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Derivation of the no expected sensitization induction level for dermal quantitative risk assessment of fragrance ingredients using a weight of evidence approach

Isabelle Lee *, Mihwa Na, Maura Lavelle, Anne Marie Api

Research Institute for Fragrance Materials, Inc. (RIFM), 50 Tice Boulevard, Woodcliff Lake, NJ, 07677, USA

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ABSTRACT

Some fragrance ingredients may have the potential to induce skin sensitization in humans but can still be safely formulated into consumer products. Quantitative Risk Assessment (QRA) for dermal sensitization is required to determine safe levels at which potential skin sensitizers can be incorporated into consumer products. The no expected sensitization induction level or NESIL is the point of departure for the dermal QRA. Sensitization assessment factors are applied to the NESIL to determine acceptable exposure levels at which no skin sensitization induction would be expected in the general population. This paper details the key steps involved in deriving a weight of evidence (WoE) NESIL for a given fragrance ingredient using all existing data, including *in vivo*, *in vitro*, and *in silico*. Read-across can be used to derive a NESIL for a group of structurally similar materials when data are insufficient. When sufficient target and read-across data are lacking, exposure waiving threshold (the DST) may be used. We outline the process as it currently stands at the Research Institute for Fragrance Materials Inc. (RIFM) and provide examples, but it is dynamic and is bound to change with evolving science as new approach methodologies (NAMs) are actively incorporated.

1. Introduction

Chemicals, including fragrance ingredients, with the potential to cause skin sensitization can be safely formulated into consumer products at levels not expected to induce skin sensitization. Exposure-based quantitative risk assessment (QRA) for induction of dermal sensitization is applied to fragrance ingredients with sensitization potential to determine safe levels at which they can be used in different product types. The dermal sensitization QRA was developed with the aim of preventing the acquisition or induction of skin sensitization, as opposed to elicitation, because the factors associated with induction are currently better understood than the latter phase. Nonetheless, with the prevention of the induction, elicitation of skin sensitization may be eliminated or reduced. A proposal for assessing the risk of induction of skin sensitization to fragrance materials in different product categories, quantitative risk assessment 1 (QRA1), was first published in 2008 (Api et al., 2008) and updated to quantitative risk assessment 2 (QRA2) in 2020 (Api et al., 2020).

Fragrance ingredients determined to be sensitizers based on weight of evidence (WoE) from all available data - *in silico*, *in vitro*, and *in vivo* (human and animal) - require the application of QRA2 for the protection of consumers. In RIFM's WoE approach, a no-observed-effect level (NOEL) for the induction of skin sensitization is confirmed for sensitizers through the human repeat insult patch test. Since the human repeat insult patch test is a confirmatory test, RIFM has proposed renaming it as the "confirmation of no induction in humans" (CNIH) (Na et al., 2020). A NOEL confirmed in a CNIH, conducted according to the RIFM protocol (Politano and Api, 2008), is primarily used to set the NESIL. The NESIL is the critical benchmark or point of departure for the application of the dermal sensitization QRA. Na et al. described how all the available skin sensitization data could be used to set a NESIL based on WoE (to be published). It may be possible in the future to use the categories described in this paper to establish a NESIL without using a CNIH.

Most fragrance ingredients are structurally simple, low molecular weight, predominantly semi-volatile substances consisting of carbon, hydrogen, and oxygen. Chemical structure helps to predict transdermal absorption, metabolism and disposition, and functional groups that can influence toxicity. Structural similarities within fragrance ingredients permit making some generalizations because chemicals that share certain common structural elements typically have comparable

* Corresponding author.
E-mail address: ilee@rifm.org (I. Lee).

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physico–chemico and toxicokinetic properties and may exhibit a common mode of action. Data from one or more tested chemicals can be used to predict the toxicity of a structurally similar chemical for the same test or endpoint (Date et al., 2020). The clustering of structurally related materials allows one to reasonably predict some degree of consistency of metabolism and toxicity. Chemical structure-based clustering of the RIFM fragrance chemical inventory has been completed. Fragrance ingredients with limited or insufficient data to determine their sensitization potential or potency can be "read-across" from structurally similar chemicals with sufficient data within the same or adjacent clusters. Read-across is based on the underlying hypothesis that the toxicity of a particular chemical is a function of its molecular structure (Date et al., 2020; T. W. Schultz et al., 2015; Terry W. Schultz, Richarz and Cronin, 2019).

When limited historical data are available for a material, and no appropriate read-across analogs are available, RIFM applies exposure-based waving based on the dermal sensitization threshold (DST). The DST, the dermal exposure level below which no skin sensitization is expected for a chemical based on its reactivity, is an important tool that has resulted in a significant reduction in animal testing (Nishijo et al., 2019; Roberts et al., 2015; R. J. Safford, 2008; Robert J. Safford, Api et al., 2015; R. J. Safford, Aptula and Gilmour, 2011). Generally, fragrance ingredients with limited historical data are used in small quantities and therefore have low exposure to the general population. Further testing can be waived for low exposure materials depending on whether their levels of use fall below the reactive or non-reactive DST. The reactivity of chemicals is predicted based on expert judgment, with the aid of *in silico*, *in vitro*, and *in vivo* data.

Since 2013, RIFM has not conducted any skin sensitization studies in animals, and new testing has been limited to *in vitro* assays and CNIH. Testing is conducted to fill various data gaps for individual chemicals or chemical clusters. Defined approaches (DAs) amalgamating data from various *in vitro* sources studying the key events of the skin sensitization adverse outcome pathway (AOP) may be used to predict the sensitization potential of chemicals. An example is the "2 out of 3" DA where *in vitro* assays of the skin sensitization AOP, including protein binding (direct peptide reactivity assay (DPRA)), keratinocyte activation (KeratinoSens or LuSens), and human dendritic cell line activation activation ((hCLAT) or USENS assay), are evaluated in combination to determine hazard (OECD, 2018a, 2018b, 2020; D. Urbisch et al., 2015). These *in vitro* assays may be conducted to determine the reactivity of chemicals in a cluster or to determine hazard based on the "2 out of 3" defined approach (Kolle et al., 2019; OECD, 2019b; Urbisch et al., 2015). In the 2 out of 3 approach, chemicals with at least two positive results in tests addressing Key events 1–3 (DPRA, Keratinosens, U-SENS h-CLAT) are predicted sensitizers, while chemicals with none or only one positive outcome are predicted non-sensitizers. For sensitizers, a CNIH from the target chemical or read-across must be available to set the NESIL. If no appropriate CNIH data are available to set the NESIL, then this test may be conducted on the target chemical of interest or a read-across analog to clear the materials in a cluster. RIFM currently only uses available OECD validated *in vitro* methods for hazard identification, but several NAMs are in the development and validation stages by industry shareholders. In the future, RIFM may use NAMs, alone or in combination, to determine potency and set the NESIL for QRA.

Outlined below is a guide on the current iterative steps involved in the derivation of a NESIL for fragrance ingredients in the RIFM chemical inventory.

2. Steps involved in conducting safety assessment for skin sensitization

2.1. Step 1: Determine the potential (hazard) to induce sensitization for a target material

2.1.1. A. Look at all historical data

Identifying hazard is the first step in the safety assessment of a fragrance material. To that end, all scientific data (published and 'in house') are included and considered for the safety evaluation of fragrance ingredients. This includes the physical and chemical properties of the materials under investigation, *in silico* data such as results obtained from (Q)SAR [(Quantitative) Structure Activity Relationship] modeling, chemical categories, grouping, *in vitro* data, and existing human and/or animal data relevant to skin sensitization. The *in vitro* data, including protein binding (direct peptide reactivity assay (DPRA)), keratinocyte activation (KeratinoSens or LuSens), and human dendritic cell line activation activation ((hCLAT) or USENS assay), can also be used in determining hazard (OECD, 2018a, 2018b, 2020; Urbisch et al., 2015). These assays contribute to the identification of skin sensitization hazard, but there is insufficient evidence to date that they provide reliable indicators of potency. The OECD continues to evaluate a range of defined approaches for combining data from individual assays, which may also assist in potency determination (OECD, 2017, 2019a). Nevertheless, it remains challenging to achieve a complete replacement of *in vivo* testing for potency determination (D. Basketter et al., 2020). In addition to *in vitro* data, historical animal data, such as guinea pig studies and the local lymph node assay (LLNA) conducted according to established OECD test guidelines, are considered to predict the hazard potential of the material (OECD, 1992, 2010). Existing human data may include CNIH tests, human maximization tests (HMTs), and diagnostic patch tests. Any unequivocal reactions indicative of skin sensitization observed in these confirmatory human tests indicate that the material is a skin sensitizer; however, it should be noted that no human tests are conducted for hazard identification. Since 2008, the methodology of the CNIH has been standardized by RIFM, and the studies are performed with approval from an ethical review board (Politano and Api, 2008). A material is considered a non-sensitizer when clear negative results are available from human, animal, and/or *in vitro* studies. The absence of protein binding alerts from *in silico* tools (i.e., OECD Toolbox and Toxtree) strengthens the WoE in the evaluation of non-sensitizers. If the material's potential to induce skin sensitization is demonstrated in any one of the *in vivo* tests and/or in at least 2 of the 3 *in vitro* tests, the material is considered a skin sensitizer (Bauch et al., 2012; Kolle et al., 2019).

2.1.2. b. If historical data are insufficient or not available: Determine a suitable read-across

When existing historical data are insufficient to adequately determine the sensitizing potential of a fragrance material, i.e., conclude that the substance is not a skin sensitizer and/or derive a NESIL, the next step is to find a suitable read-across. Read-across is a critical approach used by RIFM to waive testing by using information from structurally similar analogs to bridge data gaps for target materials. The RIFM Database presents an advantage in the search for structural analogs, as it holds the best collection of data on fragrance and flavor ingredients in the world (Api, 2002). Chemicals in this database are clustered into categories/groups that make it easier to search for fragrance ingredient read-across analogs for any endpoint of interest (Date et al., 2020).

Read-across analogs are selected by expert review of chemicals with the aid of computational or *in silico* methods. Structural, reactivity, metabolic, and physico–chemico similarities are considered in the selection of read-across candidates. RIFM experts have several rules for selecting read-across addressing each endpoint. For the skin sensitization endpoint, the reactivity of a chemical towards skin proteins is the most critical chemical property assessed. The read-across analog must be

more reactive and have the same mechanism of reactivity (e.g., Michael addition, Schiff base formation, acylation, etc.) as the target chemical. These are some of the most critical, but not exhaustive, rules applied during an expert review of read-across analogs for skin sensitization. Appropriate read-across analogs for skin sensitization provide data-bridging studies conducted according to OECD test guidelines or CNIH studies conducted according to the RIFM protocol (Politano and Api, 2008).

2.1.3. C. If no read-across analogs are available: Determine if exposure to a target fragrance material is below the DST for reactive and non-reactive chemicals

In the absence of sufficient data or read-across, a material may be evaluated by utilizing the DST. The DST applies the concept of threshold of toxicological concern (TTC) to the evaluation of dermal sensitization, by establishing a level below which there is no appreciable risk for the induction of skin sensitization (R. J. Safford, 2008). This is based on a probabilistic analysis of potency data for a diverse range of known chemical allergens. Available data on the material and materials in its cluster, as well as predictions from *in silico* tools and expert judgement, are used to determine if a material is non-reactive or reactive. If a material is considered non-reactive, a DST of 900 μg/cm^2 is applied (R. J. Safford et al., 2011), and a DST of 64 μg/cm^2 is utilized for reactive materials (Robert J. Safford et al., 2015). For reactive materials that are further classified in the high potency category (HPC), an HPC DST of 1.5 μg/cm^2 may be applied (Nishijo et al., 2019; Roberts et al., 2015); however, this threshold has yet to be utilized on fragrance ingredients in the RIFM Database. These thresholds of 900 μg/cm^2 and 64 μg/cm^2 are utilized in the dermal sensitization QRA, and when the reported 95th percentile use concentration in finished products of a material does not exceed the maximum acceptable concentration for the non-reactive or reactive DST in all QRA categories as previously described (Api et al., 2020), the safety assessment for the material can be completed. Thresholds of toxicological concern for skin sensitization are constantly under review and may be updated or refined as new data become available. If the use of a material exceeds the maximum acceptable concentrations of its respective DST, testing may be required (described in Step 4). Since exposure is critical in determining if the DST can be applied and continue to be applied, RIFM's policy is to update exposure data a minimum of every 5 years.

2.2. Step 2: Dose-response

A dose response relationship provides information on how increasing levels of exposure will produce an increasing risk of dermal sensitization. Historically, several animal models have been used to determine the dose-response relationship for a fragrance ingredient to induce sensitization. Potency, which is derived from the dose-response is crucial information for determining the NESIL. Guinea pig tests (adjuvant and non-adjuvant) have been used for many years to assess the inherent contact sensitization potential of chemicals. Some of these tests are also used to indicate potency, although the murine LLNA (OECD, 2010) became the favored animal test to identify skin sensitization hazards as well as to measure relative potency. The latter is determined from the dose-response curve to derive an EC3 value (i.e., the estimated dose of a substance required to induce a positive threshold response as derived by linear interpolation) (D. A. Basketter et al., 1999). The EC3 value has been demonstrated to closely correlate with the NOEL from human sensitization tests designed to confirm lack of induction (Gerberick et al., 2001, 2004; Griem et al., 2003; Schneider and Akkan, 2004). Dose response information determined from the LLNA is important in determining potency.

However, efforts continue to eliminate the need for any *in vivo* testing. In the European Union, the use of animal testing of cosmetics and their ingredients is banned, and consequently, non-animal tests are essential to provide the basis for hazard assessment. For each of the first 3 steps in the adverse outcome pathway for skin sensitization (AOP) (OECD, 2014), an approved *in vitro* test has become available. These include the DPRA, OECD TG 442C (OECD, 2020), the ARE-Nrf2 Luciferase Methods (KeratinoSens/LuSens, OECD TG 442D) (OECD, 2018b), and the USENS/hCLAT (OECD TG 442E) (OECD, 2018a). These assays are being used to identify skin sensitization hazards. While there is insufficient evidence that *in vitro* data alone can be used as indicators of potency, there are data to support that *in vitro* methods can used in conjunction with other data to derive potency (OECD, 2021).

2.3. Step 3: Setting a WoE NESIL

The NESIL is a benchmark that is derived from all available data, including *in silico*, *in vitro*, animal, and human data, as well as read-across through the application of the WoE approach to all the relevant data. The NESIL is expressed as a dose per unit area (e.g., μg/cm^2) value. In contact allergy, there is overwhelming empirical support for using quantity per unit area rather than other dose metrics such as concentration applied to the skin (Kligman, 1966; Magnusson and Kligman, 1969; Rees et al., 1990; Upadhye and Maibach, 1992; White et al., 1986). An in-depth review of the published studies that support the use of dose per unit area in risk assessments for induction of dermal sensitization has been published (Kimber et al., 2008).

A human sensitization test is used to confirm the lack of sensitization at an exposure level which is identified as a likely NOEL from all available data, including quantitative structure activity relationships. The test most typically conducted is the human repeat insult patch test (McNamee et al., 2008). A human repeat insult patch test that is undertaken to confirm the lack of sensitization is referred to as a CNIH. This test exaggerates exposure from normal use of fragrance ingredients in consumer products. Such tests must meet current ethical and methodological criteria and must be the remit of a properly constituted, independent, and transparent, ethical review committee (institutional review board). With the implementation of the QRA1 approach (Api et al., 2008), RIFM recommended the use of the RIFM standard CNIH protocol for the generation of confirmatory human data for use in QRA. Details of this standard protocol have been previously described (Politano and Api, 2008).

Diagnostic patch test data from dermatology clinics are not used in the determination of the NESIL. This is because these data are a measure of elicitation of allergic contact dermatitis, not induction of dermal sensitization. There are insufficient data to discern any quantitative relationship between induction and elicitation. Diagnostic patch test data can be useful to help determine the need for additional data. An expert group on skin sensitization concluded that it would not be appropriate to define elicitation thresholds as a function of skin sensitizing potency (Ezendam et al., 2012). For example, these data may indicate where current exposures to a fragrance material may be a source of clinically relevant allergic contact dermatitis. The absence of positive diagnostic patch test reactions following testing in dermatology clinics may support current exposure levels (use concentrations) for that fragrance material.

A detailed guide on how the NESIL is used as a benchmark in QRA to derive maximum acceptable concentrations for dermal exposure in different product categories has previously been published (Api et al., 2020). Briefly, the process entails application of sensitization assessment factors (SAFs) to the NESIL to account for uncertainties. The SAFs account for inter-individual variability, product composition, frequency/duration of use, skin condition to determined acceptable exposure levels per product category in which a fragrance ingredient may be used. In QRA2, we adjust the maximum acceptable concentrations by taking aggregate exposure into account.

Briefly, several criteria can assist in determining the NESIL. Using a WoE approach, all the available data for a chemical are taken into consideration. Historical animal and human (*in vivo*) data, quantitative structure activity relationships (QSAR) or *in silico* models, *in vitro* models

(including *in chemico* models) and read-across data obtained on structurally and/or mechanistically related chemicals can be applied in the derivation, and uncertainty in the underlying data are considered when deriving a human NESIL.

2.4. Step 4. Testing

When the existing data on the material under investigation is insufficient to conclude the safety assessment, generation of additional data is required. *In vitro* testing can be used to determine hazards. If the material is determined to be a skin sensitizer, a CNIH is required to set the NESIL. In circumstances where CNIH is considered essential for an assessment, a cautious approach is mandatory for the selection of the dose used for testing to minimize the likelihood of sensitizing the exposed study volunteers. All existing data on the target and structurally related analogs must be considered when selecting an appropriate dose for CNIH.

Read-across chemicals may also be important for building the overall WoE to support conclusions made, even when sufficient data is available on a particular fragrance ingredient. In cases where testing cannot be avoided, read-across analogs with insufficient data may be tested. These analogs are prioritized for testing based on the number of tests required for data-gap-filling and the number of materials or clusters that can be cleared in safety evaluation using the read-across.

The steps described above are summarized in a flowchart in Fig. 1.

All data and conclusions are reviewed by the Expert Panel for Fragrance Safety, comprising internationally known academic scientists, including dermatologists, pathologists, toxicologists, and environmental scientists (http://fragrancesafetypanel.org/), for approval before publication.

3. Case studies – WoE approach for skin sensitization analysis and NESIL derivation

The robustness of all available data on a fragrance ingredient is evaluated to establish the WoE for hazard identification, potency, and derivation of the NESIL. Greater weight is placed on studies conducted according to established OECD guidelines and CNIHs conducted according to the RIFM protocol. Structural analysis is done based on *in silico* predictions from software such as OECD toolbox, TIMES-SS, and Toxtree, as well as expert judgement by the Expert Panel for Fragrance Safety. Data from all *in vitro* assays on a chemical are also considered, but those conducted according to OECD test guidelines 442C, 442D, and 442E carry more weight. All available historical animal experiments, including those conducted in guinea pigs (Freund's complete adjuvant test (FCAT), open epicutaneous test (OET), closed epicutaneous test (CET), Draize, guinea pig maximization test (GPMT), Buehler) and mice (mouse ear swelling test and LLNA) are considered for hazard identification for a given fragrance ingredient. However, those with OECD test guidelines, i.e., the GPMT, Buehler, and LLNA, provide more WoE for safety assessment. RIFM classifies chemicals in potency categories according to the ECETOC Technical Report 87 (ECETOC, 2003) for animal studies conducted as described in their respective OECD test guidelines. Potency classification from guinea pig studies is not definitive and only

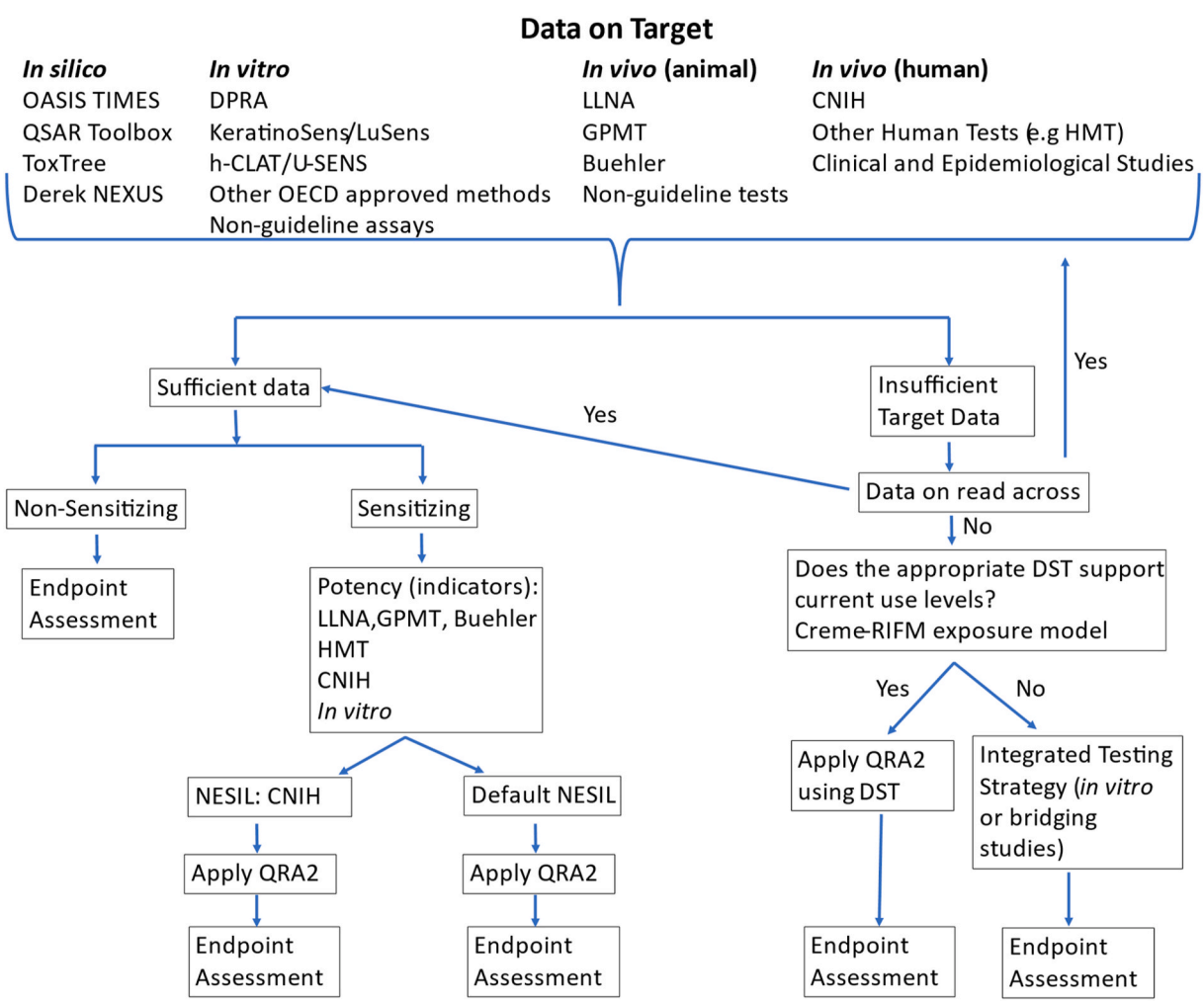

Fig. 1. Flowchart of steps involved in the derivation of a NESIL for application of QRA2.

provides a range, whereas the LLNA provides the EC3 as a specific quantitative potency value. The LLNA EC3 has been shown to correlate well with the human NOEL (Api et al., 2015); therefore, LLNA EC3 dose may be selected for testing in CNIH to confirm a NESIL. Clinical patch tests and historical HMTs are primarily used for hazard identification. While both HMTs and CNIH provide benchmarks for NOELs, only CNIH conducted according to the RIFM protocol are considered for confirming a NOEL and setting the NESIL for QRA.

Below are some case studies illustrating how the WoE approach is applied to derive a NESIL. Summarized in Table 1 are case studies of the WoE approach, while Table 2 summarizes some testing strategies and considerations made when deriving a NESIL.

4. Analysis based on target data

2-Isobutyl-4-methyltetrahydro-2H-pyran-4-ol (CAS # 63500-71-0) and 2-propanol, 1,1′,1′,1′-(1,2-ethanediyldinitrilo)tetrakis- (CAS # 102-60-3) were both determined to be non-sensitizing based on WoE from historical animal data conducted according to OECD guidelines, and lack of skin sensitization reactions in the available human studies. 2-Isobutyl-4-methyltetrahydro-2H-pyran-4-ol also has additional *in vitro* data to support that it is a non-sensitizerl based on the 2 out of 3 *in vitro* defined approach. Additionally, both chemicals were predicted *in silico* to be non-reactive to skin proteins directly (Toxtree 3.1.0; OECD Toolbox v 4.2), confirming the *in vivo* and *in vitro* analysis.

3,3,5,5-Tetramethyl-4-ethoxyvinylcyclohexanone (CAS # 36306-87-3) was determined to be a non-sensitizer based on the 2 out of 3 *in vitro* defined approach. There were no direct protein binding alerts predicted *in silico* (Toxtree 3.1.0; OECD Toolbox v 4.2), but a radical reaction alert was predicted with the autoxidation simulator in the OECD Toolbox. A negative HMT and OET are also available on this material. According to the RIFM framework, the negative HMT and OET alone would not be sufficient to determine hazard but provide WoE to support the non-sensitizer evaluation from the *in vitro* studies.

Citral (5392-40-5) was found to be a sensitizer based on 2 of 3 *in vitro* studies, animal tests, HMT, and CNIH. Given that the LLNA EC3 correlates well with the human NOEL, the mean EC3 dose of 1414 μg/cm^2 was selected and was confirmed to be negative in the CNIH. The NESIL for citral was set at 1400 μg/cm^2.

In rare circumstances (such as low volume of use or low exposure levels), the EC3 value (or weighted mean when more than one study exists) can be used to define a default NESIL based on potency considerations (Gerberick et al., 2001). This approach requires expert judgment. α-Butylcinnamaldehyde (CAS # 7492-44-6) annual volume of use was reported to be between 1 and 100 kg according to a 2015 volume of use survey of the fragrance industry (IFRA, 2015) and its 95th percentile total chronic systemic exposure (dermal, oral, and inhalation) was <0.00001 μg/kg/day (Creme RIFM Aggregate Exposure Model version 3.0). This chemical was found to be sensitizing in 2 LLNAs with a weighted mean EC3 value of 11.08% (2775 μg/cm^2). A CNIH test was not conducted, and a default NESIL of 1000 μg/cm^2 was defined for this material, based on the potency consideration by Gerberick et al., (2001). The default NESIL was used instead of the reactive DST because there were two historical LLNAs on this material. There is good correlation between LLNA EC3 and human potency (Gerberick et al., 2001, 2004; Griem et al., 2003; Schneider and Akkan, 2004).

Analysis based on read-across.

Data available on propyl alcohol (CAS # 71-23-8) were not sufficient to determine hazard. Even though this material was negative in a GPMT and Buehler test, the data were deemed insufficient due to the limited number of test animals in the GPMT and the unreported number of animals in the Buehler study. The read-across analog butyl alcohol (CAS # 71-36-3) had sufficient data to confirm that propyl alcohol is a non-sensitizer. *cis*-3-Nonen-1-ol (CAS # 10340-23-5) had no historical data but was determined to be a non-sensitizer based on read-across to *cis*-3-hexenol (CAS # 928-96-1), which had sufficient data.

2,6-Dimethyl-5-heptenal (CAS # 106-72-9) was determined to be a sensitizer with an LLNA EC3 of 8500 μg/cm^2, but due to lack of a CNIH, the NESIL was set at 10000 μg/cm^2 based on read-across to 2,6,10-trimethylundeca-5,9-dienal (CAS # 54082-68-7). No skin sensitization studies were available for 3,7-dimethyl-3,6-octadienal (CAS # 55722-59-3), but this chemical was determined to be a sensitizer with a NESIL of 7000 μg/cm^2 based on read-across to citronellal (CAS # 106-23-0), which had sufficient data.

5. DST exposure-based waving

2-Decanone (CAS # 693-54-9) had no skin sensitization data available, while 4-hexen-1-ol, 5-methyl-2-(1-methylethenyl)- (CAS # 58461-27-1) and (Z)-2-Penten-1-ol (CAS # 1576-95-0) had insufficient data to determine hazard. These materials were determined to be non-reactive with *in silico* structural analysis and assessment by the Expert Panel for Fragrance Safety, but no appropriate read-across analog with sufficient data was found. The exposure to these materials falls under the non-reactive DST of 900 μg/cm^2, so they were deemed safe under the current declared levels of use.

2-Furanmethanethiol formate (CAS # 59020-90-5) and furfuryl thioacetate (CAS # 13678-68-7) had no skin sensitization studies available, but they were determined to be reactive based on structural analysis. 1-Octen-3-ol (CAS # 3391-86-4) was positive in 2 out of 3 *in vitro* assays, while p-tolyl acetate (CAS # 140-39-6) was found to be sensitizing in the HMT. Since no appropriate read-across was found for these materials, and their exposure was below the reactive DST of 64 μg/cm^2, they were concluded to be safe under the current declared levels of use.

6. Testing strategies

The primary alcohol cluster (Table 2) was found to be sensitizing based on positive guinea pig tests on several chemicals in the cluster and a positive LLNA. However, none of the materials in the cluster had sufficient data to set a NESIL, and no appropriate read-across analogs were available. Two Chemicals in the cluster (heptyl alcohol and 1-decanol) were selected for testing in the DPRA to determine reactivity in order to choose a representative chemical for further testing in the CNIH. Both heptyl alcohol and 1-decanol had minimal reactivity in the DPRA with mean cysteine and lysine depletion of 0.80% and 0.38%, respectively. The DPRA results provided supporting evidence, that the chain length of the alcohol did not impact the reactivity. Heptyl alcohol was selected for further testing in the CNIH, and the NESIL of 9400 μg/cm^2 was selected. All the other 9 chemicals in the cluster read-across to heptyl alcohol. Only 1 chemical was tested to clear 9 structurally related chemicals by read-across.

A similar case to the primary alcohol cluster is the cinnamyl ester cluster (Table 2). In this cluster, cinnamyl acetate was initially presumed to be a sensitizer because a structurally similar alcohol, cinnamyl alcohol (CAS # 104-54-1), is a sensitizer. However, while *in vitro* data suggested that cinnamyl alcohol is reactive, cinnamyl acetate is not. Therefore, based on structural evaluation, cinnamyl esters were separated from the alcohols and then cinnamyl acetate was selected as read-across for its cluster. Cinnamyl acetate was determined to be a non-sensitizer based on 2 out of 3 *in vitro* studies. This conclusion was supported by a CNIH study conducted at 3424 μg/cm^2 according to RIFM's standard protocol. Cinnamyl acetate was selected as the read-across for all the chemicals in the cluster, and no further testing was required for the cluster.

6-Methyl-3,5-heptadien-2-one (CAS # 1604-28-0) was predicted to be a sensitizer based on positive predictions observed in the *in vitro* KeratinoSens and h-CLAT and supporting evidence from *in silico* protein binding alerts. A CNIH was conducted at 1299 μg/cm^2 on 6-methyl-3,5-heptadien-2-one, based on estimation from the negative LLNA dose at 5% (1250 μg/cm^2), but sensitization reactions were observed in 3/110 subjects. The CNIH was repeated at a lower dose of 118 μg/cm^2

Table 1
Summary of case study data.

CAS	Name	NESIL (μg/cm^2)	LLNA(%, μg/cm^2)	CNIH (μg/cm^2)	HMT (μg/cm^2)	GPMT and Buehler	Other animal studies	DPRA	[a]KS	[b]HC	Target protein binding alerts (TB 4.2)	Autoxidation protein binding alerts (TB 4.2)	Metabolite protein binding alerts (TB 4.2)	Parent Predicted SkinSens (TIMES 2.28.1)	Metabolite Predicted SkinSens (TIMES 2.28.1)	Toxtree 3.1.0
Non-Sensitizers Based on Sufficient Target Data																
63500-71-0	2-Isobutyl-4-methyltetrahydro-2H-pyran-4-ol	NS	Neg up to 30%, 7500	2 studies: Neg @ 4408 (n = 110, 1:3 EtOH: DEP); Neg @ 5000 (n = 50, petrolatum)		Neg @ 100% (n = 20 test, 20 control) Neg @ 99% (n = 20 test, 10 control)		Neg	Neg	Pos	No alert found	No alert found	No alert found	Non-sensitizer	NA	No alert
102-60-3	2-Propanol, 1,1′,1′,1’-(1,2-ethanediyldinitrilo) tetrakis-	NS				Neg at 25% (n = 20)					No alert found	No alert found	No alert found	Non-sensitizer	Non-sensitizer	No alert
Non-Sensitizer Based on 2 Out of 3 Negative *In Vitro* Studies on Target																
36306-87-3	3,3,5,5-Tetramethyl-4-ethoxyvinylcyclohexanone	NS			Neg @ 6900 (n = 25)		Neg OET	Neg	Neg		No alert found	Radical reactions	No alert found	Non-sensitizer	Non-sensitizer	No alert
Sensitizers Based on Sufficient Target Data																
1604-28-0	6-Methyl-3,5-heptadien-2-one	110	Neg up to 5%, 1250	Multiple studies: Neg @ 118 (n = 105, 1:3 EtOH: DEP); Pos @ 1299 (n = 3/110, 1:3 EtOH: DEP); Pos @ 2710 (n = 6/48, DEP)			Pos OET; Pos FCAT	Pos	Pos	Pos	Michael Addition	Michael Addition; Schiff base formation	Michael Addition	Strong sensitizer	Strong sensitizer	Michael Acceptor
5392-40-5	Citral	1400	Multiple studies: Mean EC3 = 5.6%, 1414	Multiple studies: Neg @ 1400 (n = 101, 1:3 EtOH: DEP); Pos @ 3876 (n = 8, SDA39C)	Multiple studies: Lowest Pos @ 2760	Pos Pos	Pos OET; Pos CET	Pos	Pos	Pos	Schiff base formation	Schiff base formation	Schiff base formation	Strong sensitizer	Non-sensitizer	Schiff base formation
Sensitizer Based on Default NESIL																
7492-44-6	alpha-Butylcinnamaldehyde	1000	EC3 = 9.1%, 2275		Neg @ 5520						Michael Addition	Michael Addition	Michael Addition	Weak sensitizer	Non-sensitizer	Michael Acceptor
Read-across																
Non-Sensitizers																
71-23-8	Propyl alcohol	NS; RA to 71–3				Neg @ 100% (15 test,					No alert found	No alert found	Schiff base formation	Non-sensitizer	Non-sensitizer	No alert found

(*continued on next page*)

Table 1 (*continued*)

CAS	Name	NESIL (μg/cm^2)	LLNA(%, μg/cm^2)	CNIH (μg/cm^2)	HMT (μg/cm^2)	GPMT and Buehler	Other animal studies	DPRA	[a]KS	[b]HC	Target protein binding alerts (TB 4.2)	Autoxidation protein binding alerts (TB 4.2)	Metabolite protein binding alerts (TB 4.2)	Parent Predicted SkinSens (TIMES 2.28.1)	Metabolite Predicted SkinSens (TIMES 2.28.1)	Toxtree 3.1.0
						6 control) Neg @ 10360% (no n)										
71-36-3	Butyl alcohol	NS; RA for 71-23-8	Neg up to 20%, 5000		Neg @ 2760			Neg	Neg	Neg	No alert found	No alert found	Schiff base formation	Non-sensitizer	Non-sensitizer	No alert found
10340-23-5	3-nonen-1-ol, (z)-;(z)-non-3-en-1-ol;(3z)-non-3-en-1-ol; 3-nonen-1-ol, (3z)-;cis-3-nonen-1-ol	NS; RA to 928-96-1									No alert found	No alert found	Schiff base formation	Non-sensitizer	Non-sensitizer	No alert found
928-96-1	cis-3-Hexenol	NS; RA for 10340-23-5	Neg up to 100%, 25000	Neg @ 1.25% (n < 50)	Neg @ 2760		Neg OET				No alert found	No alert found	Schiff base formation	Non-sensitizer	Non-sensitizer	No alert found
Sensitizers																
55722-59-3	3,7-Dimethyl-3,6-octadienal	7000; RA to 106-23-0									Schiff base formation	Schiff base formation Radical reactions	Schiff base formation	Strong sensitizer	Strong sensitizer	Schiff base formation
106-23-0	Citronellal	7000; RA for 55722-59-3	Neg up to 30%, 7500	Neg @ 7086 (n = 110, 1:3 EtOH: DEP)	Neg @ 2760	Pos (n = 8 test) Pos @10% (n = 10 test)	Pos OET; Neg Draize; Pos FCAT; Pos CET	Neg	Pos	Pos	Schiff base formation	Schiff base formation Radical reactions	Schiff base formation	Weak sensitizer	Weak sensitizer	Schiff base formation
106-72-9	2,6-Dimethyl-5-heptenal	10000; RA to 54082-68-7	EC3 = 34%, 8500		Neg @ 2760						Schiff base formation	Schiff base formation Radical reactions	Schiff base formation	Weak sensitizer	Weak sensitizer	Schiff base formation
54082-68-7	2,6,10-Trimethylundeca-5,9-dienal	10000; RA for 106-72-9	EC3 = 42.3%, 10575	Neg @ 10039, (n = 108, 1:3 EtOH:DEP)							No alert found	Radical reactions Schiff base formation	No alert found	Non-sensitizer	Weak sensitizer	Schiff base formation
DST																
Non-Reactive DST																
693-54-9	2-Decanone	900									No alert found	No alert found	No alert found	Non-sensitizer	Non-sensitizer	No alert found
58461-27-1	4-Hexen-1-ol, 5-methyl-2-(1-methylethenyl)-	900			Neg @ 3450						No alert found	Radical reactions	Schiff base formation	Non-sensitizer	Weak sensitizer	No alert found
1576-95-0	(Z)-2-Penten-1-ol	900		Neg @ 193.8 (n = 38, SDA 39c)							No alert found	Michael Addition	Michael Addition	Non-sensitizer	Strong sensitizer	No alert found
Reactive DST																
59020-90-5	2-Furanmethanethiol formate	64									SN2	SN2	SN2	Non-sensitizer	Strong sensitizer	SN2; Acyl Transfer agent
	Furfuryl thioacetate	64									SN2	SN2	SN2			

(*continued on next page*)

Table 1 (*continued*)

CAS	Name	NESIL (μg/cm^2)	LLNA(%, μg/cm^2)	CNIH (μg/cm^2)	HMT (μg/cm^2)	GPMT and Buehler	Other animal studies	DPRA	[a]KS	[b]HC	Target protein binding alerts (TB 4.2)	Autoxidation protein binding alerts (TB 4.2)	Metabolite protein binding alerts (TB 4.2)	Parent Predicted SkinSens (TIMES 2.28.1)	Metabolite Predicted SkinSens (TIMES 2.28.1)	Toxtree 3.1.0
13678-68-7														Strong sensitizer	Strong sensitizer	SN2; Acyl Transfer agent
3391-86-4	1-Octen-3-ol	64		Neg @ 388 (n = 40, ethanol)	Neg @ 6900			Pos	Pos		No alert found	No alert found	Michael Addition	Non-sensitizer	Non-sensitizer	No alert found
140-39-6	p-Tolyl acetate	64			Neg @ 6900		Neg OET [2]				Acylation	Acylation	Acylation Michael Addition	Strong sensitizer	Weak sensitizer	Acyl Transfer agent

Abbreviations: NESIL = No expected sensitization induction level; LLNA = Local lymph node assay; CNIH = Confirmation of no induction in humans; HMT = Human maximization test; DPRA = Direct protein reactivity assay; KS = KeratinoSens; HC = human Cell Line Activation Test (h-CLAT); Neg = Negative; Pos = Positive; RA = Read-across; OET = Open epicutaneous test; FCAT = Freund's complete adjuvant test.

(approximately 10 times lower than the positive dose) in 105 subjects, resulting in no reactions indicative of sensitization. The NESIL was therefore set at 110 μg/cm^2 based on this CNIH conducted according to the RIFM protocol. This case emphasizes the importance of confirmatory human studies to set the NESIL and using predictive tools and read-across to determine whether LLNA is truly predictive of a NOEL in humans.

Some exceptions have been noted where the LLNA EC3 does not correlate well with the human NOEL. Benzaldehyde (CAS # 100-52-7) was not found to be sensitizing when tested up to 25% (6250 μg/cm^2) in the LLNA. However, in addition to *in vitro* tests and historical animal tests demonstrating that it is a sensitizer, benzaldehyde was found to be sensitizing in a CNIH tested at 5905 μg/cm^2 in 6 out of 88 subjects. The NESIL for benzaldehyde was therefore set at 590 μg/cm^2 after confirmation in a CNIH study. We hypothesize that this disparity may be accounted for by the differences in test chemical application methods. In the LLNA, the test chemical is placed by open application to the ear while CNIH studies use closed patches; therefore, the test chemical may volatize in the LLNA but not in the CNIH. A similar example is hexen-2-al (CAS # 6728-26-3) which had an average EC3 of 4.05% (1012 μg/cm^2) from 2 separate LLNAs, but was found to be sensitizing in a CNIH at 236 μg/cm^2. A NESIL of 18 μg/cm^2 was determined, which is much lower than the EC3. In contrast to benzaldehyde and hexen-2-al, hexyl salicylate was an extreme sensitizer in the LLNA with an EC3 of 0.18% (45 μg/cm^2) but was not sensitizing in a CNIH study when tested at 30% (35,433 μg/cm^2). This finding is true for other salicylates in the RIFM inventory. These case studies emphasize the importance of the weight approach, where all the available data must be considered to determine skin sensitization hazard and potency for a given chemical. These examples also showcase why it is critical to perform confirmatory testing in humans to ensure the safety of fragranced products.

7. Discussion

The NESIL is the point of departure (PoD) for the dermal sensitization QRA. Deriving this PoD is an iterative process that involves assessing the quality of all available data (historical human and animal [*in vivo*], *in vitro*, and *in silico*) on a fragrance ingredient, determining a read-across, exposure waving based on the DST, and/or integrated testing strategies to determine a WoE NESIL. All fragrance ingredients in RIFM's inventory are evaluated on a five-year rotating basis to ensure that previously made conclusions still hold based on newly available exposure data. Revised safety assessments are published if new relevant data become available. We have outlined how RIFM currently evaluates fragrance ingredients through a stepwise process to derive a NESIL for QRA. This dynamic may change as we actively seek to incorporate NAMs and tools into our safety assessment process.

The RIFM Database and other publicly available channels, such as the European Chemicals Agency (ECHA), provide a wealth of historical data upon which the safety assessment of fragrance ingredients is built. Exposure data are primarily obtained from the Creme-RIFM Aggregate Exposure Model (Comiskey et al., 2017), which incorporates survey data on the use of fragrance ingredients from the fragrance industry. When there are limited or no skin sensitization data available on an ingredient and the gap cannot be bridged by read-across or the DST approach cannot be applied, integrated testing strategies (ITS) can be applied to determine hazard. There has been significant progress in incorporating new *in vitro* data into the risk assessment process demonstrated by OECD test guideline 497 (OECD, 2021). However, challenges still exist in determining human potency using *in vitro* methods in order to confirm a NESIL to be used for QRA purposes. Some *in vitro* assays such as SENS-IS (Cottrez et al., 2015) provide valuable insight into the potency of a sensitizer and could potentially be used to set a default NESIL, but using NAMs to determine potency is an active area of research, and more work is still needed. NAMs based on the integration of *in silico* and *in vitro* data are under development, but will be useful for deriving a NESIL without

Table 2
Summary of data illustrating testing strategies.

CAS	Name	NESIL (μg/cm^2)	LLNA (%, μg/cm^2)	CNIH (μg/cm^2)	HMT	GPMT and Buehler	Other animal studies	DPRA	KS	HC	Target protein binding alerts (TB 4.2)	Autoxidation protein binding alerts (TB 4.2)	Metabolite protein binding alerts (TB 4.2)	Parent Predicted SkinSens (TIMES 2.28.1)	Metabolite Predicted SkinSens (TIMES 2.28.1)	Toxtree 3.1.0
Testing Primary alcohols *in vitro* (DPRA) to determine the reactivity of materials to test in CNIH to clear																
111-70-6	Heptyl alcohol	Data; NESIL = 9400	EC3 = 38%, 9500	Neg @ 9400 (n = 110, 1:3 EtOH: DEP)	Neg @ 690	Neg (n = 20, 100% topical induction and 10% challenge)	Neg OET [2], Neg Draize	Neg (Mean dep 0.80%)			No alert found	No alert found	Schiff base formation	Non-sensitizer	Non-sensitizer	No alert found
111-27-3	Hexyl alcohol	NESIL = 9400; RA to 111-70-6			Neg @ 690		Neg OET [2], Neg Draize				No alert found	No alert found	Schiff base formation	Non-sensitizer	Non-sensitizer	No alert found
111-87-5	1-Octanol	NESIL = 9400; RA to 111-70-6			Neg @ 1380		Pos and Neg Draize, Neg OET [2], GPS intradermal injection test; possible positive-tested as a mixture				No alert found	No alert found	Schiff base formation	Non-sensitizer	Non-sensitizer	No alert found
143-08-8	Nonyl alcohol	NESIL = 9400; RA to 111-70-6			Neg @ 1380		Pos and Neg Draize, Neg OET [2]				No alert found	No alert found	Schiff base formation	Non-sensitizer	Non-sensitizer	No alert found
112-30-1	1-Decanol	NESIL = 9400; RA to 111-70-6			Neg @ 2070	Pos @ 5% and 10% (n = 10)										
Neg (n = 10) [ECHA]	Pos [2] and Neg Draize, Neg OET [2], Neg FCAT	Neg (Mean dep 0.38%)			No alert found	No alert found	Schiff base formation	Non-sensitizer	Non-sensitizer	No alert found						
112-42-5	Undecyl alcohol	RA to 111-70-6			Neg @ 2760		Pos and Neg Draize, Neg OET				No alert found	No alert found	Schiff base formation	Non-sensitizer	Non-sensitizer	No alert found
112-53-8	Lauryl alcohol	NESIL = 9400; RA to 111-70-6			Neg @ 2760		Pos and Neg Draize, Neg OET [2]				No alert found	No alert found	Schiff base formation	Non-sensitizer	Non-sensitizer	No alert found
112-70-9	1-tridecanol; tridecan-1-ol; tridecaol	NESIL = 9400;									No alert found	No alert found	No alert found	Non-sensitizer	Non-sensitizer	No alert found

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Table 2 (*continued*)

CAS	Name	NESIL (μg/cm^2)	LLNA (%, μg/cm^2)	CNIH (μg/cm^2)	HMT	GPMT and Buehler	Other animal studies	DPRA	KS	HC	Target protein binding alerts (TB 4.2)	Autoxidation protein binding alerts (TB 4.2)	Metabolite protein binding alerts (TB 4.2)	Parent Predicted SkinSens (TIMES 2.28.1)	Metabolite Predicted SkinSens (TIMES 2.28.1)	Toxtree 3.1.0
		RA to 111-70-6														
Minimizing testing by testing a representative material to use as read-across for the whole cluster																
103–54–8	**Cinnamyl acetate**	**NS; Data**		**Neg @ 3424 (n = 101, 1:3 EtOH: DEP)**	**Neg @ 3450**			**Neg**		**Neg**	**SN2**	**SN2**	**SN2**	**Weak sensitizer**	**Strong sensitizer**	**Acyl Transfer agent; Michael Acceptor; SN2**
104-65-4	Cinnamyl formate	NS; RA to 103-54-8			Neg @ 2760						No alert found	No alert found	SN2	Non-sensitizer	Strong sensitizer	Acyl Transfer agent; Michael Acceptor; SN2
103-56-0	Cinnamyl propionate	NS; RA to 103-54-8			Neg @ 2760						SN2	SN2	SN2	Weak sensitizer	Strong sensitizer	Acyl Transfer agent; Michael Acceptor; SN2
103-61-7	Cinnamyl butyrate	NS; RA to 103-54-8			Neg @ 2760						SN2	SN2	SN2	Weak sensitizer	Strong sensitizer	Acyl Transfer agent; Michael Acceptor; SN2
CNIH dose selected based on LLNA dose																
1604-28-0	6-Methyl-3,5-heptadien-2-one	110	Neg up to 5%, 1250	Multiple studies: Neg @ 118 (n = 105, 1:3 EtOH: DEP); Pos @ 1299 (n = 3/110, 1:3 EtOH: DEP);			Pos @ 2710 (n = 6/48, DEP) Pos OET; Pos FCAT	Pos	Pos	Pos	Michael Addition	Michael Addition; Schiff base formation	Michael Addition	Strong sensitizer	Strong sensitizer	Michael Acceptor
Importance of the WoE approach: LLNA data do not always align with human NOEL																
100-52-7	Benzaldehyde	590	>25%, >6250	Neg @ 591 (n = 117, 1:3 EtOH: DEP); Pos @ 5905 (n = 4/104;	Pos @ 2760	Neg @ 7%	Pos Draize, Neg OET	Neg	Pos	Pos	No alert found	No alert found	No alert found	Non-sensitizer	Non-sensitizer	Schiff base formation

(*continued on next page*)

Table 2 (*continued*)

CAS	Name	NESIL (μg/cm^2)	LLNA (%, μg/cm^2)	CNIH (μg/cm^2)	HMT	GPMT and Buehler	Other animal studies	DPRA	KS	HC	Target protein binding alerts (TB 4.2)	Autoxidation protein binding alerts (TB 4.2)	Metabolite protein binding alerts (TB 4.2)	Parent Predicted SkinSens (TIMES 2.28.1)	Metabolite Predicted SkinSens (TIMES 2.28.1)	Toxtree 3.1.0
				1:3 EtOH: DEP)												
6728-26-3	Hexen-2-al	18	Multiple studies: (EC3 = 2.6, 5.5%) Average 4.05%, 1012	Multiple studies: Pos @ 236 (n = 6/25, 3:1 EtOH: DEP); Neg @ 23 (n = 106, 1:3 EtOH: DEP); Neg @ 18 (n = 109, 1:3 EtOH: DEP)	Neg @ 2760	Pos @ 3% (n = 20 test 12 control) Pos	Pos OET; Pos CET	Pos	Pos	Pos	Michael Addition	Michael Addition	Michael Addition	Strong sensitizer	Non-sensitizer	Michael Acceptor
6259-76-3	Hexyl salicylate	35000	EC3 = 0.18%, 45	Neg @ 35,433 (n = 103, 1:3 EtOH: DEP)	Neg @ 2070	Neg @ 10% (n = 10)	Pos Draize	Neg	Pos	Pos	No alert found	No alert found	No alert found	Non-sensitizer	Non-sensitizer	No alert found

Abbreviations: NESIL = No expected sensitization induction level; LLNA = Local lymph node assay; CNIH = Confirmation of no induction in humans; HMT = Human maximization test; DPRA = Direct protein reactivity assay; KS = KeratinoSens; HC = human Cell Line Activation Test (h-CLAT); Neg = Negative; Pos = Positive; OET = Open epicutaneous test; FCAT = Freund's complete adjuvant test.

animal testing (Natsch et al., 2018). In the future, RIFM will integrate these methods in the safety assessment program of new fragrance ingredients or those with limited historical data.

Derivation of a NESIL or QRA PoD for a bulk of fragrance ingredients that fall in the Natural Complex Substance (NCS) class will primarily depend on the development of NAMs. NCS, such as essential oils, comprise complex mixtures of chemicals with varying degrees of functionalities and the ability to induce skin sensitization. This complexity makes it challenging to develop NAMs specifically appropriate for NCS. Additionally, NAMs that may be useful for NESIL derivation are being developed largely based on the analysis of discrete chemical substances. Currently, RIFM primarily employs component-based analysis to assesses the sensitization potential of NCS, but future NAMs may permit NESIL derivation without animal testing for this class of fragrance ingredients.

CRediT authorship contribution statement

Isabelle Lee: Conceptualization, Data curation, Writing – original draft, Writing – review & editing. **Mihwa Na:** Conceptualization, Writing – original draft. **Maura Lavelle:** Conceptualization, Writing – original draft. **Anne Marie Api:** Conceptualization, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Api, A.M., 2002. Sensitization methodology and primary prevention of the research institute for fragrance materials. Dermatology 205 (1), 84–87. https://doi.org/10.1159/000063153.

Api, A.M., Basketter, D., Bridges, J., Cadby, P., Ellis, G., Gilmour, N., White, I.R., 2020. Updating exposure assessment for skin sensitization quantitative risk assessment for fragrance materials. Regul. Toxicol. Pharmacol. 118, 104805. https://doi.org/10.1016/j.yrtph.2020.104805.

Api, A.M., Basketter, D., Lalko, J., 2015. Correlation between experimental human and murine skin sensitization induction thresholds. Cutan. Ocul. Toxicol. 34 (4), 298–302. https://doi.org/10.3109/15569527.2014.979425.

Api, A.M., Basketter, D.A., Cadby, P.A., Cano, M.F., Ellis, G., Gerberick, G.F., Safford, R., 2008. Dermal sensitization quantitative risk assessment (QRA) for fragrance ingredients. Regul. Toxicol. Pharmacol. 52 (1), 3–23. https://doi.org/10.1016/j.yrtph.2007.10.008.

Basketter, D., Beken, S., Bender, H., Bridges, J., Casati, S., Corvaro, M., Rossi, L.H., 2020. 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. 117, 104767. https://doi.org/10.1016/j.yrtph.2020.104767.

Basketter, D.A., Lea, L.J., Dickens, A., Briggs, D., Pate, I., Dearman, R.J., Kimber, I., 1999. A comparison of statistical approaches to the derivation of EC3 values from local lymph node assay dose responses. J. Appl. Toxicol. 19 (4), 261–266. https://doi.org/10.1002/(sici)1099-1263(199907/08)19:4<261::aid-jat572>3.0.co;2-5.

Bauch, C., Kolle, S.N., Ramirez, T., Eltze, T., Fabian, E., Mehling, A., Landsiedel, R., 2012. Putting the parts together: combining in vitro methods to test for skin sensitizing potentials. Regul. Toxicol. Pharmacol. 63 (3), 489–504. https://doi.org/10.1016/j.yrtph.2012.05.013.

Comiskey, D., Api, A.M., Barrett, C., Ellis, G., McNamara, C., O'Mahony, C., Tozer, S., 2017. Integrating habits and practices data for soaps, cosmetics and air care products into an existing aggregate exposure model. Regul. Toxicol. Pharmacol. 88, 144–156. https://doi.org/10.1016/j.yrtph.2017.05.017.

Cottrez, F., Boitel, E., Auriault, C., Aeby, P., Groux, H., 2015. Genes specifically modulated in sensitized skins allow the detection of sensitizers in a reconstructed human skin model. Development of the SENS-IS assay. Toxicol. Vitro 29 (4), 787–802. https://doi.org/10.1016/j.tiv.2015.02.012.

Date, M.S., O'Brien, D., Botelho, D.J., Schultz, T.W., Liebler, D.C., Penning, T.M., Salvito, D.T., 2020. Clustering a chemical inventory for safety assessment of fragrance ingredients: identifying read-across analogs to address data gaps. Chem. Res. Toxicol. 33 (7), 1709–1718. https://doi.org/10.1021/acs.chemrestox.9b00518.

ECETOC, 2003. Contact Sensitisation: Classification According to Potency. Technical Report No. 87.

Ezendam, J., Vermeulen, J.P., de Klerk, A., de Jong, W.H., van Loveren, H., 2012. A quantitative approach to assess the potency of skin sensitizers in the elicitation phase. Toxicology 299 (1), 20–24. https://doi.org/10.1016/j.tox.2012.05.002.

Gerberick, G.F., Robinson, M.K., Ryan, C.A., Dearman, R.J., Kimber, I., Basketter, D.A., Marks, J.G., 2001. Contact allergenic potency: correlation of human and local lymph node assay data. Am. J. Contact Dermatitis 12 (3), 156–161. https://doi.org/10.1053/ajcd.2001.23926.

Gerberick, G.F., Vassallo, J.D., Bailey, R.E., Chaney, J.G., Morrall, S.W., Lepoittevin, J.P., 2004. Development of a peptide reactivity assay for screening contact allergens. Toxicol. Sci. 81 (2), 332–343. https://doi.org/10.1093/toxsci/kfh213.

Griem, P., Goebel, C., Scheffler, H., 2003. Proposal for a risk assessment methodology for skin sensitization based on sensitization potency data. Regul. Toxicol. Pharmacol. 38 (3), 269–290. https://doi.org/10.1016/j.yrtph.2003.07.001.

IFRA (International Fragrance Association), 2015. Volume of Use Survey, February 2015.

Kimber, I., Dearman, R.J., Basketter, D.A., Ryan, C.A., Gerberick, G.F., McNamee, P.M., Api, A.M., 2008. Dose metrics in the acquisition of skin sensitization: thresholds and importance of dose per unit area. Regul. Toxicol. Pharmacol. 52 (1), 39–45. https://doi.org/10.1016/j.yrtph.2008.01.010.

Kligman, A.M., 1966. The identification of contact allergens by human assay: III. The maximization test: a procedure for screening and rating contact sensitizers. J. Invest. Dermatol. 47 (5), 393–409. https://doi.org/10.1038/jid.1966.160.

Kolle, S.N., Natsch, A., Gerberick, G.F., Landsiedel, R., 2019. A review of substances found positive in 1 of 3 in vitro tests for skin sensitization. Regul. Toxicol. Pharmacol. 106, 352–368. https://doi.org/10.1016/j.yrtph.2019.05.016.

Magnusson, B., Kligman, A.M., 1969. The identification of contact allergens by animal assay. The Guinea pig maximization test. J. Invest. Dermatol. 52 (3), 268–276. https://doi.org/10.1038/jid.1969.42.

McNamee, P.M., Api, A.M., Basketter, D.A., Frank Gerberick, G., Gilpin, D.A., Hall, B.M., Robinson, M.K., 2008. A review of critical factors in the conduct and interpretation of the human repeat insult patch test. Regul. Toxicol. Pharmacol. 52 (1), 24–34. https://doi.org/10.1016/j.yrtph.2007.10.019.

Na, M., Ritacco, G., O'Brien, D., Lavelle, M., Api, A.M., Basketter, D., 2020. Fragrance skin sensitization evaluation and human testing: 30-year experience. Dermatitis. https://doi.org/10.1097/der.0000000000000684.

Natsch, A., Emter, R., Haupt, T., Ellis, G., 2018. Deriving a No expected sensitization induction level for fragrance ingredients without animal testing: an integrated approach applied to specific case studies. Toxicol. Sci. 165 (1), 170–185, 10.1093/toxsci/kfy135.%J.Toxicological Sciences.

Nishijo, T., Miyazawa, M., Saito, K., Otsubo, Y., Mizumachi, H., Sakaguchi, H., 2019. The dermal sensitization threshold (DST) approach for mixtures evaluated as negative in in vitro test methods; mixture DST. J. Toxicol. Sci. 44 (1), 23–34. https://doi.org/10.2131/jts.44.23.

OECD, 1992. Test No. 406: Skin Sensitisation. Retrieved from. www.oecd.org.

OECD, 2010. Test No. 429: Skin Sensitisation. Retrieved from. www.oecd.org.

OECD, 2014. The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins. Retrieved from. www.oecd.org.

OECD, 2017. 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. Retrieved from. www.oecd.org.

OECD, 2018a. Test No. 442D. In: *Vitro* Skin Sensitisation. Retrieved from. www.OECD.org.

OECD, 2018b. Test No. 442E. In: *Vitro* Skin Sensitisation. Retrieved from. www.oecd.org.

OECD, 2019a. Draft OECD Guideline Defined Approaches for Skin Sensitisation. Retrieved from. www.oecd.org.

OECD, 2019b. Draft OECD Guideline Defined Approaches for Skin Sensitisation. Retrieved from. www.oecd.org.

OECD, 2020. Test No. 442C. In: *Chemico* Skin Sensitisation. Retrieved from. www.oecd.org.

OECD, 2021. *Guideline No. 497: Defined Approaches on Skin Sensitisation*, OECD Guidelines for the Testing of Chemicals, Section 4. OECD Publishing, Paris. https://doi.org/10.1787/b92879a4-en.

Politano, V.T., Api, A.M., 2008. The Research Institute for Fragrance Materials' human repeated insult patch test protocol. Regul. Toxicol. Pharmacol. 52 (1), 35–38. https://doi.org/10.1016/j.yrtph.2007.11.004.

Rees, J.L., Friedmann, P.S., Matthews, J.N., 1990. The influence of area of application on sensitization by dinitrochlorobenzene. Br. J. Dermatol. 122 (1), 29–31. https://doi.org/10.1111/j.1365-2133.1990.tb08236.x.

Roberts, D.W., Api, A.M., Safford, R.J., Lalko, J.F., 2015. Principles for identification of high potency category chemicals for which the dermal sensitisation threshold (DST) approach should not be applied. Regul. Toxicol. Pharmacol. 72 (3), 683–693. https://doi.org/10.1016/j.yrtph.2015.03.001.

Safford, R.J., 2008. The Dermal Sensitisation Threshold- a TTC approach for allergic contact dermatitis. Regul. Toxicol. Pharmacol. 51 (2), 195–200. https://doi.org/10.1016/j.yrtph.2008.02.010.

Safford, R.J., Api, A.M., Roberts, D.W., Lalko, J.F., 2015. Extension of the Dermal Sensitisation Threshold (DST) approach to incorporate chemicals classified as reactive. Regul. Toxicol. Pharmacol. 72 (3), 694–701. https://doi.org/10.1016/j.yrtph.2015.04.020.

Safford, R.J., Aptula, A.O., Gilmour, N., 2011. Refinement of the Dermal Sensitisation Threshold (DST) approach using a larger dataset and incorporating mechanistic chemistry domains. Regul. Toxicol. Pharmacol. 60 (2), 218–224. https://doi.org/10.1016/j.yrtph.2011.03.009.

Schneider, K., Akkan, Z., 2004. Quantitative relationship between the local lymph node assay and human skin sensitization assays. Regul. Toxicol. Pharmacol. 39 (3), 245–255. https://doi.org/10.1016/j.yrtph.2004.02.002.

Schultz, T.W., Amcoff, P., Berggren, E., Gautier, F., Klaric, M., Knight, D.J., Cronin, M.T., 2015. A strategy for structuring and reporting a read-across prediction of toxicity. Regul. Toxicol. Pharmacol. 72 (3), 586–601. https://doi.org/10.1016/j.yrtph.2015.05.016.

Schultz, T.W., Richarz, A.-N., Cronin, M.T.D., 2019. Assessing uncertainty in read-across: questions to evaluate toxicity predictions based on knowledge gained from case studies. Comput. Toxicol. 9, 1–11. https://doi.org/10.1016/j.comtox.2018.10.003.

Upadhye, M. R., & Maibach, H. I. (1992). Influence of area of application of allergen on sensitization in contact dermatitis. 27(5), 281-286. doi:https://doi.org/10.1111/j.1600-0536.1992.tb03280.x.

Urbisch, D., Mehling, A., Guth, K., Ramirez, T., Honarvar, N., Kolle, S., Sakaguchi, H., 2015. Assessing skin sensitization hazard in mice and men using non-animal test methods. Regul. Toxicol. Pharmacol. 71 (2), 337–351. https://doi.org/10.1016/j.yrtph.2014.12.008.

White, S.I., Friedmann, P.S., Moss, C., Simpson, J.M., 1986. The effect of altering area of application and dose per unit area on sensitization by DNCB. Br. J. Dermatol. 115 (6), 663–668. https://doi.org/10.1111/j.1365-2133.1986.tb06646.x.