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

Mihwa Na, PhD,* Devin O’Brien, MS,* Maura Lavelle, MS,* Isabelle Lee, PhD,* G. Frank Gerberick, PhD† and Anne Marie Api, PhD*

**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. 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). Dose per unit area is the well-established dose metric for skin sensitization, 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 μg/cm², whereas others require exposure up to 10,000 μg/cm². Historically, categorization of the sensitization potency of chemicals was based primarily on LLNA data, precisely the EC3 value. 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. In addition to LLNA data, other data may exist, including human micrograms of allergen per square centimeter of the exposed skin.

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 prove the accuracy of determining the potency categorization of skin allergens. 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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{21,22} Recently, an improved approach has been published, which is commonly referred to as QRA2.\textsuperscript{23} A solid understanding of a chemical's skin sensitization potency is critical to conducting sound risk assessments.\textsuperscript{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 (HRRIPT) is required.\textsuperscript{23} Beginning in 2020, the acronym CNIH (confirmation of no induction in humans) was suggested and implemented in place of HRRIPT to highlight the confirmatory nature of this 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.\textsuperscript{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.\textsuperscript{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 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 factors critical in the CNIH 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 considerations, such as Api et al.3,14,38 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 mL 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 mL 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 mL 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 result 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 described39,40 and addresses the first key event of the skin sensitization adverse outcome pathway (AOP).41

\[
\frac{0.2 \times (3.0 \times 10^5 \mu g)}{2.54 \text{ cm}^2} = 23622 \mu g/cm^2
\]
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. The DPRA data were collected from the RIFM Database and other publications. 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. 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 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. KeratinoSens data were collected from the RIFM Database and other publications. 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) 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. The h-CLAT data were collected from RIFM Database and other publications. 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 (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). 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). 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 twenty six 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, 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
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 μg/cm², 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 μg/cm², which is at the upper end of the extreme category. 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.

2-Methoxy-4-methylphenol (CAS 93-51-6) was placed in a moderate category. The CNIH NOEL is 110 μg/cm², which is in the strong sensitizer range. There was no human LOEL. The EC3 value of 1450 μg/cm² (5.8%) indicated that the maximum human NOEL could be higher than 110 μg/cm². 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, μg/cm² | LLNA EC3 Dose Range, μg/cm² |
|------------------|--------------------|-----------------------------|
| Extreme          | <25                | <25                         |
| Strong           | 25–500             | 25–<250                     |
| Moderate         | 500–2500           | 250–<2500                   |
| Weak             | >2500–10,000       | 2500–25,000                 |
| Very weak        | >10,000            | Negative                    |
| Nonsensitizer    | Negative           |                             |

*Adapted from Api et al.*

†Defined based on the guidance from European Center for Ecotoxicology and Toxicology of Chemicals Technical Report 87.
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/cm² and the EC3 of 1700 μg/cm² (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/cm² and a similar LOEL of 8858 μg/cm². 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-2H-pyran-4-yl acetate (CAS 131766-73-9) was categorized as a very weak sensitizer. It has a human NOEL of 11,000 μg/cm², whereas its human LOEL is not known. The LLNA was negative with the highest tested dose of 7500 μg/cm² (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-2H-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-2H-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/cm² 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/cm² (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/cm², 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/cm². 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 sensitizers.
| 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                 | <1250, estimated 625 | High                  | Positive          | Positive | Michael Addition | Strong sensitizer            | Nonsensitizer               |
| 111-80-8   | Methyl-2-nonynoate | Strong                   | 24                  | 118                 |                   | 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 | Strong sensitizer            | Nonsensitizer               |
| 93893-89-1 | 3-Methyl-5-phenylpent-2-enenitrile | Strong                   | 275                 | N/A                 | 192.5             | Minimal              | Negative          | Positive | Michael Addition | Strong sensitizer            | Nonsensitizer               |
| 17373-89-6 | 2-Hexylidene cyclopentanone | Strong                   | 300                 | 500                 | 600               | No data              | Positive          | No data  | Michael Addition | Strong sensitizer            | Nonsensitizer               |
| 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                 | Positive          | Negative | 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 phenoxacetate | 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 | Strong sensitizer             | Strong sensitizer            |
| 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-Propylideneanthalide | Moderate              | 945                 | 2760                | 350; <1250        | Low                  | Negative          | Positive | Acylation        | Strong sensitizer            | Nonsensitizer               |
| 1885-38-7  | Cinnamyl nitrile | Moderate                | 1063                | 1250                | NC (250)          | Minimal              | Positive          | Positive | No alert found | Nonsensitizer               | Strong sensitizer            |
| 68883-20-5 | Menthadiene-7-methyl formate | Moderate             | 1063                | 6900                | NC (250)          | Low                  | Positive          | Positive | No alert found | Nonsensitizer               | Weak sensitizer             |

(Continued on next page)
| 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 | 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                                                    | Schiff base formation        | 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                                                    | N/A, Nonsensitizer           | Strong sensitizer |
| 103-50-4   | Dibenzyl ether                                                                | Moderate                  | 2362                | N/A                | 1575             | Minimal                  | Positive      | Positive                                                    | No alert found               | Nonsensitizer |
| 90028-68-5 | Oakmoss absolute, low atranol                                                 | Moderate                  | 700                 | N/A                | 3775 [6]; NC (7500); NC (12,500); NC (625) | Low          | Negative      | Positive                                                    | No alert found               | Nonsensitizer |
| 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, Weak sensitizer |
| 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-Isoproplyphenyl) 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 |
| 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                                                    | Michael Addition              | Strong sensitizer, No metabolites predicted |
| 6485-40-1  | R-Carvone                                                                     | Weak                      | 2657                | 18898              | 2950 [2]         | Low                      | Positive      | Positive                                                    | Michael Addition              | Strong sensitizer |
| 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                                         | Category | Test Concentration | Test Type | Alert | Sensitizer | Notes                          |
|---------|----------------------------------------------|----------|-------------------|-----------|-------|------------|--------------------------------|
| 104-54-1 | Cinnamic alcohol                             | Weak     | 2953              | Low       | Positive | Positive   | No alert found                |
| 122760-84-3 | Tricyclo[3.3.1.1.(3.7)]decan-2-one, 4-methyl-8-methylene-Butanamide, 2-ethyl-N-methyl-N-(3-methylphenyl)- | Weak     | 3000              | Low       | Positive | Positive   | No alert found                |
| 40468-88-30-0 | Tricyclo[3.3.1.1.(3.7)]decan-2-one, 4-methyl-8-methylene-Butanamide, 2-ethyl-N-methyl-N-(3-methylphenyl)- | Weak     | 3250              | Minimal   | Negative | Positive   | No alert found                |
| 123-11-5 | p-Methoxybenzaldehyde                        | Weak     | 3543              | Moderate  | Negative | Positive   | No alert found                |
| 101-39-3 | α-Methylcinnamaldehyde                       | Weak     | 3543              | Low       | Positive | Positive   | Strong sensitizer             |
| ...     | ...                                          | ...      | ...               | ...       | ...     | ...        | ...                           |
| 82654-98-6 | Vanillyl butyl ether                         | Weak     | 3543              | Low       | Positive | Positive   | No alert found                |
| 402-31-7 | Longifolene                                   | Weak     | 3543              | Minimal   | Positive | Positive   | Nonsensitizer                 |
| 91-64-5 | Coumarin                                     | Weak     | 3543              | Minimal   | Positive | Positive   | Nonsensitizer                 |
| ...     | ...                                          | ...      | ...               | ...       | ...     | ...        | ...                           |
| 100-51-6 | Benzyl alcohol                               | Weak     | 5905              | Low       | Negative | Positive   | No alert found                |
| ...     | ...                                          | ...      | ...               | ...       | ...     | ...        | ...                           |

(Continued on next page)
| 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 | keratinSens h-CLAT | Protein Binding Alerts for Skin Sensitization, Toolbox 4.2 | Parent Prediction, TIMES-SS | Metabolite Prediction, TIMES-SS |
|------------|-------------------------------------|---------------------------|---------------------|---------------------|------------------|------------------------|----------------|----------------|-------------------------------------------------------------|-----------------------------|-------------------------------|
| 1335-66-6  | Isocyclocitralf§                    | 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             | Weak sensitizer               |
| 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               | Weak sensitizer               |
| 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   | 1,1-Pentadecalactone               | Very weak                 | 5509                | N/A                 | NC (12,500); 5187 [2] | Minimal                | Negative       | Positive       | Positive                                                    | 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| 1,3,3-Trimethylbenzenepropanol      | Very weak                 | 9917                | N/A                 | NC (25000); 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                  |
| 1205-17-0  | α-Methyl-1,3-benzodioxole-5-propionaldehyde | Very weak                 | 11810               | 16667               | 4100             | Moderate               | Positive       | Schiff base formation                                       | Weak sensitizer             | Weak sensitizer               |
| 4707-47-5  | Methyl atrarate                     | Very weak                 | 11810               | N/A                 | 4750; NC (6250)   | Inconclusive           | Positive       | No alert found                                             | Nonsensitizer               | Weak sensitizer               |
| 131766-73-9| Tetrahydro-4-methyl-2-propyl-2H-pyran-4-y acetate | Very weak                 | 11019               | N/A                 | NC (7500)        | Minimal                | Negative       | No alert found                                             | Nonsensitizer               | Nonsensitizer                  |
| 23911-56-0 | 1-(3-Methyl-2-benzofuranyl) ethanone | Very weak                 | 11019               | N/A                 | NC (7500)        | Minimal                | Positive       | No alert found                                             | Nonsensitizer               | Nonsensitizer                  |
| CAS Number | Chemical Name                        | Sensitization | Sensitization | Sensitization | Sensitization | Sensitization |
|------------|-------------------------------------|---------------|---------------|---------------|---------------|---------------|
| 106-24-1   | Geraniol                            | Very weak     | Nonsensitizer | No alert found| Minimal       | Positive      |
| 3370-4-61-9| 6-Methylenedioxy-1,2,3,3-tetrahydro-2H-furan-2-one | Very weak     | Nonsensitizer | No alert found| Minimal       | Positive      |
| 118-58-1   | Benzyl salicylate                   | Very weak     | Nonsensitizer | No alert found| Minimal       | Positive      |
| 13828-37-0 | c-4-Isopropenyl()                   | Very weak     | Nonsensitizer | No alert found| Minimal       | Positive      |
|           | cyclohexane-1,3-dione               | Very weak     | Nonsensitizer | No alert found| Minimal       | Positive      |
| 13257-44-8 | 2-Norborn-1-1,1-dimethyl acetal     | Very weak     | Nonsensitizer | No alert found| Minimal       | Positive      |
| 101-86-0   | Hexyl cinnamaldehyde               | Very weak     | Nonsensitizer | No alert found| Minimal       | Positive      |
| 106-22-9   | Citronellol                         | Very weak     | Nonsensitizer | No alert found| Minimal       | Positive      |
| 6259-76-3  | Hexyl salicylate                   | Very weak     | Nonsensitizer | No alert found| Minimal       | Positive      |
|           | 1-(1,2,3,4,5,6,7,8-Octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)ethanone§ | Very weak     | Nonsensitizer | No alert found| Minimal       | Positive      |
| 120-51-4   | Benzyl benzoate                    | Very weak     | Nonsensitizer | No alert found| Minimal       | Positive      |
| 127-5-15   | α-iso-limonene§                    | Very weak     | Nonsensitizer | No alert found| Minimal       | Positive      |
| 5989-27-7  | Octanoic acid                      | Very weak     | Nonsensitizer | No alert found| Minimal       | Positive      |
|           | Ocimum gratissimum aromatic acid   | Very weak     | Nonsensitizer | No alert found| Minimal       | Positive      |
| 68300-15-8 | 2-Methyl-2-cyclopentanol           | Very weak     | Nonsensitizer | No alert found| Minimal       | Positive      |
| 18479-58-8 | Dihydromyrcenol                    | Very weak     | Nonsensitizer | No alert found| Minimal       | Positive      |
| 105-93-9   | Ethylene brassylate                | Very weak     | Nonsensitizer | No alert found| Minimal       | Positive      |

*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-ol. 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 HMTs 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, but other studies with minor variations in the protocol were 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.
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/cm², which falls into the moderate sensitizer range. No human NOEL 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/cm², in the moderate sensitizer range. In an LLNA, it did not induce skin sensitization when tested up to 12,500 μg/cm² (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/cm², in the very weak sensitizer range. Both materials have multiple LLNA data, with EC3 values greater than 2500 μg/cm². 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.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 a 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/cm² 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.5,19 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.14 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.14,27 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.ideaiproject.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.86,82–85

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

The authors thank Dr. David A. Basketter and Dr. Cindy A. Ryan for the valuable discussions and suggestions.

REFERENCES

1. Api AM, Parakhia R, O’Brien D, et al. Fragrances categorized according to relative human skin sensitization potency. Dermatitis 2017;28(5):299–307.
2. Gerberick GF, Ryan CA, Kern PS, et al. A chemical dataset for evaluation of alternative approaches to skin-sensitization testing. Contact Dermatitis 2004; 50(5):274–288.
3. Kern PS, Gerberick GF, Ryan CA, et al. Local lymph node data for the evaluation of skin sensitization alternatives: a second compilation. Dermatitis 2010;21(1):8–32.
4. Friedmann PS. The relationships between exposure dose and response in induction and elicitation of contact hypersensitivity in humans. Br J Dermatol 2007;157(6):1093–1102.
5. Kimber I, Dearman RJ, Basketter DA, et al. Dose metrics in the acquisition of skin sensitization: thresholds and importance of dose per unit area. Regul Toxicol Pharmacol 2008;52(1):39–45.
6. Basketter DA, Alepee N, Ashikaga T, et al. Categorization of chemicals according to their relative human skin sensitizing potency. Dermatitis. 2014; 25(1):11–21.
26. Date MS, O’Brien D, Botelho DJ, et al. Clustering a chemical inventory for the derivation of EC3 values from local lymph node assay dose responses. J Appl Toxicol 1999;19(4):261–266.

27. Politano VT, Api AM. The Research Institute for Fragrance Materials’ human repeated insult patch test protocol. Regul Toxicol Pharmacol 2008;52(1):35–38.

28. Schwartz L. The skin testing of new cosmetics. J Soc Cosmet Chem 1951;2:321–324.

29. Schwartz L. Twenty-two years’ experience in the performance of 200,000 prophetic patch tests. South Med J 1960;53:478–483.

30. Shelanski HA, Shelanski MV. A new technique of human patch tests. Proc Sci Sect Toilet Goods Assoc 1953;19(46):4–7.

31. Shelanski H. Experience with and considerations of the human patch test method. J Cosmet Sci 1951;2(5):324–331.

32. Marzulli FN, Maibach HI. Contact allergy: predictive testing in man. Contact Dermatitis 1976;2(1):1–17.

33. Marzulli FN, Maibach HI. Further studies of effects of vehicles and elicitation concentration in experimental contact sensitization testing in humans. Contact Dermatitis 1980;6(2):131–133.

34. Stotts J. Planning, conduct and interpretation of human predictive sensitization patch tests. Crit Rev Toxicol 1980;1(1):41–53.

35. Kligman AM. The identification of contact allergens by human assay. 3. The maximization test: a procedure for screening and rating contact sensitzers. Contact Dermatitis 1966;4(5):393–409.

36. Kligman AM, Epstein W. Updating the maximization test for identifying contact allergens. Contact Dermatitis 1975;1(4):231–239.

37. OECD. Test No. 429: Skin Sensitisation: Local Lymph Node Assay. OECD Guidelines for the Testing of Chemicals, Section 4. Paris, France: OECD Publishing; 2010.

38. Gerberick GF, Ryan CA, Kern PS, et al. Compilation of historical local lymph node data for evaluation of skin sensitization alternative methods. Dermatitis. 2005;16(4):157–202.

39. Gerberick GF, Vassallo JD, Bailey RE, et al. Development of a peptide reactivity assay for screening contact allergens. Toxicol Sci 2004;81(2):332–343.

40. Gerberick GF, Vassallo JD, Foerttsch LM, et al. Quantification of chemical peptide reactivity for screening contact allergens: a classification tree model approach. Toxicol Sci 2007;97(2):417–427.

41. OECD. The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins. OECD Series on Testing and Assessment, No168. Paris, France: OECD Publishing; 2014.

42. OECD. Test No. 442C: In Chemico Skin Sensitisation. OECD Guidelines for the Testing of Chemicals, Section 4. Paris, France: OECD Publishing; 2021.

43. Natsch A, Gfeller H, Rothaupt M, et al. Utility and limitations of a peptide reactivity assay to predict fragrance allergens in vitro. Toxicol In Vitro 2007;21(7):1220–1226.

44. Natsch A, Gfeller H. LC-MS-based characterization of the peptide reactivity of chemicals to improve the in vitro prediction of the skin sensitization potential. Toxicol Sci 2008;106(2):464–478.

45. Natsch A, Ryan CA, Foerttsch L, et al. A dataset on 145 chemicals tested in alternative assays for skin sensitization undergoing prevalidation. J Appl Toxicol 2013;33(11):1337–1352.

46. Takenouchi O, Fukui S, Okamoto K, et al. Test battery with the human cell line activation test, direct peptide reactivity assay and DEREK based on a 139 chemical data set for predicting skin sensitizing potential and potency of chemicals. J Appl Toxicol 2015;35(11):1318–1332.

47. Bauch C, Kolle SN, Ramirez T, et al. Putting the parts together: combining in vitro methods to test for skin sensitizing potentials. Regul Toxicol Pharmacol 2012;63(3):489–504.

48. Urbisch D, Mehlng A, Guth K, et al. Assessing skin sensitization hazard in mice and men using non-animal test methods. Regul Toxicol Pharmacol 2015;71(2):337–351.
49. Emter R, Ellis G, Natsch A. Performance of a novel keratinocyte-based reporter cell line to screen skin sensitizers in vitro. *Toxicol Appl Pharmacol* 2010;245(3):281–290.

50. OECD. Test No. 442D: *In Vitro Skin Sensitisation: ARE-Nr2 Luciferase Test Method*. OECD Guidelines for the Testing of Chemicals, Section 4. Paris, France: OECD Publishing; 2018.

51. Otsubo Y, Nishijo T, Miyazawa M, et al. Binary test battery with KeratinoSens and h-CLAT as part of a bottom-up approach for skin sensitization hazard prediction. *Regul Toxicol Pharmacol* 2017;88:118–124.

52. Ashikaga T, Yoshida Y, Hirota M, et al. Development of an in vitro skin sensitization test using human cell lines: the human Cell Line Activation Test (h-CLAT). I. Optimization of the h-CLAT protocol. *Toxicol In Vitro* 2006;20(5):767–773.

53. Ashikaga T, Sakaguchi H, Sono S, et al. A comparative evaluation of in vitro skin sensitisation tests: the human cell-line activation test (h-CLAT) versus the local lymph node assay (LLNA). *Altern Lab Anim* 2010;38(4):275–284.

54. OECD. Test No. 442E: *In Vitro Skin Sensitisation assays addressing the Key Event on activation of dendritic cells on the Adverse Outcome Pathway for Skin Sensitisation*. OECD Guidelines for the Testing of Chemicals, Section 4. Paris, France: OECD Publishing; 2018.

55. Nukada Y, Ashikaga T, Sakaguchi H, et al. Predictive performance for human skin sensitizing potential of the human cell line activation test (h-CLAT). *Contact Dermatitis* 2011;65(6):343–353.

56. ECETOC. *Contact Sensitization: Classification According to Potency Technical Report No. 87*. Brussels, Belgium: European Centre for Ecotoxicology & Toxicology of Chemicals; 2003.

57. Schnuch A, Uter W, Geier J, et al. Sensitization to 26 fragrances to be labelled according to current European regulation. Results of the IVVD and review of the literature. *Contact Dermatitis* 2007;57(1):1–10.

58. Hausen BM. Contact allergy to balsam of Peru. II. Patch test results in 102 patients with selected balsam of Peru constituents. *Am J Contact Dermat* 2001;12(2):93–102.

59. RIFM. Skin sensitization in the Guinea-Pig of Clarycet. 1992.

60. Gerberick GF, Cruse LW, Ryan CA, et al. Use of a B cell marker (B220) to discriminate between allergens and irritants in the local lymph node assay. *Toxicol Sci* 2002;68(2):420–428.

61. de Groot AC, Coenraads PJ, Bruynzeel DP, et al. Routine patch testing with fragrance chemicals in the Netherlands. *Contact Dermatitis* 2000;42(3):184–185.

62. Romaguera C, Grimalt F. Statistical and comparative study of 4600 patients tested in Barcelona (1973–1977). *Contact Dermatitis* 1980;6(5):309–315.

63. Api AM, Belósito D, Botelho D, et al. RIFM fragrance ingredient safety assessment, 1-(3-methyl-2-benzofuranyl)ethanone, CAS Registry Number 23911-56-6. *Food Chem Toxicol* 2021;153 Suppl.1:112300.

64. RIFM. Cutaneous Test-Patch Test. Unpublished work. RIFM report number 52954. 1997.

65. Api AM, Belósito D, Biserta S, et al. RIFM fragrance ingredient safety assessment, 2-methyldecanenitrile, CAS Registry Number 69300-15-8. *Food Chem Toxicol* 2021;153 Suppl.1:112296.

66. RIFM. Delayed Dermal Sensitization Study in Guinea Pig. Unpublished work. RIFM report number 31689. 1989.

67. Api AM, Belósito D, Bhatia S, et al. RIFM fragrance ingredient safety assessment, Linalool, CAS registry number 78-70-6. *Food Chem Toxicol* 2015;82(suppl):S29–S38.

68. Skold M, Borje A, Matura M, et al. Studies on the autoxidation and sensitizing capacity of the fragrance chemical linalool, identifying a linalool hydroperoxide. *Contact Dermatitis* 2002;46(5):267–272.

69. Skold M, Borje A, Harambasic E, et al. Contact allergens formed on air exposure of linalool. Identification and quantification of secondary and secondary oxidation products and the effect on skin sensitization. *Chem Res Toxicol* 2004;17(12):1697–1705.

70. Karlberg AT, Boman A, Melin B. Animal experiments on the allergenicity of d-limonene—the citrus solvent. *Ann Occup Hyg* 1991;35(4):419–426.

71. Uter W, Geier J, Frosh P, et al. Contact allergy to fragrances: current patch test results (2005–2008) from the Information Network of Departments of Dermatology. *Contact Dermatitis* 2010;63(5):254–261.

72. Basketter D, Beken S, Bender H, et al. Building confidence in skin sensitisation potency assessment using new approach methodologies: report of the 3rd EPAA Partners Forum, Brussels, 28th October 2019. *Regul Toxicol Pharmacol* 2020;117:104767.

73. OECD. *Guidance Document on the Reporting of Defined Approaches and Individual Information Sources to be Used within Integrated Approaches to Testing and Assessment (IATA) for Skin Sensitisation*. OECD Series on Testing and Assessment, No 256. Paris, France: OECD Publishing; 2017.

74. Gilmour N, Kern PS, Alepee N, et al. Development of a next generation risk assessment framework for the evaluation of skin sensitisation of cosmetic ingredients. *Regul Toxicol Pharmacol* 2020;116:104721.

75. Kleinsteuerc NC, Hoffmann S, Alepee N, et al. Non-animal methods to predict skin sensitization (II): an assessment of defined approaches (*). *Crit Rev Toxicol* 2018;48(5):359–374.

76. Natsch A, Emter R, Haupt T, et al. Deriving a no expected sensitization induction level for fragrance ingredients without animal testing: an integrated approach applied to specific case studies. *Toxicol Sci* 2018;165(1):170–185.

77. Cottrez F, Boitel E, Auriault C, et al. Genes specifically modulated in sensitized skins allow the detection of sensitizers in a reconstructed human skin model. Development of the SENS-IS assay. *Toxicol In Vitro* 2015;29(4):787–802.

78. Cottrez F, Boitel E, Ourlin JC, et al. SENS-IS, a 3D reconstructed epidermis based model for quantifying chemical sensitization potency: reproducibility and predictivity results from an inter-laboratory study. *Toxicol In Vitro* 2016;32:248–260.

79. Zeller KS, Forreyd A, Lindberg T, et al. The GARD platform for potency assessment of skin sensitizing chemicals. *ALTEX* 2017;34(4):539–559.

80. Wareing B, Urbisch D, Kolle SN, et al. Prediction of skin sensitization potency sub-categories using peptide reactivity data. *Toxicol In Vitro* 2017;45 (Pt 1):134–145.

81. Natsch A, Haupt T, Wareing B, et al. Predictivity of the kinetic direct peptide reactivity assay (kDPRA) for sensitizer potency assessment and GHS subclassification. *ALTEX* 2020;37(4):652–664.

82. Natsch A, Emter R, Gieller H, et al. Predicting skin sensitizer potency based on in vitro data from KeratinoSens and kinetic peptide binding: global versus domain-based assessment. *Toxicol Sci* 2015;143(2):319–332.

83. Hirota M, Fukui S, Okamoto K, et al. Evaluation of combinations of in vitro sensitisation test descriptors for the artificial neural network-based risk assessment model of skin sensitization. *J Appl Toxicol* 2015;35(11):1333–1347.

84. Hirota M, Ashikaga T, Koizumi K. Development of an artificial neural network model for risk assessment of skin sensitization using human cell line activation test, direct peptide reactivity assay, KeratinoSens™ and in silico structure alert parameter. *J Appl Toxicol* 2018;38(4):514–526.

85. Jaworska JS, Natsch A, Ryan C, et al. Bayesian integrated testing strategy (BITE) to discriminate between allergens and irritants in the local lymph node assay. *Contact Dermatitis* 2015;89(12):2355–2383.

86. Kolle SN, Landsiedel R, Natsch A. Replacing the refinement for skin sensitization testing: considerations to the implementation of adverse outcome pathway (AOP)-based defined approaches (DA) in OECD guidelines. *Regul Toxicol Pharmacol* 2020;115:104713.