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Fragrances Categorized According to Relative Human Skin Sensitization Potency

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Background: **The development of non-animal alternatives for skin sensitization potency prediction is dependent upon the availability of a sufficient dataset whose human potency is well characterized. Previously, establishment of basic categorization criteria for 6 defined potency categories, allowed 131 substances to be allocated into them entirely on the basis of human information.**

Objectives: **To supplement the original dataset with an extended range of fragrance substances.**

Methods: **A more fully described version of the original criteria was used to assess 89 fragrance chemicals, allowing their allocation into one of the 6 potency categories.**

Results: **None of the fragrance substances were assigned to the most potent group, category 1, whereas 11 were category 2, 22 were category 3, 37 were category 4, and 19 were category 5. Although none were identified as non-sensitizing, note that substances in category 5 also do not pass the threshold for regulatory classification.**

Conclusions: **The combined datasets of >200 substances placed into potency categories solely on the basis of human data provides an essential resource for the elaboration and evaluation of predictive non-animal methods.**

The fundamental purpose of toxicological evaluation is to uncover substances that possess properties, rendering them a potential hazard to human health.[1] However, the identification of such substances is often meaningless unless the strength of that hazard, often termed potency, is also characterized. With respect to the toxicological hazard known as skin sensitization, the simple identification of hazard has been ensured for many decades, and the key details were well documented.[1,2] However, in recent decades, the concept of simultaneously measuring the relative potency of the identified hazard has also become central to the process of assessing the risk of skin sensitization.[3–7] It is not germane to the present work to discuss the merits (or otherwise) of the risk assessment itself, save to note that it is well characterized and transparent, such that it is capable of critical scrutiny to move it into a second-generation version.[8–10] What is pertinent is that the toxicological predictions of the relative potency of a skin sensitizer are actually meaningful in terms of the species of concern, that is, humans. To meet this challenge, a first publication (in this journal) detailed an approach to the subcategorization of chemicals into 1 of 6 potency classes, solely on the basis of human data, and then reported on the outcome for a total of 131 substances.[11] Of these, only a small minority were fragrance chemicals, so that, in an associated follow-up, human data were presented for a small number of additional fragrance chemicals.[12] In the present work, we have endeavored to extend the original series more substantially via the addition of information on a larger body of substances used as fragrances. In total, 89 chemicals were assessed because they had sufficient information to permit potency categorization using only human data. However, as a refinement to the previous publication, we have endeavored to offer a clearer explanation of the basis for individual classification, thereby enhancing the categorization outline provided in that original publication.[11] It is anticipated that this additional set of substances will further assist those working to produce nonanimal models capable of predicting the relative human potency of newly identified skin sensitizing substances.

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MATERIALS AND METHODS

The 89 substances considered are reported in Table 1, along with their chemical abstracts service (CAS) numbers. All materials were of the quality supplied to downstream users by the fragrance industry, thus ensuring that data generated using them were relevant to the real-life situation.

A decision on allocation to a category was achieved using information from experimental human studies, specifically the human repeated insult patch test (HRIPT), conducted according to the protocol previously published, or in a few instances, the human maximization test (HMT) as published by Kligman.[13,14] Most data

TABLE 1. Outline of Potency Categorization Guidance

Potency Category	HRIPT/HMT NOEL*	DPT Data†	Use Information‡
1 (extreme)	<25 μg/cm^2	>3% In most dermatology clinics	Probably low exposure concentration
2 (strong)	25–500 μg/cm^2	>1% In many dermatology clinics	Lower use concentration may raise
3 (moderate)	500–2500 μg/cm^2	Up to 1% in major dermatology clinics	by 1 category; higher use concentration
4 (weak)	>2500 μg/cm^2	Less common/frequent positive results than category 3	may drop a category
5 (very weak)	>10,000 μg/cm^2	Rarely positive except in selected patients with eczema	Possibly despite high use
6 (nonsensitizer)	Negative§	An absence of positives despite testing in many clinics	Use could be high or low

**For this purpose, the 2 types of human test are taken as equivalent; LOEL data are used only as a guide to the proximity of the NOEL to the true HRIPT induction threshold. The HRIPT is normally given more weight than the HMT because the former involves testing in larger panel sizes, typically 4 times the HMT.*
†Generally taken from multiclinic-collated information on consecutive patients with eczema. However, the lower potency categories may rely more on isolated cases.
‡Given the great rarity with which there is a clear correlation between exposure and the induction of contact allergy from DPT data, the use information on total volume of sales and, where it exists, the typical maximum use levels are used to refine the conclusions.
§In effect, this simply means that a high test concentration yielded no evidence of the induction of skin sensitization.

from these sources offer a no-effect level (NOEL), and where multiple data exist, the highest value has been taken. For a few substances, a lowest-effect level (LOEL) has been recorded. Accordingly, it is important to state that no new positive data have been generated for the purpose of this work—all of the LOEL data are derived from historic studies. The authors recognize that the conduct of new human studies to determine an LOEL for the induction of contact allergy is, by definition, unethical. Human repeated insult patch test and HMT studies as conducted by Research Institute for Fragrance Materials are of equivalent sensitivity and thus taken as interchangeable. The limited LOEL data provide a guide concerning the extent to which the NOEL data are close to the true threshold.

Indications concerning potential categorization were modified by information derived from a survey of diagnostic patch test (DPT) data from published clinical literature, with the existence of such information typically being indicated by the recording of a patch test concentration.[15] Particular account also was taken of the important and comprehensive review of fragrance allergy already completed by the European Commission independent advisory body, the Scientific Committee on Consumer Safety (SCCS).[16]

To assist in understanding the process of potency categorization solely on the basis of human data, an outline of the criteria used is provided in Table 1. It is worth reinforcing here the key point that larger NOEL values equate to lower skin sensitization potency. Thus, where there are multiple values, unless there is compelling information to suggest a different strategy, the *higher* value should always be used. The converse argument would always apply to LOEL values, where the *smaller* value must be adopted. As always, the final decision on a category will have considered all of the available evidence. This includes DPT data, where this exists, judged against the use volume information. Diagnostic patch test data can be taken from the clinical literature and, for some of the materials here, from the SCCS review already mentioned.[16]

RESULTS

The outcome of the analysis on this set of fragrance substances is contained in Table 2. None of the substances were allocated to the highest, category 1, although for 2 materials, *trans*-2-hexenal and methyl 2-nonynoate, the decision was borderline, and so this is discussed in more detail later. Ultimately, along with 9 others, they were assigned to category 2. For the remainder, 22 were assigned to category 3, 37 were assigned to category 4, and 19 were assigned to category 5. None were assigned to category 6, the true nonsensitizers. To facilitate the understanding of the rationale, several of these are discussed to provide an exposition of how the criteria described in Table 1 and the previous publication are applied.[10] None of the substances was regarded as entirely nonsensitizing; thus, category 6 was not represented.

For a first example, *trans*-2-hexenal is considered. It has an HRIPT NOEL of 24 μg/cm^2, which is only less than the threshold for category 1 (Table 1). However, the HRIPT LOEL is almost 10-fold higher, suggesting that the true NOEL is higher than the category 1 threshold. There is no HMT information to add to the mix; the remaining source of information for consideration is therefore DPT data. In this case, it is very sparse. A patch test concentration of 1% is suggested.[15] However, a search on PubMed reveals an absence of any data, an outcome consistent with the conclusions of a European Commission advisory body report.[16] Consequently, the decision must be that *trans*-2-hexenal is most appropriately placed into category 2. A similar logic was applied to methyl 2-nonynoate, supported by the occurrence of only a single positive patch test reaction in the literature.[23]

In comparison, the next example, farnesol, is somewhat less clear-cut. The HRIPT NOEL is close to category 3, but it is clearly in category 4. However, it is a well-known human contact allergen that is used in routine diagnostic testing as a component of fragrance mix II.[17,18] The frequency of positive patch tests for a fragrance component that has rather low use volume was regarded as sufficient evidence to elevate farnesol into category 3.

1,2,3,4,5,6,7,8-Octahydro-8,8-dimethyl-2-naphthaldehyde was placed into category 3 on the basis of the view that the HRIPT, which in this case involved only more than 100 volunteers, would not be overridden by the HMT, which used only a quarter of the number and recorded an NOEL that was not too far from the category 3/4 border. Had the HMT value been much higher, as was the case with ylang-ylang, then the decision might have been different. However, in this latter case, the fact that the HRIPT NOEL was not as low (ie, relatively close to the category 3/4

TABLE 2. List of Substances and HPC Conclusions

Fragrance Ingredient	CAS Number	NOEL HRIPT,* μg/cm²	NOEL HMT,† μg/cm²	LOEL HRIPT,† μg/cm²	Annual Use Volume,* tons	HPC	Comments‡
Oakmoss	90028-68-5	700	1724	1417	1–10	2	Cat 2 on the assumption that atranol/chloroatranol concentrations are fully controlled (IFRA guideline).
3-Methyl-5-phenylpent-2-enenitrile	93893-89-1	275	NA	NA	10–100	2	Predominantly based on HRIPT
5,6,7-Trimethylocta-2,5-dien-4-one	358331-95-0	250	NA	NA	1–10	2	Predominantly based on HRIPT
trans-α-Damascone	24720-09-0	310	138	2531	10–100	2	Predominantly based on HRIPT; limited positive DTP available
trans-2-Hexenal	6728-26-3	24	NA	236	1–10	2	HRIPT LOEL suggests a higher NOEL; although tonnage is low, DPT evidence is very sparse; thus, balance is Cat 2 rather than Cat 1.
2-Hexylidene cyclopentanone	17373-89-6	300	NA	500	<0.1	2	HRIPT and HMT nicely aligned; no DPT information found
2-Methoxy-4-methylphenol	93-51-6	118	NA	NA	0.1–1	2	Based on HRIPT; no DPT information found
6-Methyl-3,5-heptadien-2-one	1604-28-0	118	NA	1299	<0.1	2	Based on HRIPT; no DPT information found
Methyl 2-nonynoate (methyl octine carbonate)	111-80-8	24	NA	118	10–100	2	HRIPT on border of Cat 1, but adjusted because of LOEL and paucity of DPT data§
Tea leaf absolute	84650-60-2	480	NA	NA	1–10	2	Based on HRIPT; no DPT information found
Methyl 2-octynoate (methyl heptine carbonate)	111-12-6	118	NA	194	1–10	2	Predominantly based on HRIPT; very little DPT information found
Cuminaldehyde	122-03-2	1181	2760	NA	1–10	3	Predominantly based on HRIPT because positive DPT data are very rare‖
Hexyl tiglate	16930-96-4	110	8316	NA	0.1–1	3	No DPT case reports found; HMT suggests that HRIPT NOEL is too low
Methyl 2,4-dihydroxy-m-toluate	33662-58-7	620	NA	NA	<0.1	3	Predominantly based on HRIPT
1-(1-Naphthyl)ethanone	941-98-0	2598	1380	NA	1–10	3	Only 1 DPT case in the literature
1-(5,5-Dimethyl-1-cyclohexen-1-yl) pent-4-en-1-one	56973-85-4	2500	NA	NA	10–100	3	Based on HRIPT; no DPT information found
1,2,3,4,5,6,7,8-Octahydro-8, 8-dimethyl-2-naphthaldehyde	68991-97-9	551	2760	NA	10–100	3	HRIPT fits Cat 3 and dominates the HMT, which is only indicative of Cat 4.
3-(*p*-Isopropylphenyl)propionaldehyde	7775-00-0	1102	NA	NA	10–100	3	Based on HRIPT
7-Methyl-2H-benzo-1,5-dioxepin-3(4H)-one	28940-11-6	1000	NA	NA	10–100	3	Based on HRIPT
Propanedioic acid, 1-(3,3-dimethylcyclohexyl) ethyl, ethyl ester	478695-70-4	2000	NA	NA	10–100	3	Based on HRIPT
2-Methyldecanenitrile	69300-15-8	2250	NA	NA	10–100	3	Based on HRIPT
4-Hydroxy-2,5-dimethyl-3(2H)-furanone	3658-77-3	591	NA	1181	1–1	3	Based on HRIPT supported by the HMT NOEL

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TABLE 2. *(Continued)*

Fragrance Ingredient	CAS Number	NOEL HRIPT,* μg/cm²	NOEL HMT,† μg/cm²	LOEL HRIPT,† μg/cm²	Annual Use Volume,* tons	HPC	Comments‡
Farnesol	4602-84-0	2755	NA	68974	1–10	3	Added LOEL; low volume, so positive DPT data[16,17] mean category confirmed[11] rather than being placed in Cat 4
Allyl phenoxyacetate	7493-74-5	709	690	NA	10–100	3	Based on HRIPT
Cinnamyl nitrile	1885-38-7	1063	3448	1250	1–10	3	Based on HRIPT
Jasmine absolute (*Jasminum grandiflorum*)	8022-96-6; 8024-43-9; 90045-94-6; 84776-64-7	1475	NA	2069	1–10	3	Based on HRIPT, moderate tonnage, and a fair number of DPT positives
p-Mentha-1,8-dien-7-al	2111-75-3	709	690	2760	0.1–1	3	Based on HRIPT and low tonnage with absence of DPT data
Menthadiene-7-methyl formate	68683-20-5	1063	690	6900	0.1–1	3	Based on HRIPT and low tonnage with absence of DPT data
2-Methyl-butanoic acid hexyl ester	10032-15-2	696	6930	NA	1–10	3	Based on HRIPT; the HMT NOEL is insufficient to shift it to Cat 4
Phenylacetaldehyde	122-78-1	592	NA	1181	1–10	3	Based on HRIPT, lower tonnage with a few positive DPTs
3-Propylidenephthalide	17369-59-4	945	345	2760	0.1–1	3	Based on HRIPT, low tonnage, and very limited DPT data[16]
Treemoss	90028-67-4	700	6896	1417	1–10	3	HRIPT and HMT NOELs consistent with Cat 3; positive DPT data
Ethyl acrylate	140-88-5	1600	NA	NA	No data	3	Based on HRIPT NOEL; common positive DPTs (eg, 20)
Piperonal (heliotropin)	120-57-0	2952	4140	NA	100–1000	4	Based on HRIPT NOEL, supported by close NOEL, high volume but with little evidence of contact allergy[18]
Heptaldehyde, ethylene glycol acetal	1708-34-5	2780	NA	NA	0.1–1	4	Based on HRIPT
ω-Pentadecalactone	106-02-5	5510	6900	NA	100–1000	4	Negative in a survey despite moderate use[19]
Butanamide, 2-ethyl-*N*-methyl-*N*-(3-methylphenyl)-	406488-30-0	3250	NA	NA	1–10	4	Based on HRIPT
Ethyl tiglate	5837-78-5	3465	NA	NA	<0.1	4	Based on HRIPT
Formaldehyde cyclododecyl ethyl acetal	58567-11-6	3543	1380	NA	100–1000	4	Higher HRIPT NOEL dominates over HMT; lack of DPT evidence against high use volume supports Cat 4.
Methoxy dicyclopentadiene carboxaldehyde	86803-90-9	5000	NA	NA	10–100	4	HRIPT used diethylphthalate only, but this would not impact outcome
2-Isobutyl-4-methyltetrahydro-2H-pyran-4-ol	63500-71-0	4408	NA	NA	100–1000	4	Based on HRIPT

3-(4-Methyl-3-cyclohexenyl)butanol	15760-18-6	5906	NA	NA	1–10	4	Based on HRIPT
3-Phenylbutanal	16251-77-7	5905	NA	12500	10–100	4	Based on HRIPT
Longifolene	475-20-7	3543	6900	NA	100–1000	4	Based on HRIPT, supported by HMT result
β-Farnesene	18794-84-8	3780	NA	6250	1–10	4	Based on HRIPT, supported by HMT result
2-Methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal	3155-71-3	2953	NA	NA	0.1–1	4	Based on HRIPT
2,4-Dimethyl-3-cyclohexen-1-carboxaldehyde	68039-49-6	5905	6900	NA	>1000	4	Based on HRIPT, supported by HMT result
p-Methoxybenzaldehyde (anisaldehyde)	123-11-5	3543	6900	4724	>1000	4	Based on HRIPT, supported by HMT result and HRIPT LOEL
6-Methoxy-2,6-dimethylheptan-1-al	62439-41-2	5905	NA	NA	1–10	4	Based on HRIPT
α-Bisabolol	515-69-5	5510	NA	NA	<0.1	4	Based on HRIPT
Tricyclo[3.3.1.1.(3.7)]decan-2-ol, 4-methyl-8-methylene	122760-84-3	3000	NA	NA	0.1–1	4	HRIPT NOEL confirmed in diethylphthalate and alcohol vehicles
3,3-Dimethyl-5-(2,2,3-trimethyl-3-cyclopenten-1-yl)-4-penten-2-ol	107898-54-4	2598	NA	5000	100–1000	4	Based on HRIPT NOEL supported by LOEL
2-Methyldecanal	19009-56-4	5905	6900	NA	10–100	4	Based on HRIPT, supported by HMT result
Benzyl alcohol	100-51-6	5906	6897	8858	100–1000	4	Based on HRIPT NOEL supported by HMT result and HRIPT LOEL, steady flow of positive DPT results set against high tonnage
Benzyl cinnamate	103-41-3	4720	5517	NA	10–100	4	Based on HRIPT, some DPTs, and moderately high tonnage
Dibenzyl ether	103-50-4	2362	2760	NA	10–100	4	Based on HRIPT downgraded because of absence of DPTs and moderately high tonnage
Eucalyptol (cineole)	470-82-6	590	11040	NA	100–1000	4	Cat 3 from HRIPT NOEL is adjusted because of very high HMT NOEL and high use volume but limited evidence of positive DPT (eg, Vilaplana and Romaguera[20])
p-Isobutyl-α-methyl hydrocinnamal	6658-48-6	2362	5520	NA	10–100	4	Cat 4 as HRIPT is close to the border, the HMT has a higher NOEL, and there is no positive body DPT evidence despite moderate use
Isocyclocitral	1335-66-6	7087	2759	NA	10–100	4	Based on HRIPT, moderate tonnage, and absence of DPT testing
Isocyclogeraniol	68527-77-5	3898	NA	5000	1–10	4	Based on HRIPT NOEL supported by LOEL
Jasmine absolute (*Jasminum sambac*)	91770-14-8	8858	NA	NA	1–10	4	Based on HRIPT NOEL and positive DPTs[18]
4-Methoxy-α-methyl benzenpropanal	5462-06-6	5905	1380	NA	10–100	4	Based on HRIPT, moderate tonnage, and a DPT
1-Octen-3-yl acetate	2442-10-6	3543	NA	6900	0.1–1	4	Based on HRIPT NOEL supported by the LOEL
β,β,3-Trimethyl benzenepropanol	103694-68-4	9900	NA	NA	10–100	4	Based on HRIPT, moderate tonnage, few DPTs
p-t-Butyl-dihydrocinnamal	18127-01-0	1181	4138	NA	10–100	4	Based on HRIPT; HMT NOEL suggests Cat 4, but absence of DPT evidence to support lower category
Carvone	99-49-0	2657	NA	NA	100–1000	4	Based on HRIPT NOEL and very limited evidence of positive DPTs

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TABLE 2. *(Continued)*

Fragrance Ingredient	CAS Number	NOEL HRIPT,* μg/cm²	NOEL HMT,† μg/cm²	LOEL HRIPT,† μg/cm²	Annual Use Volume,* tons	HPC	Comments‡
Vanillyl butyl ether	82654-98-6	3543	NA	NA	1–10	4	Based on HRIPT NOEL; no DPT data
α-Methyl cinnamal	101-39-3	3543	5517		100–1000	4	Based on HRIPT NOEL; no DPT data despite large use volume
Ylang-ylang	8006-81-3; 68606-83-7; 83863-30-3	1772	6897	7752	10–100	4	Based on HRIPT, where LOEL suggests NOEL may be higher, supported by HMT NOEL, moderate tonnage, and evidence of DPT positives[21,22]
Anisyl alcohol	105-13-5	3448	NA	NA	10–100	4	Based on HRIPT NOEL; limited DPT data to substantiate
1-(3-Methyl-2-benzofuranyl)ethanone	23911-56-0	11019	NA	NA	1–10	5	No DPT data, very high HRIPT NOEL
trans-Anethole	4180-23-8	5510	1380	NA	100–1000	5	Anethole was Cat 5 with CAS of 104-46-1[11]; human use volume is huge, but DPT data are typically negative
Tetrahydro-4-methyl-2-propyl-2H-pyran-4-yl acetate	131766-73-9	11019	NA	NA	0.1–1	5	No DPT data, very high HRIPT NOEL
Isobornyl acetate	125-12-2	6496	6900	NA	100–1000	5	Based on high HRIPT NOEL, enhanced by large volume of use and no contradictory evidence from DPTs
3-Methylcyclopentadecenone (muscone)	82356-51-2	10000	NA	NA	10–100	5	Very high HRIPT NOEL; no DPT data to contradict
Citronellal	106-23-0	7086	2760	NA	10–100	5	High HRIPT NOEL supported by HMT, moderate use volume and absence of DPT data to contradict
5-Cyclotetradecen-1-one, 3-methyl-,(5E)-	259854-70-1	10000	NA	NA	1–10	5	Very high HRIPT NOEL; DPT data do not contradict
1,1,3-Trimethyl-3-phenylindane	3910-35-8	10630	NA	NA	10–100	5	Very high HRIPT NOEL; DPT data do not contradict
α-Methyl-1,3-benzodioxole-5-propionaldehyde	1205-17-0	4016	13800	15000	100–1000	5	HRIPT LOEL suggests that the NOEL is underestimated, supported by HMT NOEL; few positive DPTs despite high use
Methyl dihydrojasmonate	24851-98-7	10000	13800	NA	>1000	5	High HRIPT NOEL supported by HMT, large use volume, and limited positive DPT data
6,7-Dihydro-1,1,2,3,3-pentamethyl-4(5H)-indanone	33704-61-9	12121	NA	NA	100–1000	5	Very high HRIPT NOEL; no DPT data to contradict
Methyl atrarate	4707-47-5	11810	6900	NA	100–1000	5	Very high HRIPT NOEL supported by HMT, large use volume, and limited DPT data

2-Nonyn-1-al dimethyl acetal	13257-44-8	23620	2760	NA	0.1–1	5	Very high HRIPT NOEL supported by HMT and limited DPT data
cis-4-(Isopropyl)cyclohexanemethanol	13828-37-0	17,717	NA	NA	100–1000	5	Very high HRIPT NOEL; no contradiction from DPT data
Dihydromyrcenol	18479-58-8	23622	2760	NA	>1000	5	Very high HRIPT NOEL supported by HMT and limited DPT data
Ethylene brassylate	105-95-3	23622	20700	NA	>1000	5	Very high HRIPT NOEL; no contradiction from DPT data
α-iso-Methylionone	127-51-5	70866	NA	NA	>1000	5	Very high NOEL, a few scattered patch test reports, high tonnage
1-(1,2,3,4,5,6,7,8-Octahydro-2,3,8,8 tetramethyl-2-naphthalenyl)ethanone	54464-57-2	47244	NA	NA	>1000	5	Based on HRIPT with rare positive DPTs but very high tonnage
dl-Citronellol	106-22-9	29528	NA	NA	>1000	5	Based on HRIPT with rare positive DPTs but very high tonnage

**Data supplied by Research Institute for Fragrance Materials and reflect use levels for the fragrance industry in recent years; no data were available for ethyl acrylate.*
†All figures relate to the induction of sensitization.
‡Unless indicated otherwise, HPC is based on the HRIPT information, backed up by HMT data when available. Diagnostic patch test results may refine the category.
§Adjustment to Cat 2 because the volume of use is not low and the DPT data were essentially absent bar a single case.[23]
‖Three cases were noted by the SCCS,[16] although they reference Rycroft,[24] who only reports positive reactions to dipentene, a different fragrance.
Cat indicates category; HPC, human information and potency category; NA, not available (ie, does not exist).

border) and that the HMT is in category 4, together with the availability of HRIPT LOEL data well into category, made the final placement of ylang-ylang into category 4 a simple decision. It is worth noting that the moderate volume of use and occasional clinical evidence of positive reactions from normal use of ylang-ylang are also perfectly consistent with category 4.

For the final example, consider formaldehyde cyclododecyl ethyl acetal. This substance was placed into category 4, although the HMT NOEL suggested category 3. However, all of these studies involve a single dose level, so we do not know whether testing in the HMT at a higher concentration might also have proven negative and delivered a higher NOEL. That this would likely be the case is suggested by the HRIPT NOEL, which is clearly in category 4. There are no DPT data to contradict this categorization decision.

The decision to place a substance into category 5 typically was prompted by an NOEL value in excess of 10,000 $\mu g/cm^2$ together with an absence of DPT data that would contradict this decision—a reasonable body of positive evidence, particularly if used volumes were not very high, would elevate a substance to category 4. However, in a couple of instances (*trans*-anethole and isobornyl acetate), NOEL values a little lower than 10,000 $\mu g/cm^2$, associated with category 4, have been combined with knowledge of a very high volume of use (for many years) and an absence of DPT results to associate the materials with category 5.[16]

DISCUSSION

Predictive toxicology is only of value if genuine human hazards are correctly identified, characterized, and assessed. It has long been recognized that in vivo methods have valuable predictive value regarding skin sensitization hazards.[2,25,26] More recently, integrated testing strategies involving nonanimal models have been presented as performing to a similar standard.[27–29] However, the characterization and assessment of identified skin sensitization hazards, particularly with respect to their relative potency, remains a weakness.[30,31] Only the LLNA (and specifically the derived EC3 value) offered an estimation of relative skin sensitization potency with some basis for demonstrating its correlation with human data.[32–34] The challenge of developing integrated testing strategies with nonanimal assays is outside the scope of this article, but for those engaged in such work, an essential need is a substantial catalog of chemicals categorized on the basis of their relative potency in humans. A first effort in this respect involving 131 chemicals has already been offered.[11] The data in the present publication extend this work with a further 89 substances, with the small overlap meaning that the total data set now totals well more than 200 materials. This combined data set offers a broad distribution into 6 potency categories, with most substances in the more difficult to predict intermediate, lower-potency, categories 3 to 5 (see Fig. 1). It is our view that, taken together, these comprise a valuable basis for the continued development of nonanimal approaches to the prediction of human skin sensitization potency.

To complete this discussion, it is essential to remind the reader of significant caveats not least that much of the categorization depends

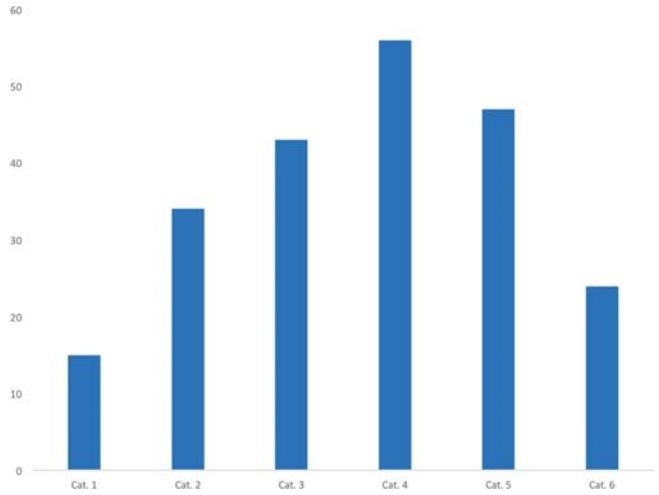

Figure 1. How the potency categories are populated. This figure combines the information from the first publication[11] with the present data to show the number of substances placed into each of the 6 categories.

on judgment. It is hoped that the reader might regard this as expert judgment, but even then, the human data on which this is based are not of the standard on which toxicological assessment would normally be founded. Human repeated insult patch test and HMT data, the primary drivers of conclusions on potency categorization, are derived from small populations of healthy volunteers. Note that the volunteers are healthy and not recruited from a specially sensitive subpopulation that impacts relative potency indications. The DPT data represent ad hoc collations of information from dermatology clinics whose original purpose was to assist in a correct diagnosis for individual patient care. All of these sources contain imperfections and uncertainties that cannot, and never will, be eliminated, thus "caveat emptor"—the use of the data must also involve an acceptance by the user that these categorizations are the best that can be achieved. Any nonanimal assay-integrated testing strategy that achieves a predictive accuracy against this data set of more than 90% is, by definition, likely to be flawed as a result of overfitting to imperfect data.

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