

Categorization of Chemicals According to Their Relative Human Skin Sensitizing Potency

David A. Basketter, DSc, FRCPath,* Nathalie Alépée, PhD,† Takao Ashikaga, PhD,‡ João Barroso, PhD,§ Nicola Gilmour, PhD,|| Carsten Goebel, PhD,¶ Jalila Hibatallah, PhD,# Sebastian Hoffmann, PhD,** Petra Kern, PhD,†† Silvia Martinozzi-Teissier, PhD,‡ Gavin Maxwell, PhD,|| Kerstin Reisinger, PhD,‡‡ Hitoshi Sakaguchi, PhD,§§ Andreas Schepky, PhD,|||| Magalie Tailhardat, PhD,¶¶ and Marie Templier, PhD##

Although adoption of skin sensitization in vivo assays for hazard identification is likely to be successful in the next few years, this does not replace their use in potency prediction. Notably, measurement of potency of skin sensitizers in the local lymph node assay has been important. However, this local lymph node assay potency measure has not been formally assessed against a range of substances of known human sensitizing potential, because the latter is lacking. Accordingly, criteria for human data have been established that characterize 6 categories of human sensitizing potency, with 1 the most potent and 5 the least potent; category 6 represents true nonsensitizers. The literature has been searched, and 131 chemicals assigned into these categories according to their intrinsic potency judged only by the available human information. The criteria and data set generated provide a basis for examination of the capacity of nonanimal approaches for the determination of human sensitization potency.

Skin sensitization represents an important toxicological end point, which, at the time of writing, has relied heavily on in vivo methods to identify and characterize potential hazards.^{1,2} Consequently, the development of in vitro test methods has been encouraged, and substantive efforts made to identify such alternatives in this area of toxicology, alongside other end points.³ These potential replacements (see References^{4–8} for reviews) seek only to deliver largely binary information, that is, a yes/no hazard identification decision on whether a substance is, or is not, a skin sensitizer.^{3,9,10} The decision criteria for this hazard identification

are those already fully set out in regulatory guidelines, such as those of the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (UN GHS).¹¹ Formal validation for this purpose is underway,^{3,12,13} and extensive catalogs of in vivo information already exist against which a test, or test strategy, can be assessed.^{9,14–18} However, the reality for toxicological safety assessment is that hazard identification is only a preliminary, albeit essential, first step. Once a skin sensitizer has been detected, it is then important to characterize that hazard by determining its relative potency. Commonly, this is achieved via the derivation of the local lymph node assay (LLNA) EC3 value, the concentration of a substance required to cause a 3-fold stimulation in lymph nodes draining the site of application, compared with that in concurrent vehicle controls.^{19,20} Furthermore, it has been shown that there is a good degree of correlation between LLNA EC3 values and intrinsic human potency^{21–26} that can lead into a quantitative risk assessment.^{2,27}

The in vitro test methods currently under validation (and others offered for that purpose) generally do not deliver a differentiated view of relative potency, certainly not beyond basic subcategorization of the UN GHS.¹¹ However, to produce a nonanimal system that does achieve a sufficient level of potency prediction, the most valuable option would be to develop and evaluate it against a reference standard data set of substances categorized according to their relative human potency. In recent years, the few efforts to develop in vitro assays that deliver insights concerning the potency of skin sensitizers normally have focused an examination of their predictive ability by comparison with LLNA EC3 values,²⁸ although 1 attempt at correlation with human

*From the *DABMEB Consultancy Ltd, Sharnbrook, Bedford, UK; †L'Oréal Research & Innovation, Aulnay Sous Bois, France; ‡Shiseido Co, Ltd, Quality Assurance Center, Kanagawa, Japan; §Cosmetics Europe, The Personal Care Association, Brussels, Belgium; ||Unilever, Safety and Environmental Assurance Centre, Bedford, Sharnbrook, United Kingdom; ¶The Procter & Gamble Company, Darmstadt, Germany; #Chanel, Neuilly sur Seine, France; **Seh Consulting + Services, Paderborn, Germany; ††The Procter & Gamble Company, Strombeek Bever, Belgium; ‡‡Henkel AG & Co KGaA, Düsseldorf, Germany; §§Kao Corporation, Safety Science Research Laboratories, Tochigi, Japan; ||||Beiersdorf, Hamburg, Germany; and ¶¶LVMH Recherche, St Jean De Braye Cedex, and ##Laboratoire Pierre Fabre, Castres, France.*

Address reprint requests to David A. Basketter, DSc, FRCPath, DABMEB Consultancy Ltd, Sharnbrook, Bedford MK44 1PR, United Kingdom.

E-mail: dabmebconsultancyLtd@me.com.

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data has been undertaken.²⁹ However, despite its evident value, the LLNA EC3 value is not a universally accurate predictor of human potency, such that it is always appropriate to consider any available additional evidence to make a weight of evidence decision.^{27,30,31}

Given the above, in the present article, the authors have undertaken 2 tasks. First, a set of 6 potency categories has been established together with definitions of how a substance can be categorized using human data. Second, available human data that permit such categorization of intrinsic human potency have been reviewed so that a substantial number of substances could be placed into 1 of these 6 predefined categories. Of course, to achieve this, a good deal of judgment has been required, so the outcome is certainly to be taken as the considered view of its authors. Nevertheless, it is argued that this data set of potency-categorized substances provides a more appropriate, human relevant, basis for development of a nonanimal based potency prediction than consideration of the LLNA EC3 value alone. Importantly, the chemicals identified cover a wide spectrum of materials of relevance to a broad range of industries.

LITERATURE SOURCES

An extensive range of substances has existing in vivo data, either for hazard and/or for relative sensitizing potency (in the mouse).^{14–18} These data, however, are not relevant for the present work except to note that it suggests that sensitizing substances cover a very wide spectrum of potency, at least 5 orders of magnitude according to the LLNA. Here, what is important is the categorization of relative sensitizing potency solely according to human information. Sources for such information comprise standard textbooks on contact dermatitis^{32–34} as well as the extensive dermatological literature focused on this topic, particularly the journals *Contact Dermatitis* and the (*American Journal of Contact Dermatitis*). Typically, this material informs on the substances that can cause skin sensitization (in other words, the existence of intrinsic hazard) and on prevalence, particularly within the subset of the general population attending dermatology clinics.

However, the intrinsic potency of the substance for humans can only be defined by understanding these data in the context of exposure and/or by the reference to historical human predictive sensitization test data.^{35–41} In addition, a recent review of fragrance allergens by a European independent expert group provides useful background information.⁴² Note has also been taken of an existing example of a small group of substances that have been categorized based on human evidence and that have already been published.⁴³

It has to be recognized that not all of the substances may be fully identified/characterized chemically in these literature sources. This is true particularly of skin sensitizers that produce that activity only as a consequence of air oxidation^{44,45} or metabolic activation.^{46–48} However, the work here is simply to categorize these substances according to what is known of their human effects. Ultimately, it will be the responsibility of those seeking to develop in vitro test methods to make use of these categories to find ways to characterize potency using data from nonanimal methods and thus to recognize and take into account the potential limitations of such information.

CATEGORIZATION CRITERIA

Table 1 provides a succinct overview of the criteria used for categorization of the sensitizing chemicals, together with some benchmark examples and details of how the human threshold value (no observed effect level [NOEL]) was determined. It is essential to keep in mind that the primary outcome of the work is assignment of substances to categories; although quantitative in nature, the NOEL is presented as contributory information and is by no means the sole determinant for categorization. Importantly, NOEL values taken from human experimentation (see above) are themselves often best estimates derived from very limited data and are not necessarily the highest possible value; that is, they are a no-effect level rather than a maximum NOEL. Even with that, NOEL values could be identified from the literature for only 46 (58%) of the 79 substances in the 4 categories that would lead to regulatory classification.

TABLE 1. Criteria for Categorization

Human Category*	Clinical Data (Benchmark Substances)	Human Test Data and NOEL
1	Extensive evidence of contact allergy in relation to degree of exposure and size of exposed population (MCI/MI, DNCB, <i>p</i> -phenylenediamine)	Where data were available, a best estimation has been made of the “NOEL” for the induction of skin sensitization in a HRIPT
2	A frequent cause of contact allergy, but of less significance compared with category 1 (formaldehyde, isoeugenol, methyltribromo glutaronitrile)	
3	A common cause of contact allergy, perhaps requiring higher exposure compared with category 2 (abietic acid, eugenol, imidazolidinyl urea)	Where a substance was nonsensitizing, this is indicated by “NS”
4	Infrequent cause of contact allergy in relation to level of exposure (benzocaine, hydroxycitronellal, resorcinol)	Where insufficient data were available, this is indicated as “ND”.
5	A rare cause of contact allergy except perhaps in special circumstances, eg, use in topical medicaments (hexylcinnamal, isopropanol, parabens)	Key references are indicated for each substance
6	Essentially absent, with at least no systematic convincing evidence of contact allergy (xylene, glycerol, sodium lauryl sulfate)	

*Extended explanations for each category can be found in the main text.

What follows here is a more detailed overview of the criteria for each category and that offers example benchmark substances for each of the 6 groups. These provide a basic guide for the placement of other substances in these categories. Despite these descriptions, it has to be recognized that there remains a great deal of expert judgment in the categorization and indeed that each category itself represents a span of potency. There will always be debate about substances close to the border of any particular category. Where exposure concentrations are mentioned (high, moderate, and low), for this present purpose, values greater than 1% are generally considered to be high, whereas low is less than 0.01% (100 ppm), with moderate falling between these limits. It may be helpful to mention that substances in categories 1 to 4 would classify as positives in UN GHS criteria.¹¹ Also, experimentally derived human thresholds are more challenging for weaker skin sensitizers due to the limited statistical power of their determination in a small group size. Finally, although it is hard to translate clinical data on prevalence into a frequency of contact allergy in the general population, there have been attempts to estimate this (summarized in Thyssen et al⁴⁹), which suggest that adopting a 10-fold factor to adjust from the former to the latter may represent a suitably conservative and workable approach. This information is then relevant for potency categorization where it permits better (expert) judgment of sensitization rate in the context of exposure.

Category 1

This group contains substances with the highest intrinsic skin-sensitizing potency. As a consequence, infrequent skin contact with a low dose is likely to sensitize a significant proportion (>1.0%) of those exposed. Where the dose level is very low, multiple exposures over a period of time may be needed to achieve such a rate. Note has been taken also of published human predictive test data, notably the human maximization test³⁶ and/or the human repeated insult patch test (HRIPT).^{37–41} Where a concentration of 1% or less induced sensitization in greater than 90% of those exposed and/or the HRIPT threshold (NOEL) typically was less than 25 $\mu\text{g}/\text{cm}^2$, the substance has been placed in this category. Benchmark substances for this group include dinitrochlorobenzene, methylchlorisothiazolinone (MCI)/methylisothiazolinone, and *p*-phenylenediamine. MCI/MI represents a good example of a category 1 skin sensitizer. Use at concentrations less than 50 ppm was associated with an epidemic of allergic contact dermatitis (ACD) (reviewed in References^{50,51}); data from the HRIPT indicate a NOEL of less than 1 $\mu\text{g}/\text{cm}^2$.²⁶ Similarly, *p*-phenylenediamine is a common cause of ACD, with a frequency in the general population of 2.7% in 1 study⁵²; the estimated HRIPT NOEL is 23 $\mu\text{g}/\text{cm}^2$.²³

Category 2

This group contains substances that are little less sensitizing than category 1, but which nevertheless possess a strong intrinsic potency. It is expected that regular contact with moderate concentrations is likely to sensitize perhaps 1% to 10% of those so exposed. High doses (both in terms of concentration and exposure) may sensitize at a

higher rate, whereas regular (very) low concentration exposure may be relatively safe. Human repeated insult patch test NOEL values typically fall within the range 25 to 500 $\mu\text{g}/\text{cm}^2$. Benchmark substances for this group include formaldehyde, isoeugenol, and methylaldibromo glutaronitrile. For example, isoeugenol is one of the commonest of fragrance sensitizers, despite exposure being limited to 0.2% historically and more recently to 0.02%⁵³; it has an HRIPT NOEL of 69 $\mu\text{g}/\text{cm}^2$.²³

Category 3

This group contains substances that may be quite well known as contact allergens, but for which a substantial degree of exposure typically is necessary to produce sensitization in 0.01% to 0.1% of those exposed; lower concentrations may sensitize only a small minority, or indeed none at all. Human repeated insult patch test NOEL values are likely to be within the range of 500 to 2500 $\mu\text{g}/\text{cm}^2$. Benchmark substances for this group include imidazolidinyl urea, colophony (abietic acid), and eugenol. For example, imidazolidinyl urea is a widely used preservative (typically up to 0.3% in leave on cosmetics), but which does not appear as a particularly frequent contact allergen diagnostically, being positive in 1% of consecutive eczema patients in Switzerland almost 20 years ago⁵¹ and positive in 1.05% of collected Spanish patients in the 2005–2009 period⁵⁴; imidazolidinyl urea has an HRIPT NOEL of 2000 $\mu\text{g}/\text{cm}^2$.²⁶

Category 4

This group contains substances that are rarely important clinical allergens, because they require considerable/prolonged exposure to higher dose levels to produce sensitization, which even then is unlikely to exceed 0.01% of those exposed. The group also includes substances where particularly intimate exposure, for example, to the anal/vulval mucosa, is associated with a distinct frequency of sensitization. Although sometimes lower, HRIPT NOEL values can exceed 2500 $\mu\text{g}/\text{cm}^2$. Benchmark substances for this group include hydroxycitronellal, benzocaine, and resorcinol. For example, hydroxycitronellal is a very widely used fragrance whose HRIPT NOEL is almost 3000 $\mu\text{g}/\text{cm}^2$ ²³ and that is a less common fragrance allergen in humans, with a 0.4% prevalence in consecutive eczema patients⁵⁵; however, use concentrations are generally in the moderate or low range. Benzocaine has a reported human experimental NOEL of 2000 $\mu\text{g}/\text{cm}^2$;²² it is used less commonly, but use concentrations can be in the high range (ie, >1%) and often are to damaged or vulnerable skin; despite this, it is not a particularly frequent general cause of contact allergy.⁵⁶ Resorcinol was judged to be in this category based on its low frequency of human sensitization, despite widespread use at high levels in hair dyes and topical skin preparations, including pharmaceuticals.⁵⁷

Category 5

This group contains substances that have a very low intrinsic ability to cause skin sensitization. Here, typically only exceptionally prolonged exposure in combination with high use levels will lead to skin sensitization, for example, routine use in medicaments

TABLE 2. List of Substances Placed in Category 1 Based on Their Relative Human Skin Sensitization Potency

Chemical	CAS No.	Human Class*	Human NOEL†	Reference‡
Atranol	526-37-4	1	ND	—
Beryllium sulfate	7787-56-6	1	11	25
Chloroatranol	57074-21-2	1	ND	—
Dimethyl fumarate	624-49-7	1	88	64
Dinitrochlorobenzene	97-00-7	1	8.8	23
Diphenylcyclopropenone	886-38-4	1	ND	—
Hydrazine	301-01-2	1	ND	—
MCI/MI	55965-84-9/96118-96-6	1	0.83	26
Mercuric chloride	7487-94-7	1	924	25
MI	2682-20-4	1	15	26
p-Phenylenediamine	106-50-3	1	10	23
Pentadecylcatechol	492-89-7	1	ND	—
Potassium dichromate	7778-50-9	1	111	25
Squaric acid dibutylester	2892-62-8	1	ND	—
Tetrachlorosalicylanilide	1154-59-2	1	ND	—

*Human class based on an analysis of human data, adapted from References²³⁻⁴³ with additions based on criteria from Table 1. Note that it is not a direct function of the NOEL (see full details of categorization criteria in the main text).

†Human predictive test threshold, the NOEL expressed in $\mu\text{g}/\text{cm}^2$ with key reference source indicated; where differing values have been reported, the lower value has been adopted.

‡The reference relates to the source of the NOEL.

ND indicates insufficient data to define a NOEL; NS, nonsensitizer.

TABLE 3. List of Substances Placed in Category 2 Based on Their Relative Human Skin Sensitization Potency

Chemical	CAS No.	Human Class*	Human NOEL†	Reference‡
2-Aminophenol	95-55-6	2	ND	—
Benzisothiazolinone	2634-33-5	2	45	26
Cinnamal	104-55-2	2	591	23
Cobalt sulfate	10124-43-4	2	ND	—
Diethyl maleate	141-05-9	2	1600	26
Dimethylaminopropylamine	109-55-7	2	ND	—
Formaldehyde	50-00-0	2	37	23
Glutaraldehyde	111-30-8	2	100	23
Glyoxal	107-22-2	2	ND	—
Gold chloride	13453-07-1	2	65	25
Hydroxyisohexyl 3-cyclohexene carboxaldehyde	31906-04-4	2	4000	62
Isoeugenol	97-54-1	2	69	23
Lauryl gallate	1166-52-5	2	ND	—
Methyl heptine carbonate	111-12-6	2	118	62
Methyldibromo glutaronitrile	35691-65-7	2	ND	—
Nickel	7440-02-0	2	154	24
Nitro-4-phenylenediamine	5307-14-2	2	ND	—
Peru balsam	8007-00-9	2	950	62
Propyl gallate	121-79-9	2	ND	—
Quaternium-15	4080-31-1	2	ND	—
Thimerosal	54-64-8	2	ND	—
Thioglycerol	96-27-5	2	661	25
Toluene diamine sulfate	615-50-9	2	ND	—

*Human class based on an analysis of human data, adapted from References²³⁻⁴³ with additions based on criteria from Table 1. Note that it is not a direct function of the NOEL (see full details of categorization criteria in the main text).

†Human predictive test threshold, the NOEL expressed in $\mu\text{g}/\text{cm}^2$ with key reference source indicated; where differing values have been reported, the lower value has been adopted.

‡The reference relates to the source of the NOEL.

ND indicates insufficient data to define a NOEL; NS, nonsensitizer.

for treatment of chronic skin conditions. For these materials, sensitization in the general population is likely to be (extremely) rare. Indeed, except in highly selected patient groups, prevalence in the dermatology clinic should not exceed 1%. Human repeated insult patch test NOEL values are variable, or indeed absent, because of the inherent inaccuracy of determination of a threshold for such a weak sensitizer in a small panel size. Benchmark substances for this group include isopropanol, propylene glycol, and hexyl cinnamal. For example, isopropanol is placed in this category because it is recognized as a rare contact allergen⁵⁸; similarly, propylene glycol is, rarely, associated with the causation of contact allergy.⁵⁹

Category 6

This group contains substances that are essentially free from skin-sensitizing activity (ie, nonsensitizers). It must be noted that this does not mean that there will never have been a clinical report of a positive patch test; for example, petrolatum is judged to belong to this group, even though there are very occasional reports of positive patch tests, despite its routine global use in patch testing without evidence of sensitization. In principle, this true nonsensitizer category should represent the largest group, but the reality is that it means that many of the substances have little human evidence, and an absence of evidence is not a compelling rationale for

inclusion in this group unless it is known that there is a very great extent of human skin exposure. Benchmark substances for this group include xylene, sodium lauryl sulfate, and glycerol.

In respect of regulatory classification for skin sensitization hazard, the GHS provides for 3 groups.¹¹ As mentioned previously, categories 5 and 6 correspond to substances that are not classified; categories 1 to 4 all classify a substance as a skin sensitizer, with categories 1 and 2 corresponding to the stronger skin sensitizers (category 1A in the UN GHS) and categories 3 and 4 corresponding to the weaker skin sensitizers (category 1B in the UN GHS). Note that the human categorization offered should not be expected perfectly to correlate with a classification based solely on the LLNA EC3 value.

THE CATEGORIZATION

Using the information sources mentioned previously and the categorization guidelines set out, 131 substances have been identified that could readily be placed into 1 of the 6 groups. These are fully detailed in Tables 2 to 7, which also include their Chemical Abstracts Service (CAS) numbers to ensure proper definition of the chemical involved. In total, 15 substances were placed into category 1, 23 into category 2, 22 into category 3, 19 into category 4, 28 into category 5, and 24 into category 6. In regulatory terms,¹¹

TABLE 4. List of Substances Placed in Category 3 Based on Their Relative Human Skin Sensitization Potency

Chemical	CAS No.	Human Class*	Human NOEL†	Reference‡
Abietic acid (colophony)	514-10-3	3	ND	—
Bacitracin	1405-87-4	3	ND	—
Benzoyl peroxide	94-36-0	3	ND	—
Bisphenol A diglycidyl ether	1675-54-3	3	ND	—
Butylglycidylether	2426-08-06	3	437	25
Chlorpromazine	50-53-3	3	1150	25
Cinnamic alcohol	104-54-1	3	3000	62
Citral	5392-40-5	3	775	23
Coumarin	91-64-5	3	3543	62
Ethylenediamine	107-15-3	3	732	25
Eugenol	97-53-0	3	1938	23
Farnesol	4602-84-0	3	2755	62
Glycerylmonothioglycolate	30618-84-9	3	ND	—
Hydroquinone	123-31-9	3	ND	—
Hydroxyethyl acrylate	818-61-1	3	ND	—
Imidazolidinyl urea	39236-46-9	3	2000	26
Mercaptobenzothiazole	149-30-4	3	2269	25
Methyl-2,3-hexanedione	13706-86-0	3	3448	23
Metol	55-55-0	3	ND	—
Penicillin	61-33-6	3	76	25
Phenyl benzoate	93-99-2	3	9448	63
Tetramethylthiuram disulfide	137-26-8	3	ND	—

*Human class based on an analysis of human data, adapted from References²³⁻⁴³ with additions based on criteria from Table 1. Note that it is not a direct function of the NOEL (see full details of categorization criteria in the main text).

†Human predictive test threshold, the NOEL expressed in $\mu\text{g}/\text{cm}^2$ with key reference source indicated; where differing values have been reported, the lower value has been adopted.

‡The reference relates to the source of the NOEL.

ND indicates insufficient data to define a NOEL; NS, nonsensitizer.

this means 38 of these substances would be regarded as stronger sensitizers (UN GHS category 1A), and 41 as weaker sensitizers (UN GHS category 1B), whereas 53 would not be classified. In vivo data, such as LLNA EC3 values, have been excluded because they did not inform this analysis. However, for the purposes of completeness, these values have been included for information in Appendix 1. The primary purpose of this work, to produce a reasonably distributed and substantial set of substances assigned into 6 distinct potency categories, based solely on human evidence has been delivered.

COMMENTARY

It is appropriate to mention and also to comment on a number of aspects of the work reported herein. Perhaps most importantly, it is to recognize that it is simply not possible to produce a list of this kind underpinned by a solid foundation of incontrovertible quantitative human data. Many of the substance categorizations rely on an element of judgment, and many depend also on human investigations carried out some decades ago and whose quality is now hard to establish. Even where information appears to be quantitative, such as the HRIPT NOEL or the % frequency of positive diagnostic patch test information, the data are subject to a number of variables and, especially with the latter figures, have to be considered in the context of exposure, itself a difficult thing to quantify. Nevertheless, without making use of this information, the

list in Tables 2 to 7 would become trivially small. All would agree that 2,4-dinitrochlorobenzene is a powerful human sensitizer and should appear in the highest category; similarly, all would agree that sodium lauryl sulfate is a skin irritant and should appear in the lowest category. However, it is not possible to develop and to rigorously evaluate the capacity of in vitro methods for the prediction of human skin-sensitizing potency without a much more extensive basis on which to conduct that evaluation. Local lymph node assay EC3 values have their merit, but even these are to all intents and purposes given their credibility only by comparison to human data.^{20–26}

A second matter to consider is the set of substances placed in categories 5 and 6. These would not classify according to regulatory criteria.¹¹ Nevertheless, particularly in category 5, there are many substances recognized at least as occasional skin sensitizers. Even in category 6, there are several substances for which positive diagnostic patch tests have been recorded (eg, glycerol, octanoic acid, petrolatum, sodium lauryl sulfate, tocopherol, xylene). It is beyond the scope of the present work to engage here in a detailed review of the literature for each of these. What has to be recognized is that where there are extensive skin exposure and exceedingly rare positive patch tests, then it is reasonable to suppose that either the patch test was not a true allergic reaction and/or that the positive result represents the highly idiosyncratic response of an individual, which therefore does not accurately reflect how the population as a whole responds to that substance.

TABLE 5. List of Substances Placed in Category 4 Based on Their Relative Human Skin Sensitization Potency

Chemical	CAS No.	Human Class*	Human NOEL†	Reference‡
Amylcinnamal	122-40-7	4	23622	26
Amylcinnamyl alcohol	101-85-9	4	3543	27
Aniline	62-53-3	4	2463	25
Benzocaine	94-09-7	4	2000	24
Bronopol	52-51-7	4	ND	—
Carvone	99-49-0	4	2657	62
Chlorhexidine gluconate	55-56-1	4	ND	—
Ethyl acrylate	9003-32-1	4	1600	26
Ethyleneglycol dimethacrylate	97-90-5	4	ND	—
Geraniol	106-24-1	4	3875	26
Hexyl salicylate	6259-76-3	4	35433	62
Hydroxycitronellal	107-75-5	4	2953	23
Iodopropynyl butyl carbamate	55406-53-6	4	ND	—
Kanamycin	8063-07-08	4	1874	25
p-tert-Butyl- α -methylhydrocinnamal	80-54-6	4	3750	24
Linalool	78-70-6	4	13793	23
Methylmethacrylate	80-62-6	4	ND	—
Neomycin sulfate	1404-04-2	4	15625	25
Resorcinol	108-46-3	4	ND	—

*Human class based on an analysis of human data, adapted from References^{23–43} with additions based on criteria from Table 1. Note that it is not a direct function of the NOEL (see full details of categorization criteria in the main text).

†Human predictive test threshold, the NOEL expressed in $\mu\text{g}/\text{cm}^2$ with key reference source indicated; where differing values have been reported, the lower value has been adopted.

‡The reference relates to the source of the NOEL.

ND indicates insufficient data to define a NOEL; NS, nonsensitizer.

TABLE 6. List of Substances Placed in Category 5 Based on Their Relative Human Skin Sensitization Potency

Chemical	CAS No.	Human Class*	Human NOEL†	Reference‡
Anethole	104-46-1	5	ND	—
4-Aminobenzoic acid	150-13-0	5	ND	—
Anisyl alcohol	105-13-5	5	3448	62
Benzaldehyde	100-52-7	5	590	62
Benzalkonium chloride	8001-54-5	5	ND	—
Benzyl benzoate	120-51-4	5	20690	24
Benzyl salicylate	118-58-1	5	17717	62
Butylated hydroxytoluene	128-37-0	5	ND	—
Butylene glycol	107-88-0	5	ND	—
Cetrimide	57-09-0	5	ND	—
Cetylpyridinium chloride	773-52-6	5	ND	—
Citronellol	106-22-9	5	29528	62
Diethanolamine	111-42-2	5	ND	—
Hexylcinnamal	101-86-0	5	23622	26
Hydrocortisone	50-23-7	5	ND	—
Isopropanol	67-63-0	5	ND	—
Isopropyl myristate	110-27-0	5	ND	—
Limonene (not oxidized)	138-86-3, 5989-27-5	5	ND	—
Methyl salicylate	119-36-8	5	ND	—
Pentachlorophenol	87-86-5	5	2155	25
Phenoxyethanol	122-99-6	5	ND	—
Propyl paraben	94-13-3	5	ND	—
Propylene glycol	57-55-6	5	60000	23
Pyridine	110-86-1	5	41051	25
Sorbic acid	110-44-1	5	ND	—
Triclosan	3380-34-5	5	ND	—
Triethanolamine	102-71-6	5	ND	—
Vanillin	121-33-5	5	1181	62

*Human class based on an analysis of human data, adapted from References²³⁻⁴³ with additions based on criteria from Table 1. Note that it is not a direct function of the NOEL (see full details of categorization criteria in the main text).

†Human predictive test threshold, the NOEL expressed in $\mu\text{g}/\text{cm}^2$ with key reference source indicated; where differing values have been reported, the lower value has been adopted.

‡The reference relates to the source of the NOEL.

ND indicates insufficient data to define a NOEL; NS, nonsensitizer.

Next, the nature of the available literature means that the chemicals in these lists contain a bias toward consumer product ingredients, including preservatives and fragrances. This bias also arises because with this type of product the nature and extent of skin exposure are often better understood. Despite this, other categories are represented, including metals, acrylates, rubber chemicals, medicaments, and some general industrial chemicals. However, it is much more important that the chemicals cover the spectrum of potency, which they do, and that they encompass the types of chemistry (mechanistic applicability domains) known to be associated with the causation of ACD.⁶⁰ The key domains include Schiff base formers, Michael acceptor electrophiles, the $\text{S}_{\text{N}}\text{Ar}$ electrophile domain, the $\text{S}_{\text{N}}2$ electrophile domain, the acyl transfer electrophile domain, and the nonelectrophilic non-proelectrophilic domain; for any of these, there may also exist inactive precursors that require metabolic or air oxidation to produce the primary reactive species. Many of the aldehydes sensitize via a

Schiff base mechanism, although some also involve Michael acceptor mechanisms, for example, cinnamal; ethylene glycol dimethacrylate and diethyl maleate are other examples of Michael acceptors; butylglycidylether and benzyl benzoate are examples of $\text{S}_{\text{N}}2$ reactors^{60,61}; tetrachlorosalicylanilide, imidazolidinyl urea, and mercaptobenzothiazole are examples of the acylation mechanism.

Lastly, and as mentioned elsewhere, this analysis inevitably involves a considerable degree of expert judgment. Thus, 1 part of the purpose in presenting this human skin sensitization potency database is to permit others to enhance it, both by the addition of further substances and/or by the correction of errors (including those that will become apparent as our knowledge increases).

CONCLUSIONS

The successful conduct of safety assessment for potentially sensitizing substances without the use of in vivo data requires that

TABLE 7. List of Substances Placed in Category 6 Based on Their Relative Human Skin Sensitization Potency

Chemical	CAS No.	Human Class*	Human NOEL
Aluminum chloride	7446-70-0	6	NS
Ascorbic acid	50-81-7	6	NS
Benzene	71-43-2	6	NS
Butanol	71-36-3	6	NS
Dextran	9004-54-0	6	NS
Diethyl phthalate	84-66-2	6	NS
Diethyl toluamide	94271-03-1	6	NS
Dimethylsulfoxide	67-68-5	6	NS
Glucose	50-99-7	6	NS
Glycerol	56-81-5	6	NS
Hexachlorophene	70-30-4	6	NS
Hexane	110-54-3	6	NS
Lactic acid	50-21-5	6	NS
Octanoic acid	124-07-2	6	NS
Petrolatum	8009-03-08	6	NS
Phenol	108-95-2	6	NS
Retinoic acid	302-79-4	6	NS
Salicylic acid	69-72-7	6	NS
Sodium lauryl sulfate	151-21-3	6	NS
Sorbitol	50-70-4	6	NS
Tocopherol	59-02-9	6	NS
Tween 80	9005-65-6	6	NS
Xylene	1330-20-7	6	NS
Zinc oxide	1314-13-2	6	NS

*Human class based on an analysis of human data, adapted from References 23–43 with additions based on criteria from Table 1. Note that it is not a direct function of the NOEL (see full details of categorization criteria in the main text).
NS indicates nonsensitizer.

those nonanimal models deliver an appreciation of hazard characterization, specifically information on the relative potency of identified sensitizers. Currently, in vitro models that are undergoing formal validation aim to provide only hazard identification information, but not hazard characterization potency data.^{3–8} To assess whether these, or other, nonanimal models can provide the information necessary to determine the potency of a sensitizer, perhaps via an integration of the information in a structured weight of evidence methodology, it is vital to have a sufficient body of human potency data/categorization against which such an approach can be evaluated. The work herein reports on the collation of a database of 131 substances categorized into 6 potency categories that can be used for this purpose. Of these, 79 would be identified as possessing a skin sensitization hazard, whereas 52 would not be classified. However, such information is largely insufficient for risk assessment. Hence, the aim here has been to achieve a reference standard data set based on categorization solely according to information on their intrinsic human potency. This categorized set of 131 substances is offered in fact as a step in that direction, available for use, but also for constructive criticism, correction, and supplementation. What sets it apart is that it is

founded wholly on human data/information, accepting that such commonly has imperfections and/or difficulties associated with its use and interpretation, which demand a considerable use of expert judgment. However, whatever the limitations of this database may be, it is our view that it represents a more relevant option for the development and definition of nonanimal alternatives for potency prediction than does a comparison with LLNA EC3 values alone.

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APPENDIX 1. Alphabetical List of Substances and Their LLNA EC3 Values

4-Aminobenzoic acid	NS
2-Aminophenol	0.4
Abietic acid (colophony)	15
Amylcinnamal	11
Amylcinnamyl alcohol	25
Anethole	2.3
Aniline	89
Anisyl alcohol	5.9
Atranol	0.6
Benzaldehyde	25
Benzalkonium chloride	NS
Benzisothiazolinone	2.3
Benzocaine	22
Benzoyl peroxide	0.3
Benzyl benzoate	17
Benzyl salicylate	2.9
Beryllium sulfate	8.6
Bisphenol A diglycidyl ether	1.5
Butanol	NS
Butylglycidylether	31
Carvone	13
Chloroatranol	0.4
Chlorpromazine	0.14
Cinnamal	2
Cinnamic alcohol	21
Citral	13
Citronellol	44
Cobalt sulfate	0.2
Coumarin	30

APPENDIX 1. (Continued)

Dextran	NS
Diethanolamine	40
Diethylmaleate	2.1
Diethyl toluamide	NS
Diethyl phthalate	NS
Dimethylaminopropylamine	2.2
Dimethylfumarate	0.35
Dimethylsulfoxide	72
Dinitrochlorobenzene	0.08
Diphenylcyclopropanone	0.05
Ethyl acrylate	28
Ethylenediamine	2.2
Ethyleneglycol dimethacrylate	35
Eugenol	13
Farnesol	4.8
Formaldehyde	0.4
Geraniol	26
Glutaraldehyde	0.1
Glycerol	NS
Glyoxal	1.4
Gold chloride	0.3
Hexane	NS
Hexyl salicylate	0.18
Hexylcinnamal	8
Hydrocortisone	NS
Hydroquinone	0.1
Hydroxycitronellal	20
Hydroxyethyl acrylate	1.4
Hydroxyisohexyl 3-cyclohexene carboxaldehyde	17
Imidazolidinyl urea	24
Iodopropynyl butyl carbamate	0.9
Isoeugenol	1.2
Isopropanol	NS
Isopropyl myristate	44
Kanamycin	NS
Lactic acid	NS
Lauryl gallate	0.3
Lilial (p-ter-butyl-a-methylhydrocinnamal)	19
Limonene (not oxidized)	69
Linalool	30
MCI/MI	0.05
Mercaptobenzothiazole	1.7
Mercuric chloride	0.4
Methyl heptene carbonate	0.5
Methyl salicylate	NS
Methyl-2,3-hexanedione	26
Methyldibromo glutaronitrile	0.9
MI	0.4
Methylmethacrylate	90
Neomycin sulfate	6
Nickel	0.6
Nitro-4-phenylenediamine	0.4
Octanoic acid	NS
p-Phenylenediamine	0.11

(Continued on next page)

APPENDIX 1. (Continued)

Penicillin	30
Pentachlorophenol	20
Phenol	NS
Phenoxyethanol	NS
Phenyl benzoate	5.2
Potassium dichromate	0.08
Propyl gallate	0.3
Propyl paraben	NS
Propylene glycol	NS
Pyridine	72
Resorcinol	6.3
Salicylic acid	NS
Sodium lauryl sulfate	14
Tetrachlorosalicylanilide	0.04
Tetramethylthiuram disulphide	6
Thioglycerol	3.6
Tocopherol	7.4
Toluene diamine sulfate	0.2
Tween 80	NS
Vanillin	NS
Xylene	96

NS indicates tested and found to be a nonsensitizer in the LLNA.

EC3 data were not found for the following substances:

Aluminum chloride
 Ascorbic acid
 Bacitracin
 BenzeneBronopolButylated hydroxytoluene
 Butylene glycol
 Cetrimide
 Cetylpyridinium chloride
 Chlorhexidine gluconate
 Dextran
 Glucose
 Glycerylmonothioglycolate
 Hexachlorophene
 Metol
 Pentadecylcatechol
 Peru balsam
 Petrolatum
 Quaternium 15
 Retinoic acid
 Sorbic acid
 Sorbitol
 Squaric acid dibutylester
 Thimerosal
 Thioglycerol
 Triclosan
 Triethanolamine
 Zinc oxide