Weight of Evidence Approach for Skin Sensitization Potency Categorization of Fragrance Ingredients

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Background: Reliable human potency data are necessary for conducting quantitative risk assessments, as well as development and validation of new nonanimal methods for skin sensitization assessments. Previously, human skin sensitization potency of fragrance materials was derived primarily from human data or the local lymph node assay.

Objectives: This study aimed to define skin sensitization potency of fragrance materials via weight of evidence approach, incorporating all available human, animal, in vitro, in chemico, and in silico data.

Methods: All available data on 106 fragrance materials were considered to assign each material into 1 of the 6 defined potency categories (extreme, strong, moderate, weak, very weak, and nonsensitizer).

Results: None of the 106 materials were considered an extreme sensitizer, whereas a total of 6, 23, 41, and 26 materials were categorized as strong, moderate, weak, and very weak sensitizers, respectively. Ten materials lacked evidence for the induction of skin sensitization.

Conclusions: Skin sensitization potency categorization of the 106 fragrance materials based on the described weight of evidence approach can serve as a useful resource in evaluation of nonanimal methods, as well as in risk assessment.

Abbreviations: AOP: adverse outcome pathway, CNIH: confirmation of no induction in humans, DPRA: direct peptide reactivity assay, h-CLAT: human cell line activation test, HMT: human maximization test, HRIPT: human repeated insult patch test, LLNA: local lymph node assay, LOEL: lowest observed effect level, NCS: natural complex substance, NESIL: no expected sensitization induction level, NOEL: no observed effect level, OECD: Organization for Economic Cooperation and Development, QRA: quantitative risk assessment, SI: stimulation index, RIFM: Research Institute for Fragrance Materials, WoE: weight of evidence

Some fragrance materials have been identified as contact allergens, and they are known to express varying degrees of sensitizing potency.[1] For consumers, clinicians, industry, and regulatory authorities, this allergenic potency is of considerable interest and importance. Determining the potency of skin allergens quantitatively is critical for assessing their risk of inducing skin sensitization in consumer products. The potency range of known allergens can encompass at least 5 orders of magnitude. This is consistent with the range of human no observed effect levels (NOELs) and EC3 values from the local lymph node assays (LLNAs).[2,3] Dose per unit area is the well-established dose metric for skin sensitization,[4,5] which is expressed as the total amount of allergen, typically in micrograms of allergen per square centimeter of the exposed skin. There are known allergens capable of inducing sensitization at exposure levels less than 1 $\mu g/cm^2$, whereas others require exposure up to 10,000 $\mu g/cm^2$.[1,6] Historically, categorization of the sensitization potency of chemicals was based primarily on LLNA data, precisely the EC3 value.[7–9] The EC3 value, calculated from the LLNA dose-response curve, is the concentration required to induce a positive threshold response, that is, a stimulation index (SI) of 3.[8] The rationale for using EC3 values for potency categorization is that a reasonable degree of correlation has been shown between LLNA potency data and the available predictive human data.[10–14] In addition to LLNA data, other data may exist, including human data, which, when taken into consideration, can significantly improve the accuracy of determining the potency categorization of skin allergens.[15,16]

The level of topical exposure to a chemical required to induce skin sensitization is needed for risk assessment purposes. That threshold level of exposure is driven by the skin sensitization potency of the chemical, which is the quantity of chemical needed to

induce sensitization.[5] The concept of a risk assessment approach that relies on establishing a threshold level for the induction of skin sensitization has been described previously.[12,17–20] In 2008, a first methodological scheme for a skin sensitization quantitative risk assessment (QRA) of fragrance materials was published and subsequently implemented.[21,22] Recently, an improved approach has been published, which is commonly referred to as QRA2.[23] A solid understanding of a chemical's skin sensitization potency is critical to conducting sound risk assessments.[23,24] The QRA process for skin sensitization involves deriving a no expected sensitization induction level (NESIL) and applying sensitization assessment factors to the NESIL to account for various areas of uncertainty to determine an acceptable exposure level. At this level, the risk of inducing skin sensitization is negligible. To establish the NESIL of a skin sensitizer, a human NOEL from a well-conducted human repeated insult patch test (HRIPT) is required.[23] Beginning in 2020, the acronym CNIH (confirmation of no induction in humans) was suggested and implemented in place of HRIPT to highlight the confirmatory nature of HRIPTs conducted by the Research Institute for Fragrance Materials (RIFM).[16] When CNIH data on a given skin sensitizer are unavailable, a read-across analog can be used to derive the NESIL, where available.[25,26] In the absence of an appropriate read-across analog, the exposure is benchmarked to the dermal sensitization threshold. If the current exposure exceeds the dermal sensitization threshold, the generation of additional data is recommended.[25]

The goal of this article is to use a weight of evidence (WoE) approach to set skin sensitization potency categories (extreme, strong, moderate, weak, very weak, and nonsensitizer) for well-tested fragrance materials using all available data that could be evaluated to infer the chemical's skin sensitization potency. To achieve this goal, a great deal of expert judgment is required to analyze the available data. The decision-making process and the data considered are described in this article. Herein, 106 fragrance materials were assigned skin sensitization potency categories based on the review of all available information, including human, LLNA, in silico chemistry predictions, in chemico, and in vitro data. In some instances, other historical in vivo data (guinea pig), exposure use levels, and/or human diagnostic patch test data were used as secondary input data to aid in assigning an appropriate skin sensitization potency category.

Previous efforts have focused on the categorization of fragrance ingredients using primarily LLNA or human data.[1–3,6,9] Human testing is never used to identify the skin sensitization hazard of fragrance materials. It is also not used to identify "the lowest observed effect level (LOEL)," a threshold level at which a material induces skin sensitization. Rather, human testing is typically conducted at a single dose to confirm a NOEL, and the NOEL can be close or well below the threshold of the induction of sensitization. A historical LOEL, in addition to NOEL, can help derive the threshold, but LOELs are not always available. For this reason, the NOELs from human studies alone may not correlate well with the actual potency of a given material.

It is the authors' opinion that using a WoE approach, which considers and evaluates all available skin sensitization data, is a more robust

and accurate way for determining the potency categorization of fragrance ingredients for humans. This comprehensive WoE categorization approach may also aid in development of new alternative methods (in vitro, in silico, in chemico) for determining the skin sensitization potency of new or existing chemicals.

MATERIALS AND METHODS

The Data Set

Human, animal, in vitro, in chemico, and in silico data on 106 fragrance materials were evaluated to allocate each material a WoE potency category. These materials were chosen based on the availability of existing in vivo data. One hundred of these materials are discrete chemicals with known structure, whereas 6 are natural complex substances (NCSs). Natural complex substances are fragrance ingredients of botanical origin such as essential oils and absolutes. These are essentially complex mixtures of multiple chemicals.

Table 2 shows the data set evaluated in this study, which includes data that were available before December 2019 in the RIFM Database (consisting of publicly available and proprietary data, https://rifmdatabase.rifm.org), as well as in publicly available information sources such as ECHA (https://echa.europa.eu/) and PubMed (https://www.ncbi.nlm.nih.gov/pubmed).

Human NOELs obtained from CNIHs and/or human maximization tests (HMTs) were available for all assessed materials. These NOELs represent maximum levels tested without inducing skin sensitization in participating subjects but may not be the highest threshold levels at which skin sensitization is not induced. When available, higher weight was given to CNIHs conducted according to the standard protocol described hereinafter, because they involved more subjects.[27] In addition, the ethanol-based vehicles were used in the CNIHs, which is more relevant to the typical use of fragrance materials than other vehicles. Human LOELs obtained from CNIHs and/or HMTs were available for 35 materials, and these were approximately 1.2- to 13-fold higher than the respective NOELs. It should be noted that no new human data were generated for the current work.

Local lymph node assay data were available for 105 materials. Positive responses were noted for 66 materials, and their EC3 values were considered for potency categorization. It should also be noted that no new animal data were generated for the current work.

The induction of skin sensitization is initiated by covalent binding of the substance to skin proteins. Based on the chemical structure, protein binding alerts of 100 materials and their mechanistic domains of the reactivity were predicted using an in silico tool, Organization for Economic Cooperation and Development (OECD) QSAR toolbox 4.2 (http://www.qsartoolbox.org) and OASIS TIMES-SS (http://www.oasis-lmc.org). The chemical reactivity predictions were not available for the remaining 6 materials, because they are NCSs.

In chemico and in vitro data are also summarized (Table 2). Direct peptide reactivity assay (DPRA), KeratinoSens, and h-CLAT data were available on 104, 106, and 104 materials, respectively.

Human Testing Methods

Confirmation of No Induction in Humans

The HRIPT was introduced in the 1950s.[28–31] Since the publication of these early articles, there have been efforts over the intervening years to develop more robust scientific protocols for the performance and interpretation of the HRIPT.[15,27,32–34] The factors critical in the conduct and interpretation of an HRIPT include understanding the vehicle/matrix effects, amount of test material applied, patch type/technique, test subject number, and what is known about the allergenic potency of the test materials being evaluated.[15] Human repeated insult patch testing is conducted primarily as a confirmatory test focused on selecting test material concentrations that are not expected to induce a skin sensitization response. The term CNIH was proposed to refer to the HRIPTs conducted specifically for confirmatory purposes. The CNIH studies are conducted after receiving institutional review board approval. Most CNIH studies cited in this work were conducted according to the protocol published by Politano et al,[27] but other studies with minor variations in the protocol were also included. Throughout the study, 0.3 mL (liquid) of test material in a vehicle of 1:3 ethanol:diethyl phthalate was applied to occlusive 25-mm Hill Top Chamber patches. The test fragrance material concentration used in CNIH depends on detailed preceding toxicological evaluation and is always built on a WoE approach, but for most substances reported herein, it has depended on relative potency information from the LLNA.[22,24,27] The amount of fragrance material per unit area of skin is used to quantify the dosage in these studies, as it has been previously shown to be the most relevant metric to skin sensitization.[5] The dose per unit area can be easily calculated by dividing the amount of test material by the size of the patch used. For instance, in a study with α-amyl cinnamic aldehyde (Table 2), 0.3 mL (almost equal to 3.0×10^5 μg) of 20% fragrance material was applied using a Hill Top Chamber. An area of 2.54 cm^2 was covered by the fragrance material using this patch system. The dose per unit area in this study was calculated as follows:

$$\frac{0.2 \times \left(3.0 \times 10^5 \mu g\right)}{2.54 \, cm^2} = 23622 \, \mu g/cm^2$$

In addition to the test material, saline and/or vehicle control patches were applied in parallel. Induction patches were applied to skin between the scapula and spinal midline for 24 hours, followed by a 24-hour rest period, and retreatment of the same site for a total of 9 induction applications over 3 weeks. A 2-week rest period followed the final induction patching. The challenge phase consisted of a single 24-hour patch to a naive test site; the site was scored 24, 48, and 72/96 hours after application. The interpretation of the results is done according to the interpretation guidelines described by McNamee et al.[15] Typically, intense erythema, papules, and/or edema covering the entire test area that persist throughout the challenge scoring phase are considered skin sensitization reactions. Occasionally, a rechallenge may be needed to confirm the nature of questionable skin reactions. Typically, at least 100 subjects must finish the study for the data to be considered sufficient. More than a dozen inclusion/exclusion

criteria were used to identify appropriate volunteers, and they are described by Politano et al.[27] The CNIH data were used to establish a NOEL and, in some cases, a LOEL. The CNIH data used in this article were sourced from the RIFM Database, a comprehensive source of regulatory, identity, and toxicological data on more than 6000 materials, including 3000 fragrance materials, and publications such as the studies by Api et al[1] and Na et al.[16]

Human Maximization Tests

Human maximization tests as published by Kligman[35,36] are conducted by applying a test material in a vehicle (usually petrolatum) under occlusion to the same site on the volar aspects of the forearms of approximately 25 volunteers for 5 alternate-day, 48-hour periods. Patch sites may be pretreated for 24 hours with aqueous sodium lauryl sulfate under occlusion. After a 10- to 14-day rest period, challenge patches are applied under occlusion to fresh sites for 48 hours. Challenge applications may be preceded by 60-minute applications of sodium lauryl sulfate under occlusion. Challenge scoring occurs upon patch removal and 24 hours thereafter.

Animal Testing Method

Local Lymph Node Assay

Local lymph node assays were typically conducted according to OECD 429 and Good Laboratory Practice guidelines.[37] In some instances, a dose-range-finding pretest was completed. For the main study, groups of mice (n = 5) were dosed topically on the dorsum of each ear with 25 μL of test material in a vehicle, usually 1:3 ethanol:diethyl phthalate. Each group received a selected test concentration or vehicle or positive control, typically α-hexylcinnamaldehyde. During the induction phase, 25 μL of test material or vehicle or α-hexylcinnamaldehyde was applied to each ear for 3 consecutive days. After 2 days of rest, each animal received a single intravenous injection of 250 μL of saline containing 20 μCi of 3H-TdR. Approximately 5 hours later, auricular lymph nodes were excised and lymphocyte proliferation quantified by beta scintillation counting. The SI was obtained by calculating the ratio of disintegrations per minute of the treated group divided by the disintegrations per minute of the vehicle control group. In cases where none of the selected concentrations produce an SI greater or equal to 3, the response is considered negative up to the highest concentration tested. If the SI is equal to or greater than 3, the result is considered positive. Linear interpolation of the dose-response data was used to derive the estimated concentration that is needed to elicit an SI value of 3 (EC3). If a test material has multiple EC3 values, the average of the values is used even if there is a difference in protocol among the studies, which provided the EC3 values. Data were sourced from the RIFM database and publications, such as Api et al.[3,14,38]

In Chemico and In Vitro Test Methods

Direct Peptide Reactivity Assay

The DPRA has been previously described[39,40] and addresses the first key event of the skin sensitization adverse outcome pathway (AOP).[41]

The assay is based on the link between skin protein reactivity and skin sensitization. The DPRA has been validated and formally adopted by the OECD as Testing Guideline 442C.[42] The DPRA data were collected from the RIFM Database and other publications.[9,39,43–48] Generally, the DPRA quantifies the remaining concentration of cysteine- or lysine-containing peptide after a 24-hour incubation with the test chemical at 25°C ± 2.5°C. For each test chemical, an overall average peptide depletion is calculated using the means of cysteine and lysine depletion, and the distinction of sensitizers from nonsensitizers is made based on a decision tree model.[40] Chemicals with a mean of cysteine depletion and lysine depletion less than 6.37% are considered to have minimal reactivity, those with a mean peptide depletion between 6.37% and 22.62% are considered to have low reactivity, between 22.62% and 42.27% are assigned moderate reactivity, and greater than 42.47% are assigned high reactivity. Minimal reactivity chemicals are grouped as nonsensitizers, whereas low, moderate, and high reactivity chemicals are all grouped as sensitizers.

The KeratinoSens Assay

The KeratinoSens assay is generally conducted as described by Emter et al[49] and addresses the second key event of the skin sensitization AOP. This assay measures keratinocyte activation by assessing Nrf2-mediated activation of antioxidant response element–dependent genes, with the help of the luciferase reporter gene. KeratinoSens underwent validation and has been adopted by the OECD as Testing Guideline 442D.[50] KeratinoSens data were collected from the RIFM Database and other publications.[9,45,48,49,51] Generally, cells are grown for 24 hours in 96-well plates, after which the medium is replaced with medium containing the test chemical and a final level of 1% dimethyl sulfoxide. Each chemical is tested at 12 concentrations ranging from 0.98 μM to 2 mM in 3 replicate plates, and a fourth plate is tested simultaneously to determine cytotoxicity. Cells are incubated for 48 hours with the test agent, after which luciferase activity and cytotoxicity are determined. This entire experiment is repeated at least 2 times for each chemical. Gene induction for cells treated with the test reagent is then compared with dimethyl sulfoxide controls to determine induction over a 1.5 threshold. Chemicals with a significant gene induction greater than 1.5-fold, at a concentration at which the cells maintain at least 70% viability in a minimum of 2 experiments, are rated positive.

The Human Cell Line Activation Test

The human cell line activation test (h-CLAT)[52,53] addresses the third key event of skin sensitization AOP. Dendritic cell activation is assessed by measuring induction of expression of cell surface markers CD54 and CD86 after 24-hour treatment with a test substance relative to parallel vehicle controls in human monocytic leukemia cells, THP-1 cells, as a surrogate of dendritic cells. The h-CLAT has been validated by the OECD and adopted as Test Guideline 442E.[54] The h-CLAT data were collected from RIFM Database and other publications.[9,45,47,48,51,55] A 2-fold induction of the CD54 expression and/or 1.50-fold induction of CD86 expression at relative cell viabilities of at least 50% is rated positive for dendritic cell–activating potential of a test substance.

The WoE Approach for Potency Categorization

Potency categories were assigned based on the WoE approach, considering human, animal, in silico, in chemico, and in vitro data (Fig. 1). Human data were prioritized over all nonhuman data. Human NOELs were used first in the WoE approach for potency categorization. The potency categories were assigned using ranges adapted from Api et al[1] (Table 1). Human LOELs were considered next, where available, followed by the LLNA data. The EC3 values from LLNAs are known to be robust predictors of skin sensitization potency. They were found to correlate well with the human NOEL, except for a few materials such as hexen-2-al (CAS 6728-26-3).[14] The potency based on EC3 was determined using the ranges adapted from European Centre for Ecotoxicology and Toxicology of Chemicals Technical Report 87 (Table 1).[56] The EC3 percentage values were converted to dose per unit area of skin, so they could be compared with the available human data. The LLNA potency was used as a guide to determine whether a material could be categorized as a weaker sensitizer compared with the potency based on the existing human NOEL. In addition, the in chemico, in silico, and in vitro data were used in combination to determine whether a given material has the potential to induce each of the key events for induction of skin sensitization. The absence of structural features that are reactive to skin proteins and the inability to activate the key events would indicate that the material is a very weak or nonsensitizer.

The potency decisions were made for all analyzed materials, mainly using the data listed previously. In some cases, other data were considered on a case-by-case basis to assist in the WoE decision. These supporting factors included guinea pig studies and exposure data for the material coupled with available diagnostic patch test data.

If a material lacked any positive in vivo data, lacked protein binding alerts in silico, and was predicted to be negative in 2 of the 3 in chemico and in vitro assays, the material was categorized as a nonsensitizer.

RESULTS

The WoE potency categories determined for 106 fragrance materials evaluated are summarized in Figure 2. None were considered extreme sensitizers (that is, zero of the 106 fragrance materials) whereas six were strong, twenty three were moderate, forty one were weak and 26 were very weak sensitizers, respectively. In addition, 10 materials were considered non-sensitizers, because they lacked evidence for induction of skin sensitization (Fig. 2).

The category assignment for each material and main data set considered are listed in Table 2.

Of the 106 fragrance materials, 82 materials have been previously categorized by Api et al,[1] primarily using human data. For 71% of these 82 materials, the WoE categories were the same categories as previously assigned (Fig. 3). Consideration of other available data led to a change in potency categories for the remaining 29%, compared with the
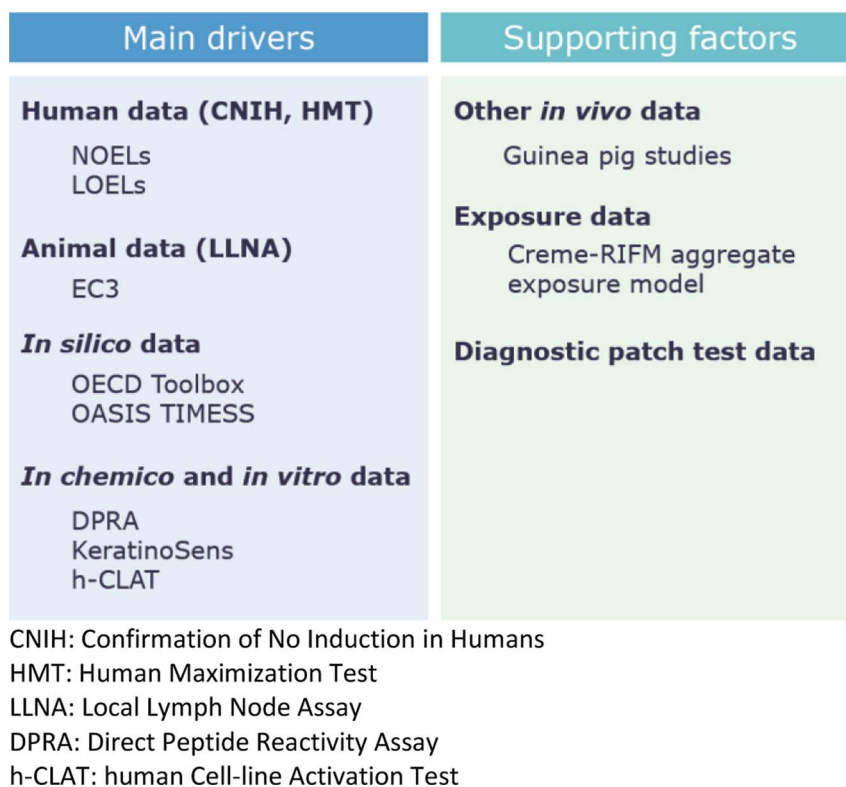
Main drivers	Supporting factors
Human data (CNIH, HMT) NOELs LOELs **Animal data (LLNA)** EC3 ***In silico* data** OECD Toolbox OASIS TIMESS ***In chemico* and *in vitro* data** DPRA KeratinoSens h-CLAT	**Other *in vivo* data** Guinea pig studies **Exposure data** Creme-RIFM aggregate exposure model **Diagnostic patch test data**

CNIH: Confirmation of No Induction in Humans
HMT: Human Maximization Test
LLNA: Local Lymph Node Assay
DPRA: Direct Peptide Reactivity Assay
h-CLAT: human Cell-line Activation Test

Figure 1. Data considered for the WoE potency categorization for induction.

previous categorization; weaker potency categories were assigned for 20.5%, whereas stronger categories were assigned for 8.5% (Fig. 3).

A few examples from Table 2 are described hereinafter to demonstrate how WoE categories were determined based on the existing data. These categories were decided based on the available evidence at the time of this study. Upon availability of new information and/or additional data, the potency category would be re-evaluated.

Cinnamic aldehyde (CAS 104-55-2) has a human NOEL and a human LOEL of 591 and 775 $\mu g/cm^2$, respectively. The NOEL can be considered a good representation of the potency, because it is close to the LOEL (1.3-fold difference). Therefore, cinnamic aldehyde was categorized as a moderate sensitizer based on the category ranges in Table 1. Cinnamic aldehyde was predicted to be a sensitizer in chemico, in vitro, and in silico. The LLNA data also support the moderate sensitizer category.

Methyl-2-nonyoate (CAS 111-80-8) was categorized as a strong sensitizer. It has a CNIH NOEL of 24 $\mu g/cm^2$, which is at the upper end of the extreme category. However, the LOEL for this material is 5-fold higher, suggesting that the true maximum NOEL might be higher than 24 $\mu g/cm^2$. It is also possible that true LOEL is lower than the available value. The EC3 from LLNA was estimated to be 625 $\mu g/cm^2$ (2.5%), supporting categorization of methyl-2-nonyoate to the strong category. In line with these in vivo data, methyl-2-nonyoate was predicted to be a strong sensitizer in the DPRA and was positive in KeratinoSens and h-CLAT. In addition, methyl-2-nonyoate was predicted to be a strong sensitizer in silico.

2-Methoxy-4-methylphenol (CAS 93-51-6) was placed in a moderate category. The CNIH NOEL is 110 $\mu g/cm^2$, which is in the strong sensitizer range. There was no human LOEL. The EC3 value of 1450 $\mu g/cm^2$ (5.8%) indicated that the maximum human NOEL could be higher than 110 $\mu g/cm^2$. The DPRA and KeratinoSens did not predict 2-methoxy-4-methylphenol to be a skin sensitizer, whereas h-CLAT predicted it to be a sensitizer, suggesting that 2-methoxy-4-methylphenol might not be a strong sensitizer. In silico analysis showed that no protein binding alerts were identified for the parent material, whereas its potential metabolite (2,5-cyclohexadien-1-one, 2-methoxy-4-methylene-) was predicted to be a strong sensitizer. In a guinea pig maximization test, 2-methoxy-4-

TABLE 1. Potency Categories and Their Dose Range

Potency Category	Dose Range,* $\mu g/cm^2$	LLNA EC3 Dose Range,† $\mu g/cm^2$
Extreme	<25	<25
Strong	25–500	25–<250
Moderate	500–2500	250–<2500
Weak	>2500–10,000	2500–25,000
Very weak	>10,000	
Nonsensitizer	Negative	

*Adapted from Api et al.[1]

†Defined based on the guidance from European Center for Ecotoxicology and Toxicology of Chemicals Technical Report 87.[56]

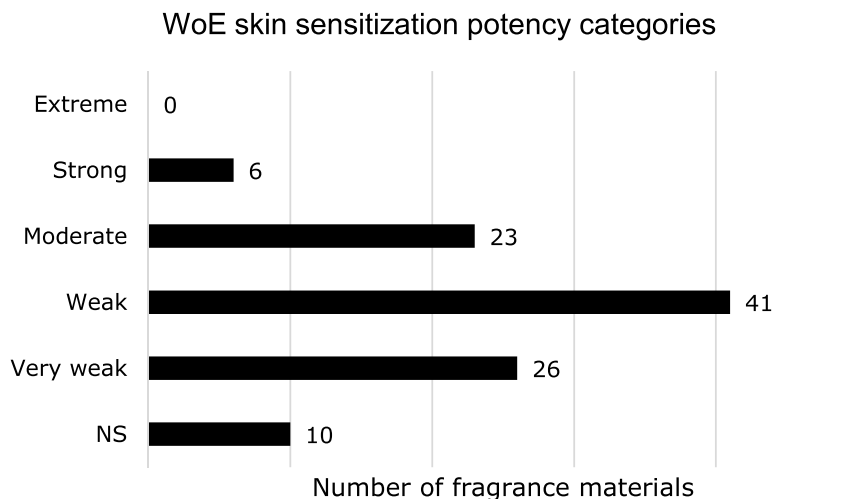## WoE skin sensitization potency categories

Figure 2. Number of materials placed in each of 6 potency categories based on the WoE approach.

methylphenol was shown to be a moderate sensitizer, supporting the moderate category.

Ylang-ylang (CAS 8006-81-3) was categorized as a moderate sensitizer. The CNIH NOEL of 1771 µg/cm^2 and the EC3 of 1700 µg/cm^2 (6.8%) values support the moderate category. In addition, ylang-ylang was predicted to be a sensitizer in KeratinoSens and h-CLAT, whereas it was negative in DPRA.

Benzyl alcohol (CAS 100-51-6) was categorized as a weak sensitizer, mainly based on a human NOEL of 5900 µg/cm^2 and a similar LOEL of 8858 µg/cm^2. The negative LLNA data and the lack of protein binding alerts suggest that benzyl alcohol is not a strong sensitizer. There are positive diagnostic patch test data in the literature. For instance, in a patch test study by Schnuch et al,[57] 1% benzyl alcohol led to skin reactions in 0.3% in 2166 patients. In another study by Hausen,[58] patch testing 102 patients with 5% benzyl alcohol led to skin reactions in 7.8% of the tested patients. However, considering the high volume of use as a fragrance ingredient in consumer products (International Fragrance Association, 2015 Volume of Use Survey), combined with the fact that benzyl alcohol is used ubiquitously in consumer products that come in close contact with the skin such as facial scrub and face wash (Creme-RIFM Aggregate Exposure Model, V3.1.3), benzyl alcohol is a weak sensitizer.

Tetrahydro-4-methyl-2-propyl-2*H*-pyran-4-yl acetate (CAS 131766-73-9) was categorized as a very weak sensitizer. It has a human NOEL of 11,000 µg/cm^2, whereas its human LOEL is not known. The LLNA was negative with the highest tested dose of 7500 µg/cm^2 (30%). Two of the 3 in chemico and in vitro tests did not predict 2-methoxy-4-methylphenol to be a skin sensitizer (Table 2). In silico, no protein binding alerts were identified on the parent or its possible metabolites. No diagnostic patch test data were available, despite its apparent use in skin-applicable products such as fine fragrances and bar soap (Creme-RIFM Aggregate Exposure Model, Version 3.1.3). These data suggested that tetrahydro-4-methyl-2-propyl-2*H*-pyran-4-yl acetate may be a nonsensitizer. However, evidence of induction of skin sensitization was observed in a guinea pig maximization test.[59] Therefore,

the potency category of tetrahydro-4-methyl-2-propyl-2*H*-pyran-4-yl acetate was adjusted to the very weak category.

Methyl salicylate (CAS 119-36-8) was categorized as a very weak sensitizer. It has limited existing human data, because no CNIH study has been conducted according to the standard protocol.[27] The HMT NOEL of 5520 µg/cm^2 is considered instead, which falls in the weak sensitizer range. No human LOEL is available for methyl salicylate. Four separate LLNAs showed that methyl salicylate is a skin sensitizer, with an average EC3 of 7341 µg/cm^2 (29.4%), whereas 3 other studies showed that it was not sensitizing at the maximum tested concentrations. In a separate study, no significant increase in B Cell Marker, B220, was observed in mice treated with methyl salicylate, suggesting that reactions observed at high doses are indicative of irritant rather than a skin sensitizer.[60] None of the 3 in chemico and in vitro tests or in silico predictions indicate that methyl salicylate is a sensitizer. However, sensitization reactions have been observed in diagnostic patch tests. In a study on diagnostic patch tests with 1825 patients using 2% methyl salicylate in petrolatum, 0.4% of patients exhibited skin sensitization reactions.[61] In another study, 0.11% of 4600 patients in total showed sensitization reactions when patched with 2% methyl salicylate in petrolatum.[62] Considering the positive data from LLNAs and the rare, but positive, reactions observed in diagnostic patch test studies, methyl salicylate is categorized as a very weak sensitizer.

1-(3-Methyl-2-benzofuranyl)ethenone (CAS 23911-56-0) was categorized as a very weak sensitizer. There are no positive in vivo data to suggest that this material is a sensitizer. It has a CNIH NOEL of 11,019 µg/cm^2, and a human LOEL is not available. In an LLNA, 1-(3-methyl-2-benzofuranyl)ethenone did not induce skin sensitization when tested up to 30%, 7500 µg/cm^2. Moreover, in a guinea pig maximization test, no reactions indicative of skin sensitization were observed.[63] In a diagnostic patch test study, no reactions indicative of skin sensitization were observed in the 48 subjects.[64] In line with in vivo data, 1-(3-methyl-2-benzofuranyl)ethenone is not predicted to be reactive to skin proteins in silico. However, it was predicted to be a skin

TABLE 2. Weight of Evidence Categorization of the Fragrance Materials and the Summary of Main Data Considered

CAS Number	Chemical Name	Category Call Based on WoE	Human NOEL*, µg/cm²	Human LOEL†, µg/cm²	LLNA, EC3, µg/cm²	DPRA, Peptide Reactivity	KeratinoSens	h-CLAT	Protein Binding Alerts for Skin Sensitization, Toolbox 4.2	Parent Prediction, TIMES-SS	Metabolite Prediction, TIMES-SS
6728-26-3	Hexen-2-al	Strong	18	236	1012 [2]	High	Positive	Positive	Michael Addition	Strong sensitizer	Nonsensitizer
111-80-8	Methyl-2-nonynoate	Strong	24	118	<1250, estimated 625	High	Positive	Positive	Michael Addition	Strong sensitizer	Nonsensitizer
111-12-6	Methyl heptine carbonate	Strong	118	194	112.5; <125; NC (250)	High	Positive	Positive	Michael Addition	Strong sensitizer	Nonsensitizer
358331-95-0	5,6,7-Trimethylocta-2,5-dien-4-one	Strong	250	N/A	400	Inconclusive	Positive	Positive	Nucleophilic addition Michael Addition	Strong sensitizer	
93893-89-1	3-Methyl-5-phenylpent-2-enenitrile	Strong	275	N/A	192.5	Minimal	Negative	Positive	No alert found	Nonsensitizer	Nonsensitizer
17373-89-6	2-Hexylidene cyclopentanone	Strong	300	500	600	No data	Positive	No data	Michael Addition	Strong sensitizer	Strong sensitizer
1604-28-0	6-Methyl-3,5-heptadien-2-one	Moderate	118	1299	NC (1250)	Low	Positive	Positive	Michael Addition	Strong sensitizer	Strong sensitizer
93-51-6	2-Methoxy-4-methylphenol	Moderate	118	N/A	1450	Minimal	Negative	Positive	No alert found	Nonsensitizer	Strong sensitizer
97-54-1	Isoeugenol	Moderate	250	775	500 [48]	High	Positive	Negative	No alert found	Nonsensitizer	Strong sensitizer
84650-60-2	Tea leaf absolute	Moderate	480	N/A	NC (1250)	High	Negative	Positive	N/A	N/A	N/A
68991-97-9	1,2,3,4,5,6,7,8-Octahydro-8,8-dimethyl-2-naphthaldehyde	Moderate	550	N/A	1050	Minimal	Negative	Positive	Schiff base formation	Weak sensitizer	Weak sensitizer
3658-77-3	4-Hydroxy-2,5-dimethyl-3(2H)-furanone	Moderate	591	1181	450	High	Negative	Positive	No alert found	Nonsensitizer	Weak sensitizer
122-78-1	Phenylacetaldehyde	Moderate	591	1181	962 [2]	Moderate	Positive	Positive	Schiff base formation	Strong sensitizer	Nonsensitizer
104-55-2	Cinnamic aldehyde	Moderate	591	775	262 [22]	High	Positive	Positive	Schiff base formation	Strong sensitizer	Nonsensitizer
33662-58-7	Methyl 2,4-dihydroxy-m-toluate	Moderate	620	N/A	2200	Inconclusive	Positive	Positive	No alert found	Nonsensitizer	Strong sensitizer
7493-74-5	Allyl phenoxyacetate	Moderate	709	N/A	775	Minimal	Positive	Negative	SN2	Strong sensitizer	Strong sensitizer
2111-75-3	p-Mentha-1,8-dien-7-al	Moderate	709	2760	2175 [2]	Moderate	Positive	Positive	Schiff base formation Michael Addition	Strong sensitizer	Nonsensitizer
90028-67-4	Treemoss, treemoss absolute	Moderate	700	1417	2162.5 [2]	High	Positive	Positive	N/A	N/A	N/A
17369-59-4	3-Propylidenephthalide	Moderate	945	2760	350; <1250	Low	Negative	Positive	Acylation	Strong sensitizer	Strong sensitizer
1885-38-7	Cinnamyl nitrile	Moderate	1063	1250	NC (2500)	Minimal	Positive	Positive	No alert found	Nonsensitizer	Nonsensitizer
68683-20-5	Menthadiene-7-methyl formate	Moderate	1063	6900	NC (2500)	Low	Positive	Positive	No alert found	Nonsensitizer	Weak sensitizer

(Continued on next page)

TABLE 2. (Continued)

CAS Number	Chemical Name	Category Call Based on WoE	Human NOEL*, µg/cm²	Human LOELt, µg/cm²	LLNA, EC3, µg/cm²	DPRA, Peptide Reactivity	KeratinoSens	h-CLAT	Protein Binding Alerts for Skin Sensitization, Toolbox 4.2	Parent Prediction, TIMES-SS	Metabolite Prediction, TIMES-SS
18127-01-0	p-tert-Butyldihydrocinnamaldehyde	Moderate	1181	N/A	1075	Low	Negative	Positive	Schiff base formation	Weak sensitizer	Nonsensitizer
5392-40-5	Citral	Moderate	1417	3876	1414 [11]	High	Positive	Positive	Schiff base formation	Strong sensitizer	Nonsensitizer
8022-96-6	Jasmine absolute (grandiflorum)	Moderate	1400	2069	1475	Low	Positive	Positive	N/A	N/A	N/A
8006-81-3	Ylang-ylang	Moderate	1771	N/A	1700	Minimal	Positive	Positive	N/A	N/A	N/A
105-13-5	Anisyl alcohol	Moderate	1771	N/A	1475	High	Negative	Positive	No alert found	Nonsensitizer	Strong sensitizer
103-50-4	Dibenzyl ether	Moderate	2362	N/A	1575	Minimal	Positive	Positive	No alert found	Nonsensitizer	Weak sensitizer
90028-68-5	Oakmoss absolute, low atranol	Moderate	700	N/A	3775 [6]; NC (7500); NC (12,500); NC (625)	High	Positive	Positive	N/A	N/A	N/A
56973-85-4	1-(5,5-Dimethyl-1-cyclohexen-1-yl)pent-4-en-1-one	Moderate	2500	N/A	747	Minimal	Positive	Positive	Michael Addition	Strong sensitizer	
100-52-7	Benzaldehyde	Weak	590	2760	NC (6250)	Minimal	Positive	Positive	No alert found	Nonsensitizer	Nonsensitizer
122-03-2	Cuminic aldehyde	Weak	1181	N/A	NC (2500)	Low	Negative	Borderline	Schiff base formation	Weak sensitizer	Weak sensitizer
7775-00-0	3-(p-Isopropylphenyl)propionaldehyde	Weak	1111	N/A	<6250, estimated 4650	Minimal	Negative	Positive	Schiff base formation	Weak sensitizer	Weak sensitizer
6658-48-6	p-Isobutyl-α-methyl hydrocinnamaldehyde	Weak	2362	N/A	<2500, estimated 2375	Minimal	Negative	Positive	Schiff base formation	Weak sensitizer	Nonsensitizer
107898-54-4	3,3-Dimethyl-5-(2,2,3-trimethyl-3-cyclopenten-1-yl)-4-penten-2-ol	Weak	2598	5000	NC (5000)	Low	Negative	Positive	No alert found	Nonsensitizer	N/A
86803-90-9	Methoxy dicyclopentadiene carboxaldehyde	Weak	2500	12500	NC (2500)	Low	Positive	Positive	Schiff base formation	Weak sensitizer	Nonsensitizer
6784-13-0	β,4-Dimethylcyclohex-3-ene-1-propan-1-al	Weak	5510	5510#	5675 [2]	Minimal	Positive	Positive	Schiff base formation	Weak sensitizer	Weak sensitizer
941-98-0	1-(1-Naphthyl)ethanone	Weak	2598	N/A	2500	Inconclusive	Negative	Positive	No alert found	Nonsensitizer	Nonsensitizer
6485-40-1	R-Carvone	Weak	2657	18898	2950 [2]	Low	Positive	Positive	Michael Addition	Strong sensitizer	No metabolites predicted
4602-84-0	Farnesol	Weak	2755	6897	1200 [2]	Minimal	Positive	Positive	No alert found	Nonsensitizer	Weak sensitizer
3155-71-3	2-Methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal	Weak	2953	N/A	2975	High	Negative	Positive	Michael Addition	Strong sensitizer	Nonsensitizer

CAS	Name										
104-54-1	Cinnamic alcohol	Weak	2953	4724	5250	Low	Positive	Positive	No alert found	Nonsensitizer	Strong sensitizer
122760-84-3	Tricyclo[3.3.1.1.(3.7)]decan-2-ol, 4-methyl-8-methylene-	Weak	3000	N/A	NC (7500)	Low	Positive	Positive	No alert found	Nonsensitizer	Nonsensitizer
406488-30-0	Butanamide, 2-ethyl-N-methyl-N-(3-methylphenyl)-	Weak	3250	N/A	6250	Minimal	Negative	Positive	No alert found	Nonsensitizer	Nonsensitizer
123-11-5	p-Methoxybenzaldehyde	Weak	3543	4724	NC (6250)	Moderate	Negative	Positive	No alert found	Nonsensitizer	Nonsensitizer
101-39-3	α-Methylcinnamaldehyde	Weak	3543	N/A	1125	Low	Positive	Positive	Michael Addition SN2	Strong sensitizer	Nonsensitizer
2442-10-6	1-Octen-3-yl acetate	Weak	3543	6900	NC (7500)	Minimal	Negative	Negative	No alert found	Weak sensitizer	Nonsensitizer
58567-11-6	Formaldehyde cyclododecyl ethyl acetal	Weak	3543	N/A	6275	Minimal	Negative	Positive	No alert found	Nonsensitizer	Weak sensitizer
82654-98-6	Vanillyl butyl ether	Weak	3543	N/A	3645	Low	Positive	Positive	No alert found	Nonsensitizer	Strong sensitizer
475-20-7	Longifolene	Weak	3543	N/A	3545 [3]	Minimal	Positive	Positive	No alert found	Nonsensitizer	Nonsensitizer
91-64-5	Coumarin	Weak	3543	8858	NC (12,500)	Minimal	Positive	Negative	No alert found	Nonsensitizer	Nonsensitizer
18794-84-8	β-Farnesene§	Weak	3700	6350	NC (7500)	Minimal	Positive	Positive	No alert found	Nonsensitizer	Weak sensitizer
68527-77-5	2,4,6-Trimethly-3-cyclohexene-1-methanol	Weak	3800	5000	NC (6250)	Minimal	Negative	Positive	No alert found	Nonsensitizer	Weak sensitizer
31906-04-4	3- and 4-(4-hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde§	Weak	4000	6259	4275	Low	Positive	Positive	Schiff base formation	Weak sensitizer	Nonsensitizer
80-54-6	p-t-Butyl-α-methylhydrocinnamic aldehyde	Weak	4125	29528	2454 [7]	Low	Negative	Positive	Schiff base formation	Weak sensitizer	Nonsensitizer
103-41-3	Benzyl cinnamate	Weak	4724	N/A	4600	Minimal	Positive	Negative	Michael Addition	Weak sensitizer	Nonsensitizer
107-75-5	Hydroxycitronellal	Weak	4960	5814	5553 [18]	Low	Positive	Positive	Schiff base formation	Weak sensitizer	Nonsensitizer
4180-23-8	trans-Anethole	Weak	5509	N/A	675	Inconclusive	Negative	Positive	No alert found	Nonsensitizer	Weak sensitizer
515-69-5	α-Bisabolol	Weak	5510	N/A	4593 [2]	Low	Negative	Positive	No alert found	Nonsensitizer	Weak sensitizer
16251-77-7	3-Phenylbutanal	Weak	5905	12500	N/A	Low	Positive	Positive	Schiff base formation	Weak sensitizer	Nonsensitizer
19009-56-4	2-Methyldecanal	Weak	5905	N/A	5900	Low	Negative	Positive	Schiff base formation	Weak sensitizer	Nonsensitizer
100-51-6	Benzyl alcohol	Weak	5905	8858	NC (12,500)	Minimal	Positive/Negative	Positive	No alert found	Nonsensitizer	Nonsensitizer
5462-06-6	4-Methoxy-α-methyl benzenepropanal	Weak	5905	N/A	5900	Minimal	Positive	Positive	Schiff base formation	Weak sensitizer	Weak sensitizer
62439-41-2	6-Methoxy-2,6-dimethylheptan-1-al	Weak	5905	N/A	NC (12,500); 6000	Low	Positive	Positive	Schiff base formation	Weak sensitizer	Nonsensitizer
15760-18-6	3-(4-Methyl-3-cyclohexenyl) butanol	Weak	5905	N/A	5000	Minimal	Positive	Positive	No alert found	Nonsensitizer	Weak sensitizer
68039-49-6	2,4-Dimethyl-3-cyclohexen-1-carboxaldehyde§	Weak	5905	N/A	3468 [5]; NC (6250)	Low	Positive	Positive	Schiff base formation	Weak sensitizer	Nonsensitizer
97-53-0	Eugenol	Weak	5906	N/A	2703 [6]	Low	Negative	Positive	No alert found	Nonsensitizer	Weak sensitizer

TABLE 2. (Continued)

CAS Number	Chemical Name	Category Call Based on WoE	Human NOEL*, µg/cm²	Human LOEL†, µg/cm²	LLNA, EC3, µg/cm²	DPRA, Peptide Reactivity	KeratinoSens	h-CLAT	Protein Binding Alerts for Skin Sensitization, Toolbox 4.2	Parent Prediction, TIMES-SS	Metabolite Prediction, TIMES-SS
1335-66-6	Isocyclocitral§	Weak	7087	N/A	1825	Moderate	Negative	Positive	Schiff base formation	Weak sensitizer	Weak sensitizer
106-23-0	Citronellal	Weak	7086	N/A	NC (7500)	Minimal	Positive	Positive	Schiff base formation	Weak sensitizer	Weak sensitizer
82356-51-2	3-Methylcyclopentadecenone	Weak	10000	N/A	1425	Low	Negative	Positive	Nucleophilic addition	Weak sensitizer	Nonsensitizer
122-40-7	Amyl cinnamic aldehyde	Weak	23622	N/A	2513 [4]	Minimal	Positive	Positive	Schiff base formation	Weak sensitizer	Nonsensitizer
470-82-6	Eucalyptol	Very weak	590	N/A	16475	Minimal	Positive	Positive	No alert found	Nonsensitizer	Nonsensitizer
28940-11-6	7-Methyl-2H-benzo-1,5-dioxepin-3(4H)-one	Very weak	1000	N/A	NC (7500)	Low	Positive	Positive	No alert found	Nonsensitizer	Nonsensitizer
1708-34-5	2-Hexyl-1,3-dioxolane	Very weak	2777	N/A	16245	Minimal	Negative	No data	No alert found	Nonsensitizer	Nonsensitizer
101-85-9	Amylcinnamyl alcohol	Very weak	3543	N/A	NC (7500); NC (6250)	Minimal	Negative	Positive	No alert found	Nonsensitizer	Weak sensitizer
121-33-5	Vanillin	Very weak	5314	N/A	NC (12,500)	Minimal	Negative	Negative	No alert found	Nonsensitizer	Weak sensitizer
106-02-5	Ω-Pentadecalactone	Very weak	5509	N/A	NC (12,500); 5187 [2]	Minimal	Negative	Positive	No alert found	Nonsensitizer	Nonsensitizer
119-36-8	Methyl salicylate	Very weak	5517	N/A	8829 [3], NC (1250), NC (6250), NC (5000)	Minimal	Negative	Negative	No alert found	Nonsensitizer	Nonsensitizer
91770-14-8	Jasmine absolute (sambac)	Very weak	8800	N/A	9100	Minimal	Positive	Positive	N/A	N/A	N/A
103694-68-4	β,β,3-Trimethyl benzenepropanol	Very weak	9917	N/A	NC (25,000); NC (7500); 7300	Minimal	Positive	Positive	No alert found	Nonsensitizer	Nonsensitizer
259854-70-1	5-Cyclotetradecen-1-one, 3-methyl-,(5E)-	Very weak	10000	N/A	4100	Minimal	Negative	Positive	Nucleophilic addition	Weak sensitizer	Nonsensitizer
3910-35-8	1,1,3-Trimethyl-3-phenylindane	Very weak	10000	N/A	10,825	Minimal	Positive	Inconclusive results (solubility)	No alert found	Nonsensitizer	Nonsensitizer
106-24-1	Geraniol	Very weak	11811	N/A	4063 [6]	Minimal	Positive	Positive	No alert found	Nonsensitizer	Weak sensitizer
33704-61-9	6,7-Dihydro-1,1,2,3,3-pentamethyl-4(5*H*)-indanone	Very weak	12121	N/A	8250	Minimal	Negative	Positive	Nucleophilic addition	Weak sensitizer	
118-58-1	Benzyl salicylate	Very weak	17715	N/A	725	Minimal	Positive	Negative	SN2 Acylation	Strong sensitizer	Nonsensitizer
13828-37-0	*cis*-4-(Isopropyl) cyclohexanemethanol	Very weak	17715	N/A	11,000	Minimal	Negative	Positive	No alert found	Nonsensitizer	Nonsensitizer
13257-44-8	2-Nonyn-1-al dimethyl acetal	Very weak	23622	N/A	NC (5000)	Minimal	Positive	Positive	No alert found	Nonsensitizer	Nonsensitizer
101-86-0	Hexyl cinnamic aldehyde	Very weak	23622	N/A	2425 [21]	Minimal	Positive	Negative	Schiff base formation	Weak sensitizer	Nonsensitizer
106-22-9	Citronellol	Very weak	29525	N/A	10875; NC (20,000)	Low	Negative	Positive	No alert found	Nonsensitizer	Weak sensitizer
6259-76-3	Hexyl salicylate	Very weak	35430	N/A	45	Minimal	Positive	Positive	No alert found	Nonsensitizer	Nonsensitizer
54464-57-2	1-(1,2,3,4,5,6,7,8-Octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)ethanone§	Very weak	47244	N/A	3783 [3]	High; low	Negative	Positive	Nucleophilic addition	Weak sensitizer	Weak sensitizer
120-51-4	Benzyl benzoate	Very weak	59050	N/A	4250; NC (12,500)	Minimal	Positive	Negative	SN2	Weak sensitizer	no metabolites predicted
127-51-5	α-iso-Methylionone§	Very weak	70860	N/A	5450	Minimal	Negative	Positive	Michael Addition	Weak sensitizer	
5989-27-5	D-Limonene	NS	10000	N/A	9215 [5]	Minimal; low	Negative	Positive	No alert found	Nonsensitizer	Weak sensitizer
78-70-6	Linalool	NS	14998	N/A	8883 [3]	Minimal	Negative	Positive	No alert found	Nonsensitizer	Weak sensitizer
124-07-2	Octanoic acid	NS	690	N/A	NC (12,500)	Minimal	Negative	Positive	No alert found	Nonsensitizer	Nonsensitizer
478695-70-4	Propanedioic acid, 1-(3,3-dimethylcyclohexyl) ethyl, ethyl ester	NS	2000	N/A	Neg up to 100%	Minimal	Negative	Positive	No alert found	Nonsensitizer	Nonsensitizer
69300-15-8	2-Methyldecanenitrile	NS	2250	N/A	Neg up to 100%	Minimal	Negative	Positive	No alert found	Nonsensitizer	Nonsensitizer
63500-71-0	2-Isobutyl-4-methyltetrahydro-2*H*-pyran-4-ol	NS	4408	N/A	NC (7500)	Minimal	Negative	Positive	No alert found	Nonsensitizer	
18479-58-8	Dihydromyrcenol	NS	5000	N/A	NC (6250)	Minimal	Negative	Positive	No alert found	Nonsensitizer	
125-12-2	Isobornyl acetate	NS	6495	N/A	NC (6250)	Minimal	Negative	Positive	No alert found	Nonsensitizer	Nonsensitizer
24851-98-7	Methyl dihydrojasmonate	NS	10000	N/A	NC (10,000)	Minimal	Negative	Positive	No alert found	Nonsensitizer	Nonsensitizer
105-95-3	Ethylene brassylate	NS	23600	N/A	NC (7500)	Minimal	Negative	Positive	No alert found	Nonsensitizer	Nonsensitizer

When more than 1 LLNA data are available, the average of EC3 from all studies was listed. The number of studies is noted in brackets. The data are indicated as not calculated (highest tested dose); the LLNA was negative up to the highest tested dose.

**Skin sensitization reactions were observed in the CNIH when 0.1% tocopherol was used as a stabilizer with β,4-dimethylcyclohex-3-ene-1-propan-1-al. However, no reactions were observed in a study where 0.1% BHT was used as a stabilizer.*

†The LOELs were based on CNIHs for 24 materials and on HMT for 7 materials.

‡The NOELs for 104 materials were based on the CNIH studies. Most CNIH studies cited in this work were conducted according to the protocol published by Politano and Api,[27] but other studies with minor variations in the protocol were also included. The NOELs for methyl salicylate (CAS 119-36-8) and octanoic acid (CAS 124-07-2) were derived from HMTs, because no CNIH studies were available on these 2 materials.

§The samples were commercial mixtures of structural isomers.

N/A, not available; NC, not calculated; NS, no evidence of skin sensitization exists.

Figure 3. Comparison of the WoE-based potency categories to the potency categories in the study by Api et al.[1]

sensitizer in KeratinoSens and h-CLAT, supporting the potency category of a very weak sensitizer rather than a nonsensitizer.

2-Methyldecanenitrile (CAS 69300-15-8) was categorized as a nonsensitizer. It has a CNIH NOEL of 2250 µg/cm^2, which falls into the moderate sensitizer range. No human LOEL is available. In an LLNA, it did not induce skin sensitization when tested up to 100%, indicating that it may not be a skin sensitizer. 2-Methyldecanenitrile was predicted to be a nonsensitizer in the DPRA and KeratinoSens, but a sensitizer in h-CLAT. In silico, it was not predicted to be reactive to skin proteins directly or through its metabolites. Moreover, 2-methyldecanenitrile did not lead to skin sensitization reactions in a guinea pig maximization test and a Buehler test.[65,66] Given the absence of positive data in human and animal tests, 2-methyldecanenitrile was placed in the nonsensitizer category. This category was supported by the negative prediction in the DPRA and KeratinoSens. This example demonstrates that a human NOEL alone does not indicate the actual potency of the tested material.

Octanoic acid (CAS 124-07-2) was categorized as a nonsensitizer. Limited human data are available for octanoic acid. Its HMT NOEL is 690 µg/cm^2, in the moderate sensitizer range. In an LLNA, it did not induce skin sensitization when tested up to 12,500 µg/cm^2 (50%), which is in the weak range. Octanoic acid was not predicted to be a sensitizer in DPRA and KeratinoSens, but it was predicted to be a sensitizer in h-CLAT. In silico, it was not predicted to be reactive to skin proteins directly or through its metabolites.

Linalool (CAS 78-70-6) and limonene (CAS 5989-27-5) were categorized as nonsensitizers. The human NOELs on both materials are greater than 10,000 µg/cm^2, in the very weak sensitizer range. Both materials have multiple LLNA data, with EC3 values greater than 2500 µg/cm^2. Some studies suggest that the positive results obtained from the LLNA are false-positives caused by irritation at high concentrations.[67] In these studies, B-cell activation marked by an increase in B220 expression is quantified to differentiate the skin irritants from skin sensitizers. In these studies, mice treated with the test articles showed B220 expression in line with a reference skin irritant, benzalkonium chloride, but different from that of a reference skin sensitizer, 1-chloro-2,4-dinitrobenzene. In additional animal studies, the oxidation products, specifically hydroperoxides of linalool and limonene were identified as key skin sensitizers.[57,68,69] In a series of experiments using guinea pig test methods conducted in parallel with analytical measurement of sample quality, Karlberg

et al[70] have demonstrated that under low level of oxidation, high purity D-limonene is nonsensitizing, whereas oxidized D-limonene is a contact allergen. Similar findings were reported for linalool.[68] In vitro, linalool was predicted to be a nonsensitizer in a DPRA and KeratinoSens, but a sensitizer in h-CLAT. Mixed DPRA results were available on limonene; 1 DPRA study was negative, whereas another study was positive. Limonene was predicted to be nonsensitizer in KeratinoSens but positive in h-CLAT. Based on chemical structure, both materials are predicted in silico not to be reactive directly to skin proteins, but their metabolites are predicted to be weak sensitizers. Both linalool and limonene are used in products that come in close contact with skin, such as fine fragrances (Creme-RIFM Aggregate Exposure Model, V3.1.3). Despite the widespread use, the occurrence of positive responses in diagnostic patch tests is low.[71]

DISCUSSION

A systematic WoE approach has been undertaken to assign skin sensitization potency categories for 100 fragrance ingredients and 6 NCSs. The goal was to develop an approach that uses an expert assessment of all available data to categorize the skin sensitization potency of these materials. The available human data were given the highest priority in this study. Still, it is clear that all data were important in making the most accurate categorization for each of the fragrance materials. The results show that none of the 106 fragrance materials were categorized as extreme sensitizer, whereas 6, 23, 41, and 26 materials were categorized as strong, moderate, weak, and very weak sensitizers, respectively. Ten materials were categorized as nonsensitizers. Many of the chemicals were easily placed in a potency category based on available human data, specifically CNIH data (eg, cinnamic aldehyde). However, in other cases, it was more challenging and required a careful review of all available data (eg, tetrahydro-4-methyl-2-propyl-2H-pyran-4-yl acetate).

The process of reviewing and determining a material's skin sensitization potency and placing it in a category requires expert judgment. It is likely that for most materials examined in this article, other skin sensitization experts would agree with the potency categorizations presented here. However, it is expected that for a few of these materials, especially materials with mixed or borderline results, other experts might place the materials in different categories but most likely with only one category difference. What is most

important is the transparency of the process and availability of the data used to make specific judgments.

In this article, we used 5 potency category ranges plus the nonsensitizer category that were previously published.[1] The potency category names and their exposure ranges compared with the LLNA EC3 ranges are presented in Table 1. Other publications present different exposure ranges for the potency categories.[9,12,72] However, the range differences are not large among the various publications. The purpose of establishing these categories is not for regulatory purposes but to conduct sound risk assessments. Currently, NESILs for the fragrance materials are derived only when a NOEL has been confirmed through a well-conducted CNIH.[25] For most compounds in this data set, a specific NESIL can be established based on available CNIH to conduct a QRA (eg, citral, *p*-mentha-1,8-dien-7-al, cinnamic aldehyde). In other cases, it may not be possible to calculate a specific NESIL because of lack of sufficient human data. In cases where sufficiency in the human data is lacking (eg, study conducted with less than 100 subjects), the potency category can be determined based on the WoE approach.[27] An option would be to use a default value for the NESIL based on the lowest value of the potency category range. For example, if a material is categorized as a moderate sensitizer, 500 μg/cm^2 is the default NESIL in the QRA.

As mentioned, human data from previously conducted studies were used as the primary source for potency categorization. The CNIH is currently an essential component in the conduct of skin sensitization QRA where it is used to establish a NESIL.[23,24] Of course, even with a deep understanding of the allergenic potency of the tested materials, there is still a risk for the test subjects to become sensitized. Therefore, studies must be reviewed and approved by an ethical review board to ensure that the subjects are fully informed. The proportion of those becoming sensitized to fragrance materials is only 0.03%, based on only 3 positive subjects of 9854 subjects over the last 11 years.[16] If ethical and relevant human data are available, they should be used in establishing potency categorization for use in risk assessment to help protect consumers and workers from developing allergic contact dermatitis.

Currently, the value of using data from nonanimal methods, either alone or in combination, is still under development. No individual validated nonanimal methods are currently viewed as a standalone test for hazard identification, so attempts have been made to develop combination strategies (Integrated Approaches to Testing and Assessment and Defined Approaches) that seek to bring together information from various sources to enhance the accuracy with which skin sensitization hazards are identified as well as to gain insight into the potency of a compound.[72,73] In recent years, there has been substantial progress in providing guidance in deriving a NESIL from in silico, in chemico, and/or in vitro data.[74–76] The details of how these nonanimal methods will fit into the determination of a NESIL, including how they will impact the uncertainties associated with such determination, remain to be seen and form part of ongoing work programs within the fragrance industry (eg, https://www.ideaproject.info/news-events/idea-workshop-on-qra-based-on-nams-building-trust.) A few recently described nonanimal methods

have been designed to help with predicting sensitizer potency to support risk assessment, including the SENS-IS assay,[77,78] the Genomic Allergen Rapid Detection assay,[79] and the kinetic DPRA.[80,81] Other approaches using data from multiple nonanimal methods have also been proposed.[46,82–85]

This article demonstrates the benefits of using a WoE approach to evaluate all available human, animal, and nonanimal data to assess a material's skin sensitization potency. For all of these fragrance materials, CNIH data were available that allowed an expert call on the material's skin sensitization potency and assignment to a category. Although the available CNIH data carried the most weight when assigning a potency category, all data were evaluated for consistency with the assessment. Using all available background data improved our ability to substantiate the sensitization category decisions made. Human diagnostic patch test data were rarely used because they inform mostly on prevalence of an allergen and not its potency. However, there are some instances in which consideration of clinical patch test results are critical to classifying a material as a sensitizer versus a nonsensitizer. The use of robust data sets that include existing human data supported by in vivo and/or nonanimal data will be very beneficial for evaluating new, innovative nonanimal approaches. The addition of nonfragrance material data sets (eg, dental materials, artificial nail monomers, hair dyes, preservatives) should be included in the evaluation, so any analysis is not overrepresented with a limited set of materials.[86] No doubt that this is a considerable challenge and requires careful consideration in extrapolating human, animal, and nonanimal approaches for the purpose of determining a NESIL and conducting sound skin sensitization risk assessment.

ACKNOWLEDGMENTS

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