many of the chemicals, it has been demonstrated that sensitizing activity in the LLNA correlates with what is known of the relative ability to induce sensitization in humans. These data provide a unique and valuable panel of chemicals with which the sensitivity, selectivity and overall accuracy of proposed alternative methods for skin sensitization can be judged.

Materials and Methods

Chemicals

The chemicals identified in this article have all been evaluated for skin-sensitization potential using the LLNA. For each chemical listed in Table 1, the CAS number and 2-D structure is specified. In addition, the molecular weight, $\log K_p$ and $\log K_{O/W}$ (octanol/water) values are indicated for each chemical. The structures were drawn with CHEMDRAW (Version 6.0 CambridgeSoft, Cambridge, MA, USA). The molecular weight (MW), $\log K_p$ and $\log K_{O/W}$ values were obtained by running the structures through the expert system DEREK (Deductive Estimation of Risk from Existing Knowledge; LHASA Limited, Leeds, UK) (13). The $\log K_p$ is calculated using the Potts & Guy equation (14), and the $\log K_{O/W}$ using the Moriguchi estimation (15).

LLNA protocol and chemicals tested. The LLNA was conducted as described elsewhere (16, 17). Briefly, groups of 4 CBA/Ca 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. 5 days following 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 killed 5 h later and the draining lymph nodes excised and pooled for each experimental group. In some laboratories, a slightly modified protocol involving groups of 5 CBA/J female mice, with pairs of lymph nodes being processed from individual mice. These minor modifications have been demonstrated previously in interlaboratory collaborative trials to be without impact on the interpretation of LLNA data (18, 19). A single-cell suspension of LNCs was prepared by mechanical disaggregation. The LNC suspension was washed ×2 in an excess of PBS and then precipitated with 5% trichloroacetic acid (TCA) at 4°C for 18 h. Pellets were resuspended in TCA and the incorporation of tritiated thymidine measured by β-scintillation counting and was reported in disintegrations per minute (d.p.m.). An SI was calculated for each allergen-treated group as the ratio of the d.p.m. or mean d.p.m. of the treated group over the d.p.m. or mean d.p.m. of the concurrent vehicle control. A substance was classified as a skin sensitizer if at 1 or more test concentrations it induced a 3-fold or greater increase in LNC proliferative activity compared with concurrent vehicle-treated controls. That is, sensitizing chemicals by definition elicit a SI of 3 or more compared with vehicle controls. The data reported here are derived from

Table 1. Classification of relative skin-sensitization potency using local lymph node assay EC3 values

EC3 value (%)	Potency classification
≥10–≤100	Weak
≥1–<10	Moderate
≥0.1–<1	Strong
<0.1	Extreme

EC3, mathematically estimated concentration of chemical required to induce a 3-fold stimulation index.

previously conducted studies in which multiple concentrations were evaluated in order to obtain a dose–response that in most cases provided information regarding the concentration of chemical required to induce a threshold positive response. References for the sources of LLNA data for each of the chemicals are summarized in Table 2.

Potency estimation in the LLNA. The approach to the estimation of relative skin-sensitization potency of chemicals in the LLNA has been described previously in detail (12). It is based upon the mathematical estimation of the concentration of chemical necessary to obtain a 3-fold increase in proliferative activity in draining lymph nodes compared with concurrent vehicle-treated controls. It is termed the estimated concentration that yields a 3-fold stimulation value (EC3). In these present investigations, existing dose–response data for 41 chemicals evaluated in the LLNA have been used to derive EC3 values. In most cases, calculation of the EC3 values was conducted by linear interpolation according to the equation:

$$EC3 = c + [(3\text{–}d)/(b\text{–}d)] \times (a\text{–}c),$$

where the data points lying immediately above and below the SI value of 3 on the LLNA dose–response plot have the co-ordinates (a, b) and (c, d), respectively (12).

For the remainder of the chemicals for which the lowest concentration tested resulted in a stimulation index of greater than 3, an EC3 value was extrapolated from the 2 lowest doses utilized. The extrapolated EC3 value is calculated by log-linear interpolation between these 2 points on a plane where the *x*-axis represents the dose level and the *y*-axis represents the SI. The point with the higher SI is denoted (a, b) and the point with the lower SI is denoted (c, d). The formula for the extrapolated EC3 value is as follows:

$$EC3 = 2\hat{}\{\log2(c) + (3\text{–}d)/(b\text{–}d) \times [\log2(a) - \log2(c)]\}.$$

By log-transformation of the data, extrapolated EC3 values will never fall below zero. This method of deriving EC3 values should only be applied when there is clear evidence of a dose–response and where the SI induced by the lowest dose of compound tested is approaching the value of 3. Despite the lack of an SI value below 3, this method provides useful information regarding likely threshold values and in some cases can prevent the need for repeat animal testing.

The relative sensitizing potencies of the chemical allergens were categorized using an arbitrary classification scheme that has recently been proposed (11, 20). The system, summarized in Table 1, is comprised of 4 sensitization potency categories based on EC3 values. Compounds that did not induce a 3-fold increase at any concentration tested are categorized as non-sensitizing. For those chemicals which did not induce a SI below 3 and EC3 values were derived by extrapolation, a potency classification was assigned only when the extrapolated EC3 value was clearly within the specified range. It must be emphasized that this particular categorization scheme has been adopted here solely for the purpose of facilitating the ranking of the relative sensitizing potency of contact allergens. Consequently, it must be acknowledged that these classes and the way in which they are defined with respect to EC3 values do not necessarily represent the only or best approach to classification of skin-sensitizing potency as a function of LLNA data. However, it is worth noting that this categorization scheme has achieved a high degree of consensus (11).

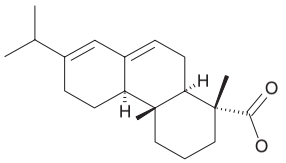
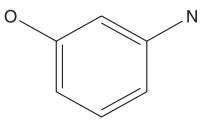
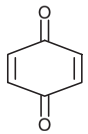
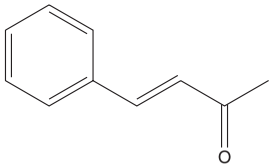
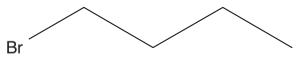
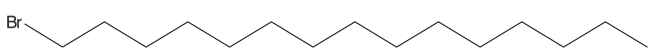
Results

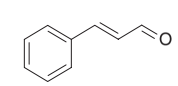
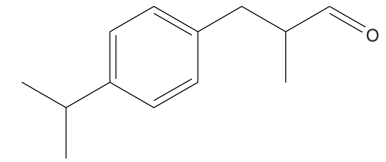
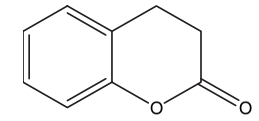
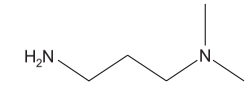
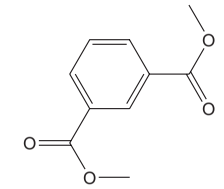
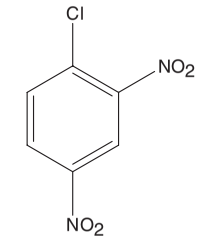
Skin-sensitization dataset: Chemical information

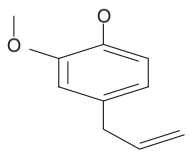
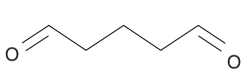
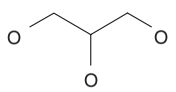
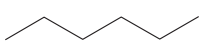
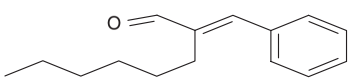
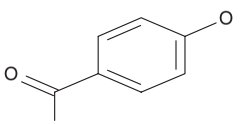
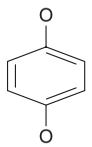
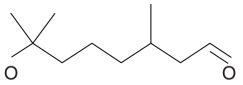
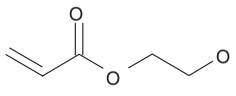
Table 2 lists 41 chemical compounds along with their respective CAS numbers and 2-D chemical structures. It is clear from reviewing the structures themselves, and from the chemical class designation assigned to each compound, that the dataset embraces the wide chemical diversity known to exist among skin allergens. For example, aldehydes, ketones, aromatic amines, quinones and acrylates are represented in the dataset. The physicochemical diversity of the allergens is reflected also by the range of $\log K_{O/W}$ values that span from −0.128 to 4.614 for 1-chloro,2,4-dinitrobenzene and abietic acid, respectively. A similar range is evident for the $\log K_p$ values. Not surprisingly, all of the allergens listed in the tables have MWs below 500 MW, which is consistent with what has been published previously (21). It is generally believed that chemical allergens have low molecular weights (<500 MW) and a $\log K_{O/W}$ of >1 that is thought to favour the penetration of the chemical across the lipid-rich stratum corneum (22).

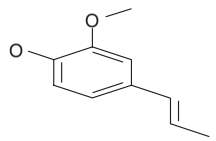
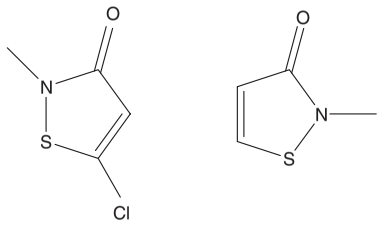
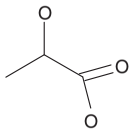
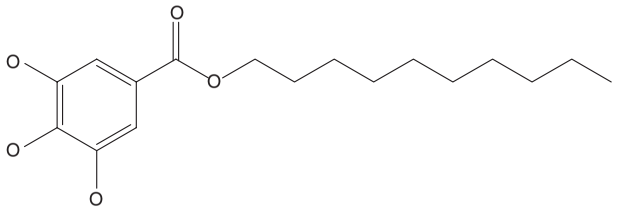
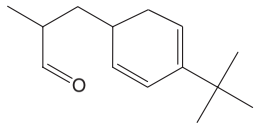
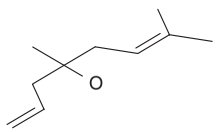
Skin-sensitization dataset: Biological data. The LLNA data for each of the 41 chemicals are summarized in Table 3. The dataset includes weak, moderate, strong and extreme skin sensitizers, as well as non-sensitizers. For some chemicals, such as those that formed the basis of the various interlaboratory trials, several different

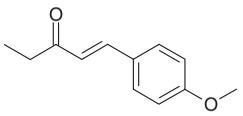
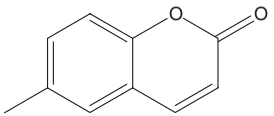
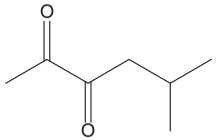
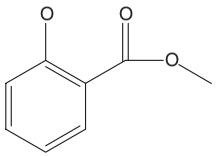
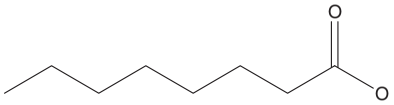
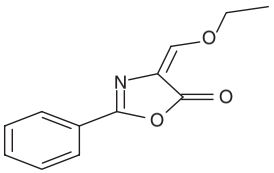
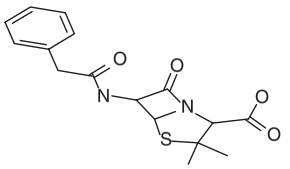
Table 2. Chemical structures and physicochemical parameters

Chemical name	CAS number	Chemical structure	MW	LogK_p	Log$K_{O/W}$	Chemical class
Abietic acid	514-10-3		302.46	−1.289	4.614	Carboxylic acid
3-Aminophenol	591-27-5		109.10	−2.555	1.17	Phenol, aromatic amine
p-Benzoquinone	106-51-4		108.10	−2.549	1.170	Ketone
Benzylidene acetone (4-phenyl-3-buten-2-one)	122-57-6		146.19	−1.808	2.540	Aliphatic aldehyde
1-Bromobutane	109-65-9		137.02	−2.264	1.819	Halogenated compound
1-Bromopentadecane	629-62-9		291.32	−1.284	4.525	Halogenated compound
Chlorobenzene	108-90-7		112.56	−1.853	2.188	Halogenated compound

Cinnamic aldehyde	104-55-2		132.16	−1.897	2.294	Aliphatic aldehyde
Cyclamen aldehyde	103-95-7		190.31	−1.553	3.278	Aliphatic aldehyde
3,4-Dihydrocoumarin	119-84-6		148.10	−2.269	1908	Coumarin
3-Dimethylaminopropylamine	109-55-7		102.20	−2.687	0.924	Aliphatic amine
Dimethylisophthalate	1459-93-4		194.2	−2.923	1.382	Aromatic ester
1-Chloro,2,4-Dinitrobenzene	97-00-7		202.55	−4.046	−0.128	Halogenated compound, Nitrobenzene

Eugenol	97-53-0		164.20	−2.192	2.154	Phenol
Formaldehyde	50-00-0		30.03	−2.672	0.326	Aliphatic aldehyde
Glutaraldehyde	111-30-8		100.10	−2.675	0.924	Aliphatic aldehyde
Glycerol	56-81-5		92.10	−3.249	0.046	Aliphatic alcohol
Hexane	110-54-3		86.18	−1.867	1.942	Miscellaneous
α-Hexylcinnamic aldehyde	101-86-0		216.33	−1.363	3.770	Aliphatic aldehyde
1,4-Dihydrobenzoic acid	99-96-7		138.12	−2.831	1.030	Carboxylic acid, Phenol
1,4-Hydroquinone	123-31-9		110.12	−2.561	1.17	Phenol, aromatic alcohol
Hydroxycitronellal	107-75-5		172.3	−2.241	2.154	Aliphatic aldehyde, aliphatic alcohol
2-Hydroxypropyl methacrylate	923-26-2		144.19	−2.868	1.030	Aliphatic ester, aliphatic alcohol

Isoeugenol	97-54-1		164.20	−2.192	2.154	Phenol
Isopropanol	67-63-0		60.10	−2.506	0.818	Aliphatic alcohol
Methylchloroisothiazolinone/methylisothiazolinone	55965-84-9		264.80	−3.528	1.136	N-ring
Lactic acid	50-21-5		90.08	−3.377	0.046	Aliphatic alcohol, Carboxylic acid
Lauryl gallate (dodecyl gallate)	1166-52-5		338.45	−2.505	3.210	Phenol, aromatic alcohol
Lilial (p-tert-butyl-a-ethyl hydrocinnamal)	80-54-6		204.30	−1.016	4.156	Aliphatic aldehyde
Linalool	78-70-6		154.25	−1.858	2.540	Aliphatic alcohol

1-(p-Methoxyphenyl)-1-penten-3-one	104-27-8		190.24	−2.002	2.646	Ketone
6-Methylcoumarin	92-48-8		160.10	−2.168	2.154	Coumarin
5-Methyl-2,3-hexanedione	13706-86-0		128.17	−2.496	1.416	Ketone, Diketone
Methyl salicylate	119-36-8		153.15	−2.742	1.276	Phenol, Aromatic ester
Octanoic acid	124-07-2		144.20	−2.420	1.662	Carboxylic acid
Oxazolone	15646-46-5		217.24	−2.715	1.874	N-ring
Penicillin G	61-33-6		333.38	−3.279	2.086	N-ring, Carboxylic acid

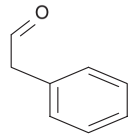
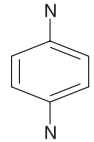
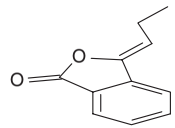
Phenylacetaldehyde	122-78-1		120.15	−1.999	2.048	Aliphatic aldehyde
1,4-Phenylenediamine	106-50-3		108.14	−2.549	1.170	Aromatic amine
β-Propionolactone	57-57-8		72.07	−2.853	0.432	Miscellaneous
3-Propylidenephthalide	17369-59-4		174.20	−2.079	2.400	Miscellaneous

Table 3. Local lymph node assay (LLNA) data and potency categorization

Chemical name*	Vehicle	LLNA percentage dose	LLNA SIs	LLNA EC3%	Potency category	Key reference
1-Bromobutane	AOO	5	1.1	NC	NS	(36)
		10	1.2			
		25	1.0			
Chlorobenzene	AOO	5	1.1	NC	NS	(36)
		10	1.7			
		25	1.6			
Diethylphthalate	AOO	25	1.0	NC	NS	(17)
		50	1.3			
		100	1.5			
Glycerol	DMF	25	1.1	NC	NS	(17)
		50	0.7			
		100	0.5			
Hexane	AOO	25	0.8	NC	NS	(37)
		50	0.8			
		100	2.2			
2-Hydroxypropyl methacrylate	AOO	10	1.1	NC	NS	(38)
		25	1.2			
		50	1.3			
4-Hydrobenzoic acid	DMSO	5	1.4	NC	NS	(36)
		10	1.5			
		25	1.3			
Isopropanol	AOO	10	1.7	NC	NS	(37)
		25	1.1			
		50	1.0			
Lactic acid	DMSO	5	1.0	NC	NS	(37)
		10	1.4			
		25	2.2			
6-Methylcoumarin	ACE	5	1.0	NC	NS	(36)
		10	1.0			
		25	1.1			
Methyl salicylate	AOO	1	1.0	NC	NS	(39)
		2.5	1.1			
		5.0	1.6			
		10	1.4			
		20	0.9			
Octanoic acid	AOO	10	0.7	NC	NS	(37)
		25	1.0			
		50	1.6			
Penicillin G	DMSO	2.5	0.8	46.4	Weak	(39)
		5	0.7			
		10	0.8			
		25	1.3			
		50	3.4			
Linalool	AOO	25	2.5	30.4	Weak	(17)
		50	4.8			
		100	8.3			
5-Methyl-2,3-hexane-dione	AOO	25	2.9	25.8	Weak	(17)
		50	6.0			
		100	14.3			
Hydroxycitronellal	AOO	10	1.7	23.0	Weak	(40)
		25	3.2			
		50	6.7			
Cyclamen aldehyde	AOO	1.0	1.4	22.3	Weak	(41)
		2.5	1.3			
		10	1.8			
		25	3.3			
		50	5.2			

Lilial (*p*-tert-butyl-a-methyl hydrocinnamal)	AOO	1.0 2.5 10 25 50	1.3 2.5 2.0 3.7 9.3	18.7	Weak	(41)
Abietic acid	AOO	5 10 25	1.5 2.0 5.2	14.7	Weak	(36)
Eugenol	AOO	2.5 5 10 25	1.6 1.5 2.4 5.5	12.9	Weak	(18)
1-(*p*-Methoxyphenyl)-1-penten-3-one	AOO	10 25 50	3.5 10.0 26.1	9.3†	*Moderate*	(17)
α-Hexylcinnamic aldehyde	AOO	2.5 5 10 25 50	1.3 1.1 2.5 10.0 17.0	8.4	Moderate	(18)
3,4-Dihydrocoumarin	AOO	2.5 5 10	1.6 2.5 6.6	5.6	Moderate	(36)
1-Bromopentadecane	AOO	5 10 25	2.9 7.8 19.6	5.1	Moderate	(36)
Benzylidene acetone	AOO	10 25 50	8.5 13.6 12.8	3.7†	*Moderate*	(17)
3-Propylidenephthalide	AOO	5 10 20	4.9 9.1 15.1	3.7†	*Moderate*	(17)
Isoeugenol	AOO	0.25 0.5 1 2.5 5	2.9 1.7 2.3 3.8 6.8	1.8	Moderate	(18)
3-Aminophenol	AOO	2.5 5 10	2.8 3.5 5.7	3.2	Moderate	(36)
Cinnamic aldehyde	AOO	0.5 1 2.5 5 10	1.4 0.9 1.9 7.1 15.8	3.1	Moderate	(41)
Phenylacetaldehyde	AOO	1 2.5 5 10 25	0.7 1.8 7.8 8.8 19.0	3.0	Moderate	(41)
3-Dimethylaminopropylamine	AOO	0.5 1 2.5 5 10	1.3 1.1 3.5 7.0 13.9	2.2	Moderate	(42)
Formaldehyde	ACE	0.093 0.185 0.37 0.925 1.85	1.1 2.3 2.3 3.9 4.0	0.7	Strong	(43)

Lauryl gallate (dodecyl gallate)	DMSO	1 10 25 50	12.1 29.7 29.3 36	0.3†	*Strong*	(P&G unpublished)
β-Propiolactone	AOO	0.025 1.0 2.5	1.5 13.0 19.9	0.2	Strong	(36)
Glutaraldehyde	ACE	0.05 0.125 0.25 0.5 1.25 2.5	1.3 4.3 7.6 11.6 17.7 18.0	0.1	Strong	(43)
1,4-Phenylenediamine	AOO	0.05 0.1 0.25 0.5 1	2.0 3.3 10.2 20.5 26.4	0.1	Strong	(44)
1,4-Dihydroquinone	AOO	0.1 0.25 0.5 1 2.5	2.8 5.8 13.7 15.2 13.1	0.1	Strong	(39)
1-Chloro, 2,4-Dinitro-benzene	AOO	0.01 0.025 0.05 0.1 0.25	1.5 1.8 2.4 8.9 38.0	0.04	Extreme	(18)
p-Benzoquinone	AOO	0.5 1 2.5	36.4 42.3 52.3	0.01†	*Extreme*	(38)
Methylchloroisothia-zolinone/methyli-sothiazolinone	AOO	0.00075 0.0015 0.0075 0.015 0.0375	0.9 1.2 4.4 9.1 8.5	0.005	Extreme	(45)
Oxazolone	ACE	0.0025 0.005 0.01 0.025 0.05	2.9 4.9 12.0 22.0 33.0	0.003	Extreme	(18)

ACE, acetone; AOO, acetone–olive oil (4 : 1); DMF, dimethylformide; DMSO, dimethylsulfide; EC3, mathematically estimated concentration of chemical required to induce a 3-fold stimulation index (SI); NC, not calculated; NS, non-sensitizing in LLNA.
*Each chemical listed in the table is associated with representative LLNA data and its specific literature citation.
†EC3 values are calculated using the log-linear extrapolation.
Potency category was determined by the following EC3 cut-off values: extreme, <0.1%; strong, 0.1–<1%; moderate, 1–<10%; weak, 10–100%. Potency categories derived from extrapolated EC3 values are given in italics.

EC3 values, albeit within a narrow range, are available. In each of these cases, the data summarized in Table 3 derive from 1 representative experiment that we feel reflects accurately the results obtained with the chemical. The specific reference for the source of the LLNA data for each chemical is indicated in Table 3. The dataset comprises 12 non-sensitizers, 8 weak sensitizers, 11 moderate sensitizers, 6 strong sensitizers and 4 extreme sensitizers, a total of 41 compounds. For the non-sensitizers, materials were included that did not give a positive response in the LLNA up to a highest dose tested of 25%. For the skin sensitizers, the range of EC3 values span from 0.003% for the extreme sensitizer, oxazolone, to 46.4% for the weak sensitizer, penicillin G. EC3 values estimated by the log-linear extrapolation method are marked with an asterisk (*) and the potency class for the chemical is shown in italics.

Discussion

During the past 25 years, there have been substantial advances in our understanding of the molecular and cellular mechanisms of skin sensitization and allergic contact dermatitis. Over the last decade, scientists from academia and industry have been seeking to apply this understanding to

the development of alternative, non-animal, test methods for skin-sensitization testing. It is an enormous challenge to reproduce accurately *in vitro* the complex immunobiological mechanisms that act in concert to permit the acquisition of skin sensitization. For example, there are numerous cell types (e.g. T lymphocytes, LCs and keratinocytes) and a plethora of immune and inflammatory mediators (including cytokines and chemokines) involved in the initiation and expression of an allergic contact dermatitis response. The key to success will be development of test method(s) that incorporate our understanding of the chemistry and biology of contact allergy.

A thorough evaluation programme is required to determine the performance characteristics of new approaches to testing, and in particular the sensitivity, selectivity and overall accuracy of a new method. This in turn requires the selection of a robust chemical dataset to interrogate and calibrate the method (23). One essential criterion is that the activity of each of the chemicals used must be supported by relevant and reliable *in vivo* data of high quality. In addition, it is important to select chemicals that display a wide range of potencies and that represent the relevant classes of chemicals and physical properties of the materials known to cause the specific endpoint. To this end, we have presented here a dataset of chemicals to be used for evaluating alternative approaches for skin-sensitization testing that meet these criteria and that encompass the chemical and biological diversity of chemicals known to cause skin sensitization in animals and/or man.

The chemicals listed in Table 2 clearly represent a diverse chemical dataset with the representatives from various chemical classes of materials including aldehydes, ketones, diketones, acrylates and aromatic amines as well as others. For each compound, the structure and CAS number is provided to aid investigators in obtaining the correct materials. It is well known that skin allergens must have a relatively low molecular weight ($\leq$500 MW) (21) and have appropriate physicochemical properties (e.g. lipophilicity) (24, 25). The chemicals selected for our dataset clearly demonstrate the physicochemical characteristics associated with skin allergens, while also showing a range of molecular weight and lipophilicity (i.e. $\log K_{O/W}$ and $\log K_p$) values.

In addition to chemical diversity, it is important to embrace biological diversity in the dataset. It was particularly relevant to encompass the enormous range of potencies known for skin allergens. It is believed that differences between contact allergens with respect to their relative skin-sensitizing potency can span 4 or more orders of magnitude (11, 26–28). We have therefore proposed chemicals that have been tested in the LLNA and that display a range of potencies (in the form of EC3 values). The approach to the assessment of relative skin-sensitization potency of chemicals in the LLNA is based upon the mathematical estimation of the concentration of chemical necessary to obtain a threshold positive response (SI = 3) of proliferative activity in draining lymph nodes (compared with concurrent vehicle-treated controls) and is termed the EC3 value (12). The preferred method for deriving EC3 values is by linear interpolation utilizing the data points (concentration and SI) immediately above and below the SI value of 3 on the LLNA dose–response curve. The robustness of this parameter has already been demonstrated (29, 30), as has its interlaboratory reproducibility (18, 19) and stability with time (29). In this article, we present a method for derivation of an EC3 value which can be applied in instances where none of the tested concentrations results in a SI below 3 and there is clear evidence of a dose–response. Use of this method can provide information regarding more likely threshold values and may obviate the need for repeat animal testing. Assignment of a potency classification is possible when the extrapolated EC3 value falls well within the specified range of EC3 values. However, if the extrapolated EC3 is close to limit of the EC3 values for any particular potency category, then repeat testing may be necessary in order to more accurately assess potency.

The LLNA EC3 values listed in Table 3 show a range of potency from 0.003% for the extreme allergen, oxazolone, to 46.4% for the weak allergen, penicillin G. The chemicals selected for the dataset are known skin allergens which have been reported to induce sensitization in animals and/or man. For each of chemicals listed in Table 3, a specific reference is given for the representative LLNA data used in the article, because many of these compounds have been tested several times in different laboratories (30). The chemicals represented in the database comprise weak, moderate, strong and extreme sensitizers, as well non-sensitizing materials, as based on using potency categorization criteria that have been developed recently by an European Centre for Ecotoxicolgy and Toxicology of Chemicals (ECETOC) Task Force (20). Of course, there is an expectation that any new assay must have the ability to detect strong allergens, such as 1-chloro,2,4-dinitrobenzene. However, there could be more latitude with the less potent allergens, especially if the particular assay being evaluated was being developed for screening purposes only.

One potential challenge for developing *in vitro* methods for skin-sensitization testing is that it is well known that some chemical allergens require biotransformation prior to their initiation of skin-sensitization response *in vivo* (24). The involvement of reactive intermediates in skin sensitization has been demonstrated with many chemicals, such as the formation of benzoquinonediimine from azo hair dyes (31), orthoquinone from isoeugenol (32) or Schiff base derivatives from cinnamal (33). Thus, we have purposely included in our dataset some chemicals that are known to undergo activation or metabolism in the skin to acquire reactivity. Such chemicals are called prohaptens. For example, eugenol and isoeugenol are considered to be prohaptens (24, 34). Based on the knowledge that some chemical allergens need to be biotransformed prior to reacting with proteins/peptides, it will be critical to the development of alternative assays to incorporate a metabolism component to address these types of molecules.

An important measure of any new *in vitro* test method will be the extent of its utility in the skin-sensitization risk assessment process. Although it would be of value to have *in vitro* methods available to assess the skin-sensitization hazard of novel chemicals, it would be more valuable to have methods available that can extrapolate that hazard as risk to humans (35). Thus, it would be useful to compare any new *in vitro* skin-sensitization test method to the murine LLNA. In addition to assessing the skin-sensitization potential (hazard), the LLNA yields important information as to the relative allergenic potency of a chemical (17, 27, 28). In addition to hazard identification, it would be advantageous that *in vitro* methods have the ability to determine the allergenic potency. On the other hand, the development of assay methods that can be used for screening purposes is a valuable tool for helping to reduce the need for animal testing.

The purpose of this article is to provide investigators with a chemical dataset for use in the evaluation of newly developed alternative test methods for skin-sensitization testing. The list of chemicals contained in the dataset represents both the chemical and biological diversity that is known to exist for chemical allergens. It is hoped that this dataset will help accelerate the development of new methods in our efforts to reduce the reliance on animals for skin-sensitization testing.

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