

Dermal Sensitization Quantitative Risk Assessment (QRA) For Fragrance Ingredients

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QRA Expert Group

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Executive Summary

Some of the chemicals in common use today may have the potential to cause dermal sensitization. However, the fact that a chemical is a skin sensitizer does not mean it cannot be formulated into consumer products at safe levels. The identification of such safe levels is achieved through dermal sensitization risk assessment.

The expert group was convened by the COLIPA Toxicology Advisory Group and the Joint COLIPA/AISE/EFFA/IFRA Perfume Safety Group to address dermal sensitization risk assessment for fragrance ingredients. The group's remit was to review approaches to such risk assessments and propose a common methodology for fragrance ingredients that are contact allergens. They were also asked to prepare clear guidance on different elements of the recommended dermal sensitization risk assessment process (e.g. uncertainty factors or sensitization assessment factors). The group expanded the remit to include preparation of guidelines to determine the known benchmarks that are used in the risk assessment process.

The group has completed its work and is recommending an exposure-based Quantitative Risk Assessment (QRA) methodology for fragrance ingredients that is described in detail in this dossier. The methodology is based on robust data and will provide the ability to conduct scientifically sound dermal sensitization risk assessments for fragrance ingredients. The overall conclusion is that this method is ready for implementation for fragrance ingredients.

The group has prepared this technical dossier in response to the questions posed. The questions posed and the group's responses are as follows:

Question 1. Are the principles of exposure-based quantitative risk assessment appropriate for evaluation of contact allergens in the fragrance industry?

- Response: Yes, The general principles of risk assessment can be applied to the induction of contact allergy as it is a threshold phenomenon. However, these general principles require tailoring to take into account unique elements of skin sensitization as a toxicity endpoint.

Question 2. Are the uncertainty factors within the exposure-based quantitative risk assessment process sufficiently founded on scientific data?

- Response: Yes, Sensitization assessment factors (SAFs; referred to as uncertainty factors in the remit) within the exposure-based quantitative risk assessment process are based on published peer-reviewed scientific data. It can be concluded that they are based on sound science.

Question 3. Can uncertainty factors be predefined for certain product categories and types of ingredients or do they have to be determined on a case-by-case basis?

Response: Yes, SAFs can be predefined for certain product types. This has already taken place within the deliberations of the QRA expert subgroup. Product categories can be further defined according to similar combinations of SAFs and exposures. Within each product category an acceptable use level of a fragrance ingredient will be defined. It should be noted that while there may be different product types in a product category, only one acceptable use level of a fragrance ingredient will be

defined for each product category. This will take place as more exposure data become available.

While the technical dossier contains all of the detailed technical information from the discussions and deliberations, the group has made the following conclusions on the proposed dermal sensitization QRA methodology and recommendations for refinement.

Key conclusions are:

- The general principles of risk assessment can be applied to the induction of contact allergy as it is a threshold phenomenon. However, these general principles require tailoring to take into account unique elements of skin sensitization as a toxicity endpoint.
- The principles of exposure-based QRA are appropriate for the evaluation of contact allergens for fragrance ingredients.
- Following the identification of a fragrance ingredient as a potential skin sensitizer, a Weight of Evidence (WOE) approach is used to determine a No Expected Sensitization Induction Level (NESIL), which introduces a more robust approach to allergen potency evaluation for use in risk assessment.
- Sensitization assessment factors (SAFs; referred to as uncertainty factors in the remit) within the exposure-based quantitative risk assessment process are based on published peer-reviewed scientific data. It can be concluded that they are based on sound science.
- SAFs can and have been predefined for certain product types. Product categories can be further defined according to similar combinations of SAFs and exposures which will lead to similar acceptable use levels of a fragrance ingredient.
- One critical element of QRA for contact allergy is the dose metric of dose per unit area for induction of contact allergy. It is essential to express the NESIL and Consumer Exposure Level (CEL) in dose per unit area.
- These tools can be used to estimate safe exposure levels and for fragrance ingredients not yet in marketed products, QRA can be used prospectively to identify acceptable levels in a range of different products.
- For fragrance ingredients already in marketed products, QRA could be used both prospectively and retrospectively. Prospective use of QRA in this context would address identifying acceptable levels in products for which IFRA Standards do not exist. Retrospective use of QRA could help to determine the acceptability or unacceptability of current IFRA Standards. Caution needs to be exercised when applying QRA retrospectively. This is on the basis that several data sources may exist and all data need to be taken into account to ensure the most appropriate acceptable exposure levels are selected.
- The use of QRA for fragrance ingredients will facilitate the establishment of IFRA Standards through better definition of product categories versus the current system of only two product categories (skin contact and non-skin contact).

- QRA can be used in combination with the clinical results from the dermatology community and company post-market surveillance data to confirm the effectiveness of fragrance ingredient use limits.
- QRA represents an important step forward in skin sensitization risk assessment, but it should be recognized that there may be refinements to the method in the future.

The group encourages further refinement as new information becomes available. Throughout this dossier, several recommendations for refinement have been identified. Such recommendations are:

- Improved exposure data would be beneficial (i.e. habits and practices, human parameter data) to further refine CEL.
- As more exposure data become available, the QRA for fragrance ingredients and product categories may need to be re-evaluated.
- As more experience is gained with use of LLNA EC3 values as an indicator of human allergenic potency, its influence in WoE NESIL determinations for use in risk assessments to calculate safe product levels of fragrance ingredients may be refined.
- As a conservative approach was taken in establishing SAFs for fragrance ingredients in different product types, additional data (e.g. the influence of evaporation, of retention factors) may lead to their refinement.
- Although it is desirable to use aggregate exposure, there are insufficient data to allow this to occur at this time. This is an area where more evaluation is needed and more data may need to be generated.

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1. Introduction

1.1. QRA Expert Group

1.1.1. Purpose

The remit of the expert group was to propose a common approach to risk assessment of fragrance ingredients that are potential contact allergens and to prepare clear guidance on the use of uncertainty factors (sensitization assessment factors) within the recommended risk assessment approach. The group expanded the remit to include preparation of guidelines to determine the known benchmarks that are used in the risk assessment process.

It should be noted that the remit of this group did not include developing methodology for assessing elicitation reactions in people who have already acquired dermal sensitization to fragrance ingredients. Nor did it include addressing skin sensitization hazard classification according to the European Directive 67/548/EEC, proposed modifications to the classification scheme (European Commission, 2003, Meeting of the Skin Sensitization Expert Group, Ispra 4-6 November 2002, ECLI/81/02 Rev. 2) or the grouping of sensitizing substances into potency classes (ECETOC, 2003; Gerberick *et al.*, 2001).

This group was commissioned by the European Cosmetic, Toiletry and Perfumery Association (COLIPA) Toxicology Advisory Group and the Joint COLIPA/ International Association for Soaps, Detergents and Maintenance Products (A.I.S.E.)/European Flavor and Fragrance Association (EFFA)/ International Fragrance Association (IFRA) Perfume Safety Group and the Research Institute for Fragrance Materials, Inc. (RIFM). These groups posed specific questions on how to manage risk assessment of fragrance ingredients that are potential contact allergens. The questions posed by these groups were:

- 1.) Are the principles of exposure-based quantitative risk assessment appropriate for evaluation of contact allergens in the fragrance industry?
- 2.) Are the uncertainty factors within the exposure-based quantitative risk assessment process sufficiently founded on scientific data?
- 3.) Can uncertainty factors be predefined for certain product categories and types of ingredients or do they have to be determined on a case-by-case basis?

1.1.2. Membership

The expert group is composed of the following individuals: Anne Marie Api (RIFM), David Basketter (SEAC, Unilever), Peter Cadby (Firmenich), Marie-France Cano (LVMH), Graham Ellis (Givaudan), Frank Gerberick (Procter & Gamble), Peter Griem (Clariant Produkte GmbH) and Pauline McNamee (Procter & Gamble). Bob Safford (SEAC, Unilever) and Cindy Ryan (Procter & Gamble) joined the group after it was initiated.

1.2. General principles of a human safety risk assessment and applicability to induction of contact allergy

The risk assessment process for non-cancer toxicity endpoints is fundamentally the same regardless of the material involved. Risk assessment traditionally focuses on extrapolation of data from experimental studies (often at high doses) to establish safe/acceptable exposure levels for humans (generally at lower levels). It allows the identified hazards of a material to be placed in the context of human exposure and permits, where appropriate, the definition of risk reduction measures. Uncertainty is inherent in the extrapolation from experimental studies to the human exposure situation. In the risk assessment process this is generally acknowledged by the application of uncertainty factors that are sufficiently conservative so as to provide confidence that the allowable human exposure will be safe for a heterogeneous population. Quantitative risk assessments are the cornerstone of health-based exposure limits and are used extensively by governments and industry for such activities such as the designation of pesticide residue limits and Acceptable Daily Intakes.

There are essentially four basic steps in the risk assessment process. These are:

Hazard identification in which the inherent ability of a material to cause an adverse effect is established;

Dose-response assessment or Hazard quantification which addresses the derivation of a No Observed (Adverse) Effect Level (NO(A)EL) for the identified hazard and establishes the relationship between the level of exposure and the probability that an adverse event will occur in the experimental species;

Exposure assessment which assesses using habits and practices data and established human parameters data, how consumers are exposed to a material in terms of amount, duration and frequency;

Risk characterization where the dose-response and exposure assessments are compared to determine the probability that an adverse effect will occur at any given exposure level in humans.

While the risk assessment methodology has been refined over time, the basic process is still the same today. A “No Observed Adverse Effect Level” (NOAEL) or equivalent is identified (usually from a laboratory animal study), and appropriate “uncertainty” or “safety factors” are applied to account for areas of data extrapolation. Using this approach, an acceptable exposure level can be determined against which actual consumer exposure can be compared.

2. Recommended QRA approach for fragrance ingredients

Although the conduct of a safety assessment has, for a long time, been a requirement of the regulatory environment in which, for example, cosmetic products are marketed, such safety assessments for chemicals that possess the ability to cause sensitization by skin contact have traditionally been done using

an ad hoc comparative risk assessment technique (Robinson *et al.*, 1989). Recently, several papers (Farage *et al.*, 2003; Felter *et al.*, 2002; Felter *et al.*, 2003; Gerberick *et al.*, 2001; Griem *et al.*, 2003; Robinson *et al.*, 2000) have been published supporting the use of alternative and potentially better quantitative risk assessment approaches.

It is only recently that the principles of exposure-based risk assessment, as an extrapolation of quantitative risk assessment methods that are widely accepted in general toxicology, have also been applied to induction of skin sensitisation. The general principles of quantitative risk assessment can be applied here, since it is known that the induction of skin sensitisation is also a threshold based phenomenon (Kimber *et al.*, 1999; Robinson *et al.*, 2000).

Two key methods were considered (Gerberick *et al.*, 2001 and Griem *et al.*, 2003) in the evaluation of a common approach to risk assessment for fragrance ingredients that are contact allergens. Both methods are based on the same fundamental principles and have significant common elements. A key difference is the use of elicitation data in the Griem *et al.* approach. It was agreed by the Expert Group that the use of diagnostic patch test data cannot be used to determine the induction no-effect level for sensitization. This is because these data are a measure of elicitation of allergic contact dermatitis. To date there are insufficient data to discern any quantitative relationship between induction and elicitation. Such information is most useful in a risk assessment approach to help determine the need for additional data, for example to indicate where current exposures to fragrance ingredients may be a source of clinically relevant positive reactions. The absence of significant clinically relevant positive reactions following testing in dermatology clinics, will provide additional data for use in the QRA approach and may provide support for current exposures to the fragrance ingredient.

3. QRA methodology for fragrance ingredients

The skin sensitization QRA approach for fragrance ingredients follows the same four steps previously outlined above for general toxicology risk assessment. It is implicit that the conduct of a dermal sensitization QRA is necessary only for those fragrance ingredients identified as dermal sensitizers.

Hazard identification – This involves the use of experimental data to determine the skin sensitization potential of the fragrance ingredient. Typically this would involve a murine Local Lymph Node Assay (LLNA), but may also involve the use of other assays such as the guinea pig maximization test or Buehler guinea pig test. Criteria that are used to define a dermal sensitizer and a non-sensitizer have been published in ECETOC, 2003.

Dose–response assessment or Hazard quantification – The dose response for induction of skin sensitization is typically determined in the first instance using animal assays such as the LLNA. Human assays such as the Human Repeat Insult Patch Test (HRIPT) may also be conducted to provide confirmation of the NOEL.

Exposure assessment – Exposure to the fragrance ingredient is determined using habits and practice data for consumer product use and human parameters data.

Risk characterization – The data from the previous steps are used to determine an acceptable exposure level to a fragrance ingredient against which the real life consumer exposure to that fragrance ingredient in a specific product type can be compared. The acceptability or unacceptability of real life exposures can then be determined accordingly.

In developing a quantitative risk assessment method for skin sensitization of fragrance ingredients, based on the above recommended approach, some new terms have been adopted and are presented below. The new terms are “No Expected Sensitizing Induction Level” (NESIL) and “Sensitization Assessment Factors” (SAFs) that replace no observed effect level (NOEL) and uncertainty factors, respectively, in general toxicology risk assessment. These terms have been adopted to take into account unique elements of quantitative risk assessment for skin sensitization. Finally, the risk characterization process for the QRA is explained and some worked examples given.

3.1. Hazard identification

3.1.1. Animal data

Historically, there are several animal models that have been used to determine the potential for a fragrance ingredient to induce sensitization. Guinea pig tests (adjuvant and non-adjuvant) have been used for many years to assess the inherent contact sensitisation potential of chemicals. These tests can assess potency to a certain extent or antigen cross-reactivity of structurally-related chemicals. More recently the murine local lymph node assay (LLNA) has been approved by the OECD and is now generally used not only to determine the potential of a material to induce contact sensitisation, but also for the measurement of the relevant sensitising potency of contact allergens. The EC3 value, determined from an LLNA study is the concentration required to induce a threshold positive response (stimulation index equal to 3). The most robust and convenient method for the routine calculation of EC3 values is to derive it by linear interpolation from the dose response data (Basketter *et al.*, 1999).

Mechanistically, the biological sequelae that take place for the immune system to mount a response are the same across mammalian species, supporting the use of the mouse model to characterize human risk. Historically, for non-cancer risk assessments, a default 10-fold uncertainty factor has been used to extrapolate from laboratory animal species to humans. The approach is somewhat different when using mouse data to estimate potency of contact sensitizers. Initial use of the LLNA data has focused on adding to the weight of evidence for ranking dermal allergens as to their relative potency. Much work has been done to correlate the dose-response data obtained in the mouse LLNA with what is known about potency in humans. The EC3 value has recently been

demonstrated to closely correlate with the NOEL from human sensitization tests designed to confirm lack of induction (Basketter *et al.*, 2000; 2005; Gerberick *et al.*, 2001; 2001a; 2004; Griem *et al.*, 2003; Schneider and Akkan, 2004).

3.2. Dose-response or Hazard quantification

3.2.1. No Expected Sensitizing Induction Level (NESIL)

The NESIL may be derived from animal and human data and would be expressed as a dose per unit area (e.g. $\mu\text{g}/\text{cm}^2$) value. Consideration of animal data is described in the section above (3.1.1) and the use of the human data is described below.

3.2.1.1. Human data

A human sensitization test is not used to determine hazard, rather it is a test to confirm the lack of sensitization at an exposure level which was identified as a NOEL in an animal model or derived as a likely NOEL from quantitative structure-activity relationships.

Human patch testing methodology has evolved over more than 50 years. In every method a number of induction exposures is followed by a rest period and then a challenge exposure, but variations exist as to patch type, number of subjects, skin site, number of induction patches, patch application time, duration and rest period prior to challenge. In all, enhancement of the skin response after challenge over that seen during early induction exposures has been the criterion by which induction of contact allergy is measured. Test volunteers are typically healthy adults who are enrolled without restriction as to sex or ethnicity. The test most typically conducted is the human repeat insult patch test (HRIPT) (McNamee *et al.*, 2006).

In HRIPTs, the size of the test population is important with regard to interpretation of findings. The sample size of test subjects must be sufficiently large so that results are valid for the population at large, yet small enough to be logistically feasible to conduct the study. To increase the sensitivity of the test whilst using such numbers of subjects, if appropriate one generally tests a higher concentration of test material than would actually be encountered in intended and foreseeable use situations among the general population. Other factors that further increase the sensitivity and reliability of the test are exaggeration through possible minor skin irritation of a test material and use of occluded patches. (McNamee *et al.*, 2006)

With implementation of the QRA approach, IFRA/RIFM are recommending the use of the RIFM standard HRIPT protocol for generation of confirmatory human data for use in QRA. Details of this standard HRIPT protocol are available from RIFM.

3.2.2. Weight of Evidence approach for determining the NESIL for fragrance ingredients

Historical data that are used to determine the sensitization potential of a material may be of variable quality and robustness. Therefore, it is recommended to use

a weight of evidence (WoE) approach using all the available data for the identification of a no-expected sensitization induction level (NESIL) that can be used in the exposure based quantitative risk assessment process.

3.2.2.1. Guidelines for applying weight of evidence approach to induction sensitization data on fragrance ingredients

A no expected sensitization induction level (NESIL) for the induction of skin sensitization will be determined for fragrance ingredients following a weight of evidence (WoE) based approach. The establishment of sound NESILs for the induction of skin sensitization is critical to the conduct of this QRA methodology.

The NESIL can be established using data from “experimental”^a animal studies, specifically the murine local lymph node assay (LLNA), and taking existing (historical) human studies into account. Historical “experimental”^a human data exist for both the Human Repeat Insult Patch Test (HRIPT) and Human Maximization Test (HMT) methods. For ethical reasons, predictive, experimental tests for skin sensitization hazard identification are no longer conducted in humans. However, there may be instances when clinical tests, specifically HRIPTs, will need to be conducted to confirm the lack of sensitizing activity of a chemical at a previously determined ‘safe’ (i.e. non-sensitizing) dose per unit area.

^a *The term “experimental” used in this technical dossier in the context of animal testing refers to animal sensitization tests conducted to determine the dermal sensitization potential (hazard). LLNA data (expressed as EC3 values) are also used to correlate to human potency of dermal allergens. The term “experimental” used in this technical dossier in the context of human testing refers to human sensitization tests conducted to confirm a NOEL and not to determine hazard.*

These guidelines have been developed specifically for fragrance ingredients and must only be applied to fragrance ingredients.

The principles of QRA are applicable to dermal sensitization risk assessment for materials other than fragrance ingredients. The use of this QRA approach for such materials (e.g. preservatives, sunscreens) can only occur once a separate and thorough review of all the elements of QRA for each class of materials in consumer products has been completed.

QRA can be used both prospectively and retrospectively. Prospective use of QRA would address identifying acceptable levels in products of raw materials identified as dermal sensitizers. For fragrance ingredients that are dermal sensitizers, prospective use of QRA is applicable when IFRA Standards do not exist. Retrospective use of QRA could help to determine the acceptability or unacceptability of raw materials already in marketed products. For fragrance ingredients specifically, this would mean the acceptability or unacceptability of limitations in current IFRA Standards. Caution needs to be exercised when applying QRA retrospectively. This is on the basis that several data sources may

exist and all data need to be taken into account to ensure the most appropriate acceptable exposure levels are selected.

The RIFM Expert Panel (REXPAN) has been the advisory body for NESIL judgments on fragrance ingredients and will be the group to continue to do this in the future. This document has been developed to provide guidelines for use in establishing WoE NESILs for fragrance ingredients; however, these are only guidelines. Scientific judgment will prevail when establishing WoE NESILs for fragrance ingredients.

When deriving a WoE NESIL, expressed as a dose per unit area, there may be cases where the level derived from a LLNA EC3 value is significantly higher or lower than the level derived from the No Observed Effect Level (NOEL) obtained in a previously conducted HRIPT or HMT. In particular, these guidelines may assist in resolving discrepancies between data generated in a LLNA and human data.

GUIDELINES

GUIDELINE #1.

From experimental investigations and on the grounds of basic immunological considerations, the quantity of chemical per unit area of the skin (e.g. $\mu\text{g}/\text{cm}^2$), is considered as the most appropriate dose metric for skin sensitization. Although this approach might be reviewed on the basis of future findings, it is currently considered the best scientific approach and is in line with the overwhelming majority of available historical data in both humans and experimental animals. Thus, NOELs, LOELs and EC3 values for sensitizing chemicals will be expressed as dose per unit area in these WoE guidelines and for skin sensitization QRA.

GUIDELINE #2.

A NOEL from a well run HRIPT, will be given precedence over NOELs from other clinical tests that were conducted in human subjects (e.g. HMT, earlier precursors to the HRIPT such as the Modified Draize Test), regardless of the NOELs indicated from those other tests. It is important to evaluate the robustness of the studies and to discriminate between the available data. A well run HRIPT is defined as one which employed a published methodology, was well documented and involved approximately 100 subjects or more.

GUIDELINE #3.

Where a Lowest Observed Effect Level (LOEL; i.e. a dose per unit area which resulted in sensitization) from other human tests exists (e.g. HMT) which is lower than the NOEL from the HRIPT, it will be considered unless there is a rationale to disregard the LOEL data. In some instances, the conduct of a confirmatory HRIPT to substantiate a NESIL may be warranted.

GUIDELINE #4.

In the absence of a NOEL from a HRIPT, a NOEL from a different predictive human test (e.g. HMT) can be used to set the NESIL, provided that it is supported by an EC3 value from a well conducted LLNA.

GUIDELINE #5.

Adjuvant tests in animals (GPMT, FCAT, MEST, etc.) and non-adjuvant tests in guinea pigs (e.g. Buehler, OET, CET) shall not be used as primary sources for defining NESILs in this context. They may be used to contribute information to determine the potency classification, according to the guidelines provided in the ECETOC, 2003 technical report No. 87, and be incorporated in a WoE approach.

GUIDELINE #6.

When only LLNA data are available (i.e. no historical human data exist), then a confirmatory HRIPT should be considered. A cautious approach will be used for selection of the dose level of fragrance ingredient in the conduct of any such confirmatory HRIPTs. Under exceptional circumstances (e.g. low volume of use,

low use level) the weighted average EC3 value (limited to two significant figures), can be used to define a NESIL.

GUIDELINE #7.

A NOEL from a well run HRIPT will (even if higher) have precedence over all other NOELs (including LLNA EC3 values). When there is a significant discrepancy between a HRIPT NOEL and a LLNA EC3 value (e.g. around an order of magnitude or more), further consideration in setting the NESIL will be required. A LLNA EC3 value that exceeds a NOEL determined by a HRIPT will not be used to define the NESIL. If the HRIPT NOEL is the lowest NOEL available, it shall take precedence in deriving the NESIL. Additional sources of data such as guinea pig studies, evaluated as described in ECETOC technical report No. 87, may provide additional evidence for the purposes of establishing a potency classification. In addition, data elucidating species differences, e.g. studies on metabolism (in the skin), skin penetration, and vehicle effects should be considered.

GUIDELINE #8.

Data from diagnostic patch test studies can not be used directly in a weight of evidence approach for the determination of NESILs for the induction of contact allergy to fragrance ingredients. These studies can be useful to help determine the need for additional data, for example for indication where current exposures to a fragrance ingredient may be a source of clinically relevant positive reactions. The absence of relevant positive reactions following testing in dermatology clinics, may provide support to current exposures to the fragrance ingredient.

3.2.2.2. WoE NESILs for selected fragrance ingredients

Animal (guinea pig and LLNA), human patch (maximization and RIPTs and others) and diagnostic patch test data for a group of 31 fragrance ingredients were reviewed in detail. This group of fragrance ingredients was chosen to include the 26 fragrance allergens that must now be labeled on cosmetic products in Europe in line with the 7th Amendment of the EU Cosmetics Directive and an additional 5 fragrance ingredients for which an IFRA Standard based on sensitization effects exists. The guidelines detailed above were applied to all the data and a WoE NESIL was identified. These NESILs are provided in Table 1.

Table 1: Fragrance Ingredients WoE NESILs.

Fragrance Ingredient	CAS No.	IFRA Standard Limit	LLNA weighted mean EC3 values ($\mu\text{g}/\text{cm}^2$) [no. studies]	Human Data			Potency Classification ²	WoE NESIL ³ ($\mu\text{g}/\text{cm}^2$) [REXPAN NOEL] ⁴
				NOEL – HRIPT (induction) ($\mu\text{g}/\text{cm}^2$)	NOEL – MAX (induction) ($\mu\text{g}/\text{cm}^2$)	LOEL ¹ (induction) ($\mu\text{g}/\text{cm}^2$)		
α -Amylcinnamaldehyde	122-40-7	NA	2942 [3]	23,622 ⁵	NA	NA	Extremely Weak	24,000 [23,622]
α -Amylcinnamyl alcohol	101-85-9	NA	>6250 [1] ⁶	3543 ⁵	NA	NA	Weak	3500 [3543]
Anisyl Alcohol	105-13-5	NA	1475 [1] ⁶	NA	3448 ⁵	NA	Weak	1500
Benzyl Alcohol	100-51-6	NA	>12,500 [1] ⁶	5906	6897	8858	Weak	5900 [5906]
Benzyl Benzoate	120-51-4	NA	>12,500 [1] ⁶	59,050 ⁵	20,690 ⁵	NA	Extremely Weak	59,000 [59,000]
Benzyl Cinnamate	103-41-3	NA	4600 [1] ⁶	4720 ⁵	5517 ⁵	NA	Weak	4700
Benzyl Salicylate	118-58-1	NA	725 [1] ⁶	17,717 ⁵	20,690 ⁵	NA	Weak	18,000
p-t-Butyl- α -methylhydrocinnamic aldehyde (BMHCA)	80-54-6	2.5%	2372 [6]	4125	NA	29,528	Weak	4100 ⁷ [29,000] ⁷
Cinnamyl Alcohol	104-54-1	0.4%	5250[1] ⁶	3000	2759	4724	Weak	3000
Cinnamaldehyde	104-55-2	0.05%	262 [23]	591	NA	775	Moderate	590 [591]
Citral	5392-40-5	NA	1414 [11]	1400	NA	3876	Weak	1400
dL-Citronellol	106-22-9	NA	10,875 [1] ⁶	29,528 ⁵	4138	NA	Extremely Weak	30,000 ⁸
Coumarin	91-64-5	NA	>6250 [1] ⁶	3543	5517	8858	Weak	3,500
Eugenol	97-53-0	0.5%	2703 [6]	5906	NA	NA	Weak	5900 [5900]
Farnesol	4602-84-0	NA	1200 [2]	2755	NA	6897 ⁹	Weak	2700 [2700]
Geraniol	106-24-1	NA	3525 [5]	11,811	NA	NA	Weak	12,000
α -Hexyl-cinnamaldehyde	101-86-0	NA	2372 [>5]	23,622 ⁵	NA	NA	Weak	24,000 [23,622]
Hydroxycitronellal	107-75-5	1%	5612 [9]	5000	NA	5906	Weak	5000 [11,811]
3 & 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde (HMPCC)	31906-04-4	1.5%	4275 [1] ⁶	4000	NA	NA	Weak	4000 [4,000]
Isoeugenol ¹⁰	97-54-1	0.02%	498 [18]	250	NA	775	Moderate	250 [250]
α -Limonene ¹¹	5989-27-5	NA	10,075 [5]	10,000 ⁵	5517 ⁵	NA	Weak	10,000 [5517]

Fragrance Ingredient	CAS No.	IFRA Standard Limit	LLNA weighted mean EC3 values ($\mu\text{g}/\text{cm}^2$) [no. studies]	Human Data			Potency Classification ²	WoE NESIL ³ ($\mu\text{g}/\text{cm}^2$) [REXPAN NOEL] ⁴
				NOEL – HRIPT (induction) ($\mu\text{g}/\text{cm}^2$)	NOEL – MAX (induction) ($\mu\text{g}/\text{cm}^2$)	LOEL ¹ (induction) ($\mu\text{g}/\text{cm}^2$)		
Linalool ¹¹	78-70-6	NA	12,650 [2]	15,000 ⁵	13,793 ⁵	NA	Extremely Weak	15,000 [13,793]
Methyl 2-octynoate (Methyl heptine carbonate)	111-12-6	0.01%	<125 [1] ⁶	118	NA	194	Strong	120 [118]
Methyl 2-nonynoate (Methyl octine carbonate)	111-80-8	0.002%	<1250 Estimated 625 [1] ⁶	24	NA	118	Strong	24 [24]
α -iso-Methylionone	127-51-5	NA	5450 [1] ⁶	70,866 ⁵	NA	NA	Weak	71,000 [70,866]
Phenylacetaldehyde	122-78-1	NA	962 [2]	591	NA	1181	Moderate	590
Oakmoss	90028-68-5	0.1%	970 [1] ⁶	700	NA	NA	Moderate	700 ¹² [700]
Treemoss	90028-67-4	0.1%	>5000 [1] ⁶	700	NA	NA	Moderate	700 ¹³ [700]
<i>trans</i> -2-Hexenal	6728-26-3	0.002%	1012 [2]	24	NA	236	Strong	24 [24]
Isocyclogeraniol	68527-77-5	NA	>6250 [1] ⁶	3898	NA	7752	Weak	3900
Cinnamyl nitrile	1885-38-7	0.125%	>2500 [1] ⁶	1476	NA	NA	Weak	1500 [1000]

All data in this Table are available from RIFM and are listed in the RIFM Database.

NOEL = No observed effect level; HRIPT = Human Repeat Insult Patch Test; MAX = Human Maximization Test; LOEL = lowest observed effect level; NA = Not Available

¹Data derived from HRIPT or Human Max tests

²Gerberick *et al.*, 2001

³WoE NESIL limited to two significant figures

⁴REXPAN HRIPT NOEL; NESILs have not been reviewed by REXPAN to date.

⁵MT-NOEL = Maximum Tested No Effect Level. No sensitization was observed in human predictive studies. Doses reported reflect the highest concentration tested, not necessarily the highest achievable NOEL.

⁶EC3 value from one LLNA, not the mean.

⁷BMHCA – HRIPT LOEL data suggest that the NOEL is likely to be in the region of 29,000 $\mu\text{g}/\text{cm}^2$. On this basis, the IFRA Joint Advisory Group (JAG) was asked to supply any sensitization data on final products containing BMHCA. May consider repeating an HRIPT at 10,000 $\mu\text{g}/\text{cm}^2$.

⁸*d*-Citronellol – IFRA Joint Advisory Group was asked to supply any sensitization data on final products containing *d*-Citronellol. May consider repeating an HRIPT at 29,500 $\mu\text{g}/\text{cm}^2$.

⁹LOEL from human maximization test, not a human repeated insult patch test.

¹⁰Isoeugenol Potency Classification is listed as "moderate" because the LOEL is 775 $\mu\text{g}/\text{cm}^2$. In addition, a moderate classification is consistent with isoeugenol's potency in animal tests.

¹¹*d*-Limonene and linalool are not contact allergens, but some hydroperoxides formed by autoxidation are known to be dermal sensitizers. In addition, *d*-limonene and linalool are known human irritants. The irritancy profile of *d*-limonene and linalool is being further investigated by RIFM.

¹²Oakmoss – Pending LLNA and a confirmatory HRIPT on new qualities of oakmoss, which contain significantly lower levels of atranol and choloratranol.

¹³Treemoss – Pending LLNA and a confirmatory HRIPT on new qualities of treemoss, which contain significantly lower levels of atranol and choloratranol.

3.2.3. Sensitization Assessment Factors for fragrance ingredients

In general toxicology uncertainty factors are applied to extrapolate from experimental to real-life exposure scenarios. These uncertainty factors are defined from inter-species variability (Travis and White, 1988; Chappell and Mordenti, 1991) and inter-individual variability (Renwick and Lazarus, 1998; Burin and Saunders, 1999; Aldridge *et al.*, 2003). In dermal sensitization risk assessments it is equally necessary to extrapolate from the experimental (defined and controlled exposure conditions) to real life consumer exposure (variable exposure controlled by the consumer).

This is achieved by the application of a Sensitization Assessment Factor (SAF). The SAF takes into account three parameters - inter-individual variability (the same as in general toxicology), vehicle/product matrix effects and use considerations (specific for dermal sensitization).

Key areas addressed are:

- 1) in-depth definition of each SAF component (sections 3.2.3.1 through 3.2.3.3);
- 2) numbers that could be assigned to each SAF component (3.2.3.4);
- 3) the rationale for assigning SAFs to a large group of consumer product types, based on the data set available to fragrance ingredients by RIFM (3.2.3.5) and
- 4) the rationale to define the large group of consumer product types included (3.2.3.6).

3.2.3.1. Inter-individual variability

The uncertainty factor or SAF for inter-individual variability allows for possible variations in the sensitivity of individuals within the human population. There can be large inter-individual differences in response to a chemical exposure due to several different parameters. These are genetic effects, sensitive subpopulations, inherent barrier function, age, gender and ethnicity. While all of these parameters are important, some have more influence than others. For example genetic effects, sensitive subpopulations and inherent barrier function (inherent dermal integrity) are more influential than age, gender and ethnicity (Felter *et al.*, 2002; Robinson, 1999).

In general toxicology, the assessment factor for inter-individual variability would be combined with a factor that addresses inter-species variability. It should be noted that inter-species variability is not taken into account in this approach for quantitative risk assessment of contact allergens when extrapolating from the experimental situation to the real life exposure scenarios. This is on the basis that the LLNA data have been correlated with the human data, which is typically not the case in general systemic toxicology.

Described briefly below are some of the factors that influence inter-individual variability.

3.2.3.1.1. Genetic effects

Genetic factors are not totally understood, but are clearly instrumental in determining individual susceptibility (Felter *et al.*, 2002). Differences in metabolic capabilities might be expected to influence an individual's susceptibility to the induction of allergic contact dermatitis. It is well established that skin enzymes, predominantly located in the epidermis, can metabolize absorbed xenobiotics via reactions analogous to those determined in the liver (Smith and Hotchkiss, 2001). An example of metabolic capability is skin allergy caused by poison ivy since this is believed to be due to the metabolic conversion of the pentadecylcatechol component of poison ivy to an orthoquinone, a known strong contact allergen (Dupuis, 1979).

3.2.3.1.2. Sensitive subpopulations

Sensitization risk assessments are no different to those for other non-genotoxic toxicity endpoints in terms of the population that is considered. It is not only the average consumer that is taken into account, but also those individuals who may be more susceptible. There are several studies that address the importance of including subpopulations, such as those with multiple allergies who may be more susceptible (Felter *et al.*, 2002; Friedmann and Moss, 1985; Moss *et al.*, 1985).

3.2.3.1.3. Inherent barrier function

One factor likely to impact individual susceptibility is overall inherent barrier function of the skin. The initial step in the induction and elicitation of allergic contact dermatitis requires that the allergen penetrate the stratum corneum. Dermal sensitization risk assessments for fragranced consumer products are conducted for healthy skin and not on diseased skin. While individuals with diseased skin (e.g. psoriasis and eczema) may use regular consumer products, it can be assumed that at least some of these individuals may be under the care of a dermatologist. However, healthy skin may have a compromised inherent barrier (e.g. dry skin). In addition, age, ethnicity and gender may have an influence on inherent barrier function in healthy skin (see sections 3.2.1.4 through 3.2.1.6). It is important to consider inherent barrier function for inter-individual susceptibility since all of the parameters detailed above indicate that inherent barrier function can be compromised and could lead to greater susceptibility for induction of contact allergy.

3.2.3.1.4. Age

The general susceptibility of infants and adults to contact allergens is essentially equal (Cassimos *et al.*, 1980). The structural and functional skin barrier properties are equal from full term infancy to late adulthood (Cunico *et al.*, 1977; West *et al.*, 1981; Holbrook KA 1982; McCormack *et al.*, 1982; Wester and Maibach 1982; Fairley and Rasmussen, 1983; Harpin and Rutter, 1983). This is in terms of epidermal thickness, density of epidermal cell layers, cellular structure, functional stratum corneum and mature skin barrier function.

Decreases in the skin barrier function can occur at either end of the age spectrum – pre-term infant (Kalia *et al.*, 1998) and geriatric (Leveque *et al.*, 1984; Ghadially *et al.*, 1995). It is important to take into account these decreases in skin barrier function in inter-individual variability although it is recognized that pre-term infants would not normally be part of such risk assessments since they are under medical care.

3.2.3.1.5. Gender

While there is some indication that females are the more reactive responder population (Jordan and King, 1977; Rees *et al.*, 1989), the weight of evidence supports that females and males react similarly to contact allergens (Felter *et al.*, 2002; Robinson, 1999). Those differences that have been noted in the general population for percentages of men and women with specific contact allergies are often attributed to exposure differences. For example, women are more prone to contact allergies to nickel (from jewelry, especially pierced ears), while men are more likely to be allergic to chromium as a result of occupational exposures (Young *et al.*, 1988).

3.2.3.1.6. Ethnicity

Weight of evidence indicates individuals of different ethnic origins are not substantially more susceptible to induction of contact allergy. In a study of induction by five common skin allergens, Kligman (1966) reported little difference in the response between highly pigmented skin and Caucasians for the strongest allergens. With less potent allergens, Kligman found that those with highly pigmented skin were increasingly resistant to the induction of sensitization compared with the response in Caucasians. Some controversy remains as to the sensitivity of Asians relative to Caucasians. In regard to percutaneous absorption of chemicals into the skin, highly pigmented skin is generally considered to be somewhat more impervious than Caucasian skin (Weigand *et al.*, 1974).

3.2.3.2. Matrix effects

The consumer can be exposed to fragrance ingredients in many different product forms (e.g. cream, shower gel, eau de toilette). These product formulations are of varying complexity ranging from a simple ethanol matrix to multi-phase creams. In the experimental situation, exposure to the fragrance ingredient is typically in a simple vehicle. In addition, some of the consumer product formulations may contain ingredients that are irritants or penetration enhancers. A vehicle can be a single moiety (e.g. water), mixtures (acetone/water, ethanol/water) or a complex product formulation presented in undiluted or diluted form. The effect of complex formulation/matrix, as a vehicle, on the physical chemical parameters and bioavailability of a test material may be substantially different from a simple vehicle. The same is true when extrapolating from the experimental situation in which a simple vehicle is used to the real life scenario

where the fragrance ingredient is typically formulated into a more complex product matrix (Felter *et al.*, 2002).

In dermal sensitization risk assessment, consideration of matrix effects encompasses extrapolation from the matrix/vehicle used to determine the EC3/NOEL in the experimental situation to the product formulation containing the fragrance ingredient to which the consumer is exposed in real life scenarios. The larger the difference between the experimental situation and real life exposure scenario, the greater the SAF will be.

The two areas within vehicle/matrix effects that are noteworthy are irritants and penetration enhancers. Both have the ability to promote the skin penetration of the fragrance ingredient. The way that it is accomplished and the importance to dermal sensitization risk assessments are briefly described below.

- *Irritants.* Dermal irritants are known to compromise the skin barrier (Robinson *et al.*, 2000). They are also known to serve as a promoter of dermal sensitization (Smith *et al.*, 2000). It is apparent that some degree of direct chemical inflammation or other concurrent trauma enhances the keratinocyte activity, produced by the applied chemical itself, by some other component of the chemical delivery system, or by some form of physical insult. This may account for the noted enhancing effect of primary skin irritation on the sensitization response (Cumberbatch *et al.*, 1993; Kligman, 1966). Smith *et al.*, 2000 proposed that one of the reasons for differences in individual responses to the same exposure to an allergen may be related to their susceptibility to skin irritation, such that those in whom the epidermal irritant response reaches a sufficient threshold level are more likely to be sensitized. It may also influence the magnitude of response by affecting other steps in the process of inducing (or eliciting) an allergic response.
- *Penetration enhancers.* Some chemicals are specifically known to affect the penetration of other chemicals through the stratum corneum (Scheuplein and Ross, 1970; Schaefer and Redelmeier, 1996). As such it remains important to understand the experimental matrix/vehicle as to its effect on the penetration of the fragrance ingredient since it will affect the bioavailability of the material in the experimental situation.

Typically there is very little information available about the bioavailability of the fragrance ingredient in either the experimental situation or real life exposure scenario. Currently, topically applied area doses (i.e., external doses for CEL and NESIL) are used in the quantitative risk assessment together with a matrix SAF to account for this area of uncertainty. It is recognized that future refinements of this risk assessment methodology for fragrance ingredients may take into account situations where more is known about the penetration of the fragrance ingredient from the experimental and product matrices.

3.2.3.3. Use considerations

Use considerations in the experimental situation are defined and controlled (e.g. site of contact, skin integrity, operator controlled, duration of exposure). On the other hand, use considerations in real life scenarios in almost all cases involve less exaggerated exposure, are more variable and are within consumer's control.

There are three key parameters for consideration when extrapolating from the controlled experimental situation to the real life scenario. They are site of contact, dermal integrity and occlusion. The larger the difference in skin site location, effect on barrier integrity and occlusion, the greater the SAF.

3.2.3.3.1. Site of contact

Regional differences in dermal absorption can be substantial. For example, Feldmann and Maibach, 1967 measured the relative regional permeability of human skin from various body sites to ¹⁴C-labelled hydrocortisone. Of eleven sites evaluated, the skin of the back (where most patch studies are conducted) was intermediate in relative permeability. The plantar foot arch was correspondingly about 12-fold less permeable than the skin of the back, while the scalp and axillae were about 2-fold higher, and the forehead was about 3-fold higher. Since the permeability on the back and the arm (the sites of contact for most experimental sensitization tests) is intermediate, it is highly likely the fragrance ingredients in consumer products will be used on body sites that may be significantly more permeable. As such the greater the difference between permeability of the skin sites in the experimental versus the real life scenario, the greater the SAF. Table 2 provides a more comprehensive list of references that describe important considerations for application to different sites of contact.

3.2.3.3.2. Barrier integrity

Barrier integrity can be inherent or can be influenced by consumer practices. Inherent barrier integrity is addressed in section 3.2.1.3. The effect that consumer practices have on barrier integrity is addressed in this section. Factors influencing dermal integrity are known to have a significant effect on dermal penetration. This might include, for example, the presence of diaper rash (Odio *et al.*, 2000) in an infant, or dermatitis in an adult (Benfeldt, *et al.*, 1999). While less dramatic, shaving has also been shown to have an influence (Edman, 1994).

3.2.3.3.3. Occlusion

Occlusion of the skin results in multiple effects, including increases in the hydration of the stratum corneum, skin temperature, microbial count, pH, and dermal irritation. The increase in hydration state, in particular, has been associated with increased dermal penetration, although occlusion does not increase the absorption of all chemicals, and the relative effect of occlusion is likely to be dependent on the lipophilicity of the chemical (Zhai and Maibach, 2001). Under most circumstances consumers are exposed to products under less than full occlusive conditions (examples of exceptions are diapers and axillary products). Conversely, most human data used to define NESILs (e.g. HRIPT NOELs) have been obtained under semi- or fully occlusive experimental

patch conditions. Typically the consumer exposure to fragrance ingredients to products involves less occlusion than that encountered in the experimental situation. In these circumstances, occlusion does not play a significant role in the assignment of the SAF. However, for those products where occlusion in the consumer exposure scenario is greater than that of the experimental situation, the SAF will be increased.

For example if the NESIL is derived from patch test data generated on the arm or back and the product is meant to be used in the axillae where the skin is easily irritated, highly follicular, occluded and may be abraded by shaving, this would increase the uncertainty factor to reflect the large differences between the experimental situation and real life scenarios here.

3.2.3.4. Defining SAF numbers

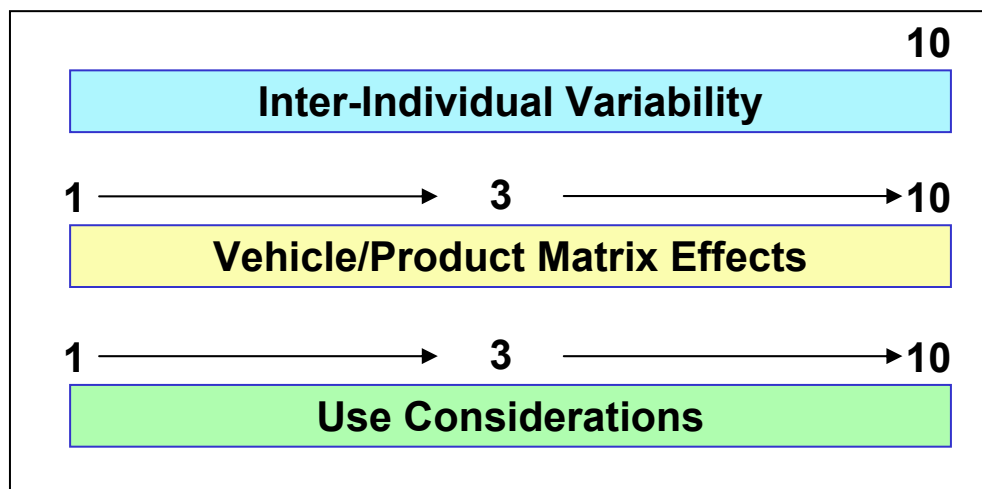
The question that is probably most apparent at this point is which number to assign each component of the SAF. For inter-individual variability, a value of 10 is assigned. This is based on well established principles of general toxicology and is meant to reflect not only the average consumer but also more susceptible sub-populations.

For matrix effects and use considerations the number that is assigned to each area is dependent upon how different the experimental situation is versus the real life scenario. For example, with vehicle effects if the vehicle in which the experimental data (used to define the WoE NESIL) is generated is the same as that to which the consumer is exposed in the finished product then a SAF of 1 would be assigned. However as mentioned earlier, it is rare that this would be the case. In general, the bigger the difference between the experimental vehicle and the consumer product, the bigger the SAF up to a maximum of 10. It is also important to take into account the effect of the product matrix on the skin since a product matrix can be radically different in chemical composition from the experimental vehicle but be expected to have no effect on the skin e.g. talcum powder versus an alcohol-based experimental vehicle.

Although any value between 1 and 10 may be assigned for the SAFs relating to matrix/product effects and use considerations, it is considered pragmatic to limit the values used to 1, 3.16 (half log of 10) and 10. It must be noted that throughout the rest of this dossier, for practical purposes the value of 3.16 is represented simply as the number 3, however, in all calculations the exact value of 3.16 is used. A value of 1 defines an experimental condition that is identical or essentially identical to the real-life scenario. A value of 10 defines an experimental condition that is unrelated or nearly unrelated to the real-life scenario. A value of 3 is used to define differences between the experimental conditions and the real-life scenarios that are greater than 1 (none or minimal differences), but less than 10 (maximal differences). These values chosen are consistent with the approach used by EPA for general risk assessment (Dourson *et al.*, 1996). SAFs less than 1 and a continuum from 1 through 10 (e.g., 2.5,

4.5, 7.5) have not been used. This lends appropriate conservatism and simplicity to the approach.

The overall SAF is a combination of the three key parameters defined above and is calculated by multiplying the inter-individual variability by vehicle/matrix effects and by use considerations. In theory, SAFs could range from 10 (inter-individual =10, vehicle/matrix=1, use considerations =1) to 1000 (inter-individual =10, vehicle/matrix=10, use considerations =10). In reality, for fragrance ingredients it is unlikely that the SAF would exceed 300. However, exceptions could include where there is mucosal contact where higher SAFs are assigned (Farage, *et al.*, 2003). The SAFs for dermal sensitization risk assessments for fragrance ingredients are specific for this toxicity endpoint and cannot be compared to the values defined for uncertainty factors in general toxicology. Figure 1 illustrates the approach to assign SAFs.



Note: for practical purposes the number 3 is the practical representation of 3.16 (half log of 10)]

Figure 1: Sensitization Assessment Factor (SAF). (Calculated by multiplying the inter-individual variability by vehicle/matrix effects and by use considerations.)

3.2.3.5. Rationale for fragrance ingredients SAFs in different product types based on RIFM data

When considering the SAFs for fragrance ingredients, the SAF of inter-individual variability was given a value of 10. It was agreed that the parameters used to determine inter-individual variability in general toxicology are equally applicable to the identification to SAFs for the induction of skin sensitization. As such, the group concluded that for the assignment of the inter-individual SAF for dermal sensitization, there is no scientific basis to change from the value of 10 used in general toxicology.

For vehicle/matrix effects based on RIFM data, the SAFs for fragrance ingredients is based on the fact that the RIFM data on fragrance ingredients is

generated using a vehicle consistently containing ethanol. Key factors in determining the SAF are:

- an evaluation of the skin effects of ethanol (drying and barrier function decrease) in the experimental situation versus the consumer product matrix.
- the presence and level of formulation ingredients that are known to be irritants in the consumer products.
- formulation differences other than the presence of ingredients that are skin irritants that would impact the integrity of the skin barrier.

For use considerations, the confirmatory human data from RIFM sponsored studies are consistently generated using the RIFM standard HRIPT protocol in which the fragrance ingredient is applied to the back or the upper arm and conducted under full occlusion for 24 hours per patch application. Other confirmatory human data previously submitted to RIFM from its members typically may have used the same skin sites but may have used semi- and/or full occlusion.

For these reasons it is considered appropriate for fragrance ingredients to establish values for the SAFs for each product type. Table 2 details the numbers assigned to each of the components of the SAF for fragrance ingredients. The table also includes the rationale for selection of the specific number and lists the literature cited references. These SAFs are specific for fragrance ingredients. SAFs for other types of ingredients may vary from these based on the considerations discussed above.

3.2.3.6. Rationale to define the scope of consumer product types reviewed

The application of the QRA for fragrance ingredients required the identification of a range of product types. The list of product types is given in Table 2, column 1. The choice of product types included is based on those products listed in the SCCNFP Notes of Guidance (SCCNFP, 2003), products surveyed by CTFA and Colipa, products included in the IFRA Standards and the experience of the industry representatives on the QRA expert subgroup. Since some of the products included in the list were selected from those covered by IFRA Standards, all of the products are not considered cosmetic products. The list of product types is not intended to be all inclusive. It is impossible, in this exercise, to cover all conceivable types of products that could contain fragrances. Future technical and market developments will certainly generate new products with matrices and recommended uses that are not covered in the Tables. The risk assessment of the exposure scenarios associated with such new products should be made by judicious choice of the nearest similar product or failing this, using the same principles described in this dossier, conduct a QRA for dermal sensitization for the new product type ensuring that the high end exposure is taken into account.

Table 2: SAFs, rationale and literature references for fragrance ingredients in different product types based on RIFM data.

Product Type	Inter-individual ¹⁻²⁷ SAF	Matrix SAF	Matrix SAF Rationale ^{6,26,28-32} (experimental** vs. real life exposure)	Use SAF	Use SAF Rationale (experimental** vs. real life exposure)	SAF
Aerosol Deodorant	10	3*	Matrix for the product not the same as the experimental** conditions.	10	The area is the underarm ³³ ; the skin is easily irritated ³⁴ , highly follicular ³³ and an area that is shaved ³⁵ . Type of occlusion is similar to that of the experimental** test conditions ³⁶ .	300
Aerosol Antiperspirant	10	3*	Matrix for the product not the same as the experimental** conditions and may contain irritating active ingredients.	10	The area is the underarm ³³ ; the skin is easily irritated ³⁴ , highly follicular ³³ and an area that is shaved ³⁵ . Type of occlusion is similar to that of the experimental** test conditions ³⁶ .	300
Stick Deodorant / Antiperspirant	10	3*	Matrix for the product not the same as the experimental** conditions and may contain irritating active ingredients.	10	The area is the underarm ³³ ; the skin is easily irritated ³⁴ , highly follicular ³³ and an area that is shaved ³⁵ . Type of occlusion is similar to that of the experimental** test conditions ³⁶ .	300
Roll-On Deodorant	10	3*	Matrix for the product not the same as the experimental** conditions.	10	The area is the underarm ³³ ; the skin is easily irritated ³⁴ , highly follicular ³³ and an area that is shaved ³⁵ . Type of occlusion is similar to that of the experimental** test conditions ³⁶ .	300
Roll-On Antiperspirant	10	3*	Matrix for the product not the same as the experimental** conditions and may contain irritating active ingredients.	10	The area is the underarm ³³ ; the skin is easily irritated ³⁴ , highly follicular ³³ and an area that is shaved ³⁵ . Type of occlusion is similar to that of the experimental** test conditions ³⁶ .	300
Cream Deodorant / Antiperspirant	10	3*	Matrix for the product not the same as the experimental** conditions and may be designed to enhance penetration. May contain irritating active ingredients.	10	The area is the underarm ³³ ; the skin is easily irritated ³⁴ , highly follicular ³³ and an area that is shaved ³⁵ . Type of occlusion is similar to that of the experimental** test conditions ³⁶ .	300
Gel Deodorant / Antiperspirant	10	3*	Matrix for the product not the same as the experimental** conditions and may contain irritating active ingredients.	10	The area is the underarm ³³ ; the skin is easily irritated ³⁴ , highly follicular ³³ and an area that is shaved ³⁵ . Type of occlusion is similar to that of the experimental** test conditions ³⁶ .	300
Deodorant Cologne (Body sprays)	10	3*	Matrix for the product not the same as the experimental** conditions.	10	The area is whole body including underarm ³³ and mucous membranes ³⁷ ; the skin is easily irritated ³⁴ , highly follicular ³³ and an area that is shaved ³⁵ . Type of occlusion is similar to that of the experimental** test conditions ³⁶ .	300
Hydroalcoholic Products applied to unshaved skin	10	3*	Matrix for the product not the same as the experimental** conditions.	3	The area is the neck, wrists, antecubital fossa that may have increased permeability ³³ .	100
Hydroalcoholic Products Applied to recently shaved skin	10	3*	Matrix for the product not the same as the experimental** conditions.	10	The area is the face with increased permeability ³³ , highly follicular ³³ and possible abrasion from shaving ³⁵ .	300

Product Type	Inter-individual ¹⁻²⁷ SAF	Matrix SAF	Matrix SAF Rationale ^{6,26,28-32} (experimental** vs. real life exposure)	Use SAF	Use SAF Rationale (experimental** vs. real life exposure)	SAF
Men's Facial Cream and balms	10	3*	Matrix for the product not the same as the experimental** conditions and may be designed to enhance penetration. May contain irritating ingredients.	10	The area is the face with increased permeability ³³ , highly follicular ³³ and possible abrasion from shaving ³⁵ .	300
Eye Products (Includes: eye shadow, mascara, eyeliner, eye make-up)	10	3*	Matrix for the product not the same as the experimental** conditions, but not expected to be more irritating.	10	The area is the eye area with increased permeability and easily irritated ³⁸ .	300
Body Creams, lotions	10	3*	Matrix for the product not the same as the experimental** conditions and may be designed to enhance penetration.	10	The area is the entire body ³³ which may include, dry skin ³⁹ , abraded skin ³⁵ (e.g. underarms, legs) and semi- occlusion, due to clothing occurs.	300
Hand Cream	10	3*	Matrix for the product not the same as the experimental** conditions and may be designed to enhance penetration.	3	The area is mainly the hands, which may include dry skin ³⁹ , there may be compromised skin due to dermatitis ³⁴ , but occlusion does not occur.	100
Women's Facial Cream/Facial Make-up	10	3*	Matrix for the product not the same as the experimental** conditions and may be designed to enhance penetration. May contain irritating ingredients.	3	The area is the face with increased permeability ³³ .	100
Make-up remover	10	3*	Matrix for the product not the same as the experimental** conditions and may be designed to enhance penetration. May contain irritating ingredients.	3	The area is the face with increased permeability ³³ .	100
Lip Products	10	3*	Matrix is very different from the experimental** test conditions, however, it is not expected to be more irritating.	10	The site is highly vascular and there is exposure to mucous membranes ⁴⁹ and possible exposure to dry or chapped lips.	300
Foot care products	10	3*	Matrix for the product is not the same as the experimental** conditions and may be designed to enhance penetration.	1	The area is the feet, which are less permeable ³³ . Type of occlusion is similar to that of the experimental** test conditions ³⁶ .	30
Shaving creams	10	3*	Matrix for the product not the same as the experimental** conditions and may be designed to enhance penetration. May contain irritating ingredients.	10	The area is the face with increased permeability ³³ and highly follicular ³³ and possible abrasion from shaving ³⁵ .	300
Depilatory	10	10	Matrix is very different from the experimental** test conditions and contains highly irritating ingredients.	3	The area is the underarm, upper part of leg and lower part of the leg ³³ .	300
Body wash/shower gels	10	3*	Matrix for the product not the same as the experimental** conditions and may be designed to enhance penetration. May contain irritating ingredients.	3	The area is the entire body ³³ which may include, dry skin ³⁹ , abraded skin ³⁵ (e.g. underarms, legs) and possible exposure to mucous membranes ^{37,40-48} .	100
Hair styling aids (mousse, gels, leave in conditioners)	10	3*	Matrix is very different from the experimental** test conditions and may contain ingredients that are irritating.	3	The area is the head which is highly follicular ³³ and the scalp which is more permeable ^{33,49} .	100

Product Type	Inter-individual SAF ¹⁻²⁷	Matrix SAF	Matrix SAF Rationale ^{6,26,28-32} (experimental** vs. real life exposure)	Use SAF	Use SAF Rationale (experimental** vs. real life exposure)	SAF
Hair sprays	10	3*	Matrix for the product not the same as the experimental** conditions.	3	The area is the head which is highly follicular ³³ and the scalp which is more permeable ^{33,49} .	100
Shampoo	10	3*	Matrix for the product is very different from experimental** conditions and may contain irritating ingredients.	3	The area is the head which is highly follicular ³³ and the scalp which is more permeable ^{33,49} .	100
Conditioner (rinse-off)	10	3*	Matrix for the product not the same as the experimental** conditions and may be designed to enhance penetration. May contain irritating ingredients.	3	The area is the head which is highly follicular ³³ and the scalp which is more permeable ^{33,49} .	100
Bar soap	10	3*	Matrix for the product not the same as the experimental** conditions and may be designed to enhance penetration. May contain irritating ingredients.	3	The area is mainly the hands, but may include the entire body ³³ which may include, dry skin ³⁹ , abraded skin ³⁵ (e.g. underarms, legs), there may be compromised skin due to dermatitis ³⁴ and possible exposure to mucous membranes ^{37,40-48} .	100
Liquid soap	10	3*	Matrix for the product not the same as the experimental** conditions and may be designed to enhance penetration. May contain irritating ingredients.	3	The area is mainly the hands, which may include dry skin ³⁹ , there may be compromised skin due to dermatitis ³⁴ .	100
Face washes, gels, scrubs	10	3*	Matrix for the product not the same as the experimental** conditions and may be designed to enhance penetration. May contain irritating ingredients.	3	The area is the face with increased permeability ³³ .	100
Bath gels, foams, mousses	10	3*	Matrix for the product not the same as the experimental** conditions and may be designed to enhance penetration. May contain irritating ingredients.	3	The area is the entire body ³³ which may include, dry skin ³⁹ , abraded skin ³⁵ (e.g. underarms, legs) and possible exposure to mucous membranes ^{37,40-48} . Bathing involves a longer time of exposure to the product than showering. Conversely, product concentration is greater when showering than bathing.	100
Aerosol air fresheners	10	3*	Matrix for the product not the same as the experimental** conditions.	3	The area is the upper extremities and the face the latter of which has increased permeability ³³ .	100
Toothpaste	10	3*	Matrix is different from the experimental** test conditions and may contain irritating ingredients.	3	The sites are the lips and mouth which are highly vascular (these areas are a mixture of keratinized and non-keratinized skin). ^{37,50-57} Data suggest the peri-oral skin (a site of concern) is highly permeable ⁵⁸ and is exposed to oral care products that may not be removed. For many products, especially for buccal cavity exposure, rapid dispersion, limited contact time and salivary dilution would indicate a lower SAF for use considerations ³⁷ .	100

Product Type	Inter-individual ¹⁻²⁷ SAF	Matrix SAF	Matrix SAF Rationale ^{6,26,28-32} (experimental** vs. real life exposure)	Use SAF	Use SAF Rationale (experimental** vs. real life exposure)	SAF
Mouthwash	10	3*	Matrix for the product not the same as the experimental** conditions but, not expected to be more irritating than the experimental** conditions.	3	The sites are the lips and mouth which are highly vascular (these areas are a mixture of keratinized and non-keratinized skin). ^{37,50-57} Data suggest the peri-oral skin (a site of concern) is highly permeable ⁵⁸ and is exposed to oral care products that may not be removed. For many products, especially for buccal cavity exposure, rapid dispersion, limited contact time and salivary dilution would indicate a lower SAF for use considerations ³⁷ .	100
Nail care	10	3*	Matrix for the product is not the same as the experimental** conditions, is highly solvent based and expected to be more irritating than the experimental** test conditions.	3	The area is the nail, which is less permeable ⁵⁹ but there may be compromised skin due to dermatitis ³⁴ .	100
Candle not in a jar	10	1	Fragrance is not freely available for release from the matrix, unlike experimental** conditions.	1	Brief contact with fingers ⁶⁰ .	10
Closed air fresheners	10	1	Enclosed product; limited contact with fragrance.	1	Closed product, only rare accidental contact may occur.	10
Feminine hygiene conventional pads, liners, interlabial pads	10	1	Matrix is different from the experimental** test conditions, however, it is not expected to be more irritating and the matrix is an inert material and not skin impactful.	10	The area is vulval mucous membrane ^{37,40-48} ; Type of occlusion is similar to that of the experimental** test conditions ³⁶ .	100
Intimate wipes	10	3*	Matrix is different from the experimental** test conditions, however, it is not expected to be more irritating.	10	The area is vulval mucous membrane ^{37,40-48} and outer labia, which are highly follicular. ³³ Type of occlusion, due to under clothing, is similar to that of the experimental** test conditions ³⁶ .	300
Tampons	10	1	Matrix is very different from the experimental** test conditions, however, it is not expected to be more irritating and the matrix is an inert material and not skin impactful.	20 ³⁷	The area is vaginal mucous membrane ^{37,40-48} includes non-keratinized mucous membrane - increased permeability. ^{52,58,61} The nature of occlusion is different, but effect is expected to be similar to that of the experimental** test conditions ³⁶ .	200
Baby diapers	10	1	Matrix is very different from the experimental** test conditions, however, it is not expected to be more irritating and the matrix is an inert material and not skin impactful.	10	The area is the baby's buttocks, groin, lower stomach and upper thighs and the skin integrity may be compromised (diaper rash) ⁶² and involve mucous membrane exposure ^{37,40-48} . There is occlusion through diaper use ⁶² .	100
Baby wipes	10	3*	Matrix is different from the experimental** test conditions, however, it is not expected to be more irritating.	10	The area is primarily the baby's buttocks, groin, lower stomach and upper thighs and the skin integrity may be compromised (diaper rash) ⁶² and involve mucous membrane exposure ^{37,40-48} . There may be occlusion through diaper use ⁶² .	300

Product Type	Inter-individual ¹⁻²⁷ SAF	Matrix SAF	Matrix SAF Rationale ^{6,26,28-32} (experimental** vs. real life exposure)	Use SAF	Use SAF Rationale (experimental** vs. real life exposure)	SAF
Baby shampoo	10	3*	Matrix for the product is very different from experimental** conditions and may contain irritating ingredients.	3	The area is the head (scalp more permeable) ³³ or possibly whole body ³³ and mucous membrane exposure (body wash) ^{37,40-48} .	100
Baby wash, bath	10	3*	Matrix for the product is very different from experimental** conditions and may contain irritating ingredients.	3	The area is possibly whole body ³³ and the skin integrity may be compromised (diaper rash) ⁶² and mucous membrane exposure (body wash) ^{37,40-48} .	100
Baby cream	10	3*	Matrix for the product is designed to enhance penetration.	10	The area is possibly whole body ³³ or head (scalp more permeable) ³³ and the skin integrity may be compromised (diaper rash) ⁶² and mucous membrane exposure (body wash) ^{37,40-48} . There may be occlusion through diaper use ⁶² .	300
Baby oil	10	3*	Matrix for the product is designed to enhance penetration.	10	The area is possibly whole body ³³ or head (scalp more permeable) ³³ and the skin integrity may be compromised (diaper rash) ⁶² and mucous membrane exposure (body wash) ^{37,40-48} . There may be occlusion through diaper use ⁶² .	300
Baby powder	10	1	Matrix is different from the experimental** test conditions, however, it is not expected to be more irritating and the matrix is an inert material and not skin impactful.	10	The area is possibly whole body ³³ and the skin integrity may be compromised (diaper rash) ⁶¹ and mucous membrane exposure ^{37,40-48} . There may be occlusion through diaper use ⁶² .	100
Handwash laundry detergent	10	3*	Matrix for the product is very different from experimental** conditions and may contain irritating ingredients.	3	Hands and lower arms ³³ . May involve skin sites with dermatitis ³⁴ .	100
Laundry pre-treatment	10	3*	Matrix for the product is very different from experimental** conditions and may contain irritating ingredients.	3	Hands and lower arms ³³ . May involve skin sites with dermatitis ³⁴ .	100
Hand dishwashing detergent	10	3*	Matrix for the product is very different from experimental** conditions and may contain irritating ingredients.	3	Hands and lower arms ³³ . May involve skin sites with dermatitis ³⁴ .	100
Hard surface cleaner	10	3*	Matrix for the product is different from experimental** conditions and may contain solvents and other irritating ingredients.	3	Hands and lower arms ³³ . May involve skin sites with dermatitis ³⁴ .	100

*For practical purposes the number 3 is the practical representation of 3.16 (half log of 10)]

**Experimental in this context is defined in Section 3.2.2.1 Guidelines for applying weight of evidence approach to induction sensitization data on fragrance ingredients

Note: Products that contain sunscreens are not addressed separately but are included in the major product types (e.g. lip creams with sunscreen are included in lip product category).

- | | | | | |
|-----------------------------------|------------------------------------|--------------------------------------|------------------------------------|---------------------------------------|
| 1. Travis and White, 1988 | 14. West <i>et al.</i> , 1981 | 27. Weigand, 1974 | 40. Britz and Maibach, 1979 | 53. Lesch <i>et al.</i> , 1989 |
| 2. Chappell and Mordenti, 1991 | 15. Holbrook, 1982 | 28. Robinson <i>et al.</i> , 2000 | 41. Britz and Maibach, 1979a | 54. Squier and Hall, 1985 |
| 3. Renwick and Lazarus, 1998 | 16. McCormack <i>et al.</i> , 1982 | 29. Smith <i>et al.</i> , 2000 | 42. Elsner and Maibach, 1990 | 55. Squier, 1986 |
| 4. Burin and Saunders, 1999 | 17. Wester and Maibach 1982 | 30. Cumberbatch <i>et al.</i> , 1993 | 43. Elsner <i>et al.</i> , 1990 | 56. Squier, 1991 |
| 5. Aldridge, <i>et al.</i> , 2003 | 18. Fairley and Rasmussen, 1983 | 31. Scheuplein and Ross, 1970 | 44. Elsner <i>et al.</i> , 1990a | 57. Sayani and Chien, 1996 |
| 6. Felter <i>et al.</i> , 2002 | 19. Hargin and Rutter, 1983 | 32. Schaefer and Redelmeier, 1996 | 45. Elsner <i>et al.</i> , 1990b | 58. Kobayashi and Tagami, 2004a |
| 7. Robinson, 1999 | 20. Kalia <i>et al.</i> , 1998 | 33. Feldmann and Maibach, 1967 | 46. Elsner <i>et al.</i> , 1990c | 59. American Beauty Association, 2000 |
| 8. Smith and Hotchkiss, 2001 | 21. Leveque <i>et al.</i> , 1984 | 34. Benfeldt <i>et al.</i> , 1999 | 47. Elsner <i>et al.</i> , 1991 | 60. Selim, 2005 |
| 9. Dupuis, 1979 | 22. Ghadially <i>et al.</i> , 1995 | 35. Edman, 1994 | 48. Farage and Maibach (2004) | 61. Thompson <i>et al.</i> , 2001 |
| 10. Friedmann and Moss, 1985 | 23. Jordan and King, 1977 | 36. Bucks, <i>et al.</i> , 1989 | 49. Zhai <i>et al.</i> , 2004 | 62. Odio <i>et al.</i> , 2000 |
| 11. Moss <i>et al.</i> , 1985 | 24. Rees <i>et al.</i> , 1989 | 37. Farage <i>et al.</i> , 2003 | 50. Kobayashi and Tagami, 2004 | |
| 12. Cassimos <i>et al.</i> , 1980 | 25. Young <i>et al.</i> , 1988 | 38. Nuutinen <i>et al.</i> , 2003 | 51. de Vries, <i>et al.</i> , 1991 | |
| 13. Cunico <i>et al.</i> , 1977 | 26. Kligman, 1966 | 39. Matts and Rawlings, 2005 | 52. Harris and Robinson, 1992] | |

3.3. Exposure

3.3.1. Dose metric

The dose metric recommended for use in dermal sensitization risk assessments for fragrance ingredients is dose/area ($\mu\text{g}/\text{cm}^2$).

It is important to know the applied versus the delivered dose since there are factors that can affect the effective amount of material delivered to the viable epidermis – evaporation, binding/sequestration in the skin, metabolism (inactivation and activation).

Throughout the skin sensitization literature, historical and current, allergen exposures are most commonly expressed in terms of percent (i.e. weight of allergen per volume applied to the skin). This leads to the assumption that in any given test system an equal percentage exposure will lead to a similar incidence and/or severity of skin sensitization.

Based upon the understanding of the immunological mechanism involved, it is logical to assume that for an immune response to be initiated, a certain number of Langerhans Cells (LC) are required to be activated in order to initiate the cascade of events to exceed a threshold of induction for skin sensitization. This would suggest that for the induction of contact allergy, the application of an amount of allergen expressed as percent weight volume is not as important as understanding both the dose applied and the surface area over which the allergen is applied. This is diagrammatically expressed in Figure 2.

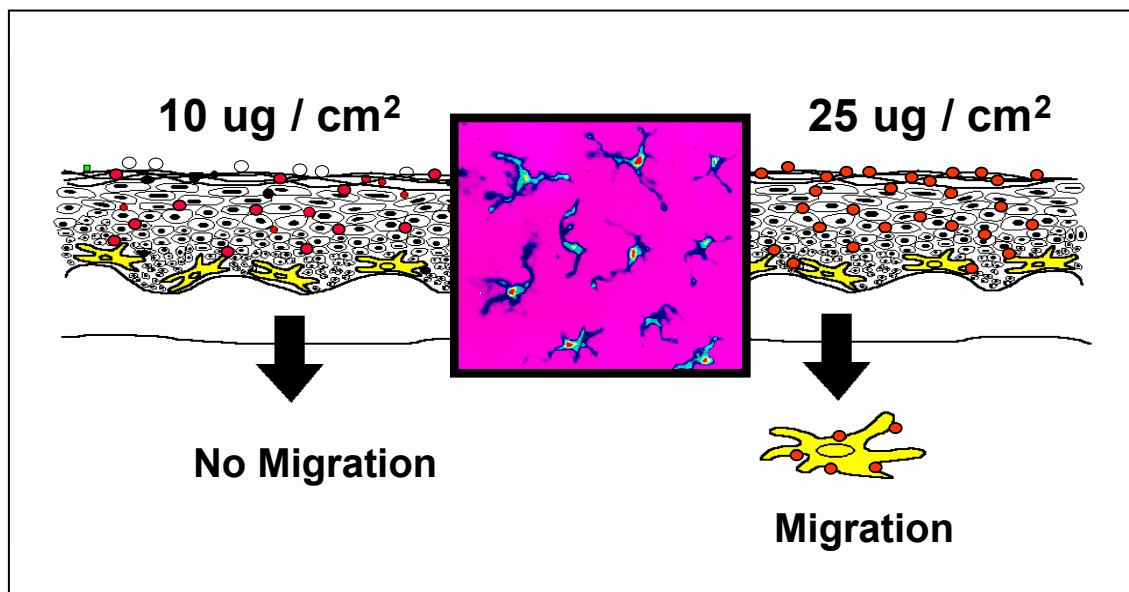


Figure 2: Importance of dose/unit area in skin sensitization.

Published data that support the use of this dose metric for the induction of skin sensitization is both robust and convincing in humans and animals. There are a number of literature references to support this position (Kligman, 1966;

Magnusson and Kligman, 1970; Friedmann and Moss, 1985; White *et al.*, 1986; Rees *et al.*, 1990; Upadhye and Maibach, 1992). A selection of the most noteworthy references is briefly reviewed below.

One of the most important pieces of work in this area was conducted by Kligman in 1966 on the development of a human skin sensitization test. There were several key parameters that were investigated during his study. These were sensitizing areas of exposure, stimulation of more than one lymph node using various sites, the use of a number of smaller patches versus one larger patch. The results of these studies are summarized below.

Using four different allergens (ammoniated mercury, monobenzyl ether of hydroquinone, nickel sulfate and neomycin sulfate) with different allergenic potencies, surface concentrations (amount per area) were kept constant and the application areas varied (see Table 3). Results indicated that an equal proportion of persons were sensitized at areas of 14 and 56 cm² for all four allergens. However, when the exposure area was significantly smaller, 0.36 cm², the rate of sensitization was reduced suggesting that a minimum surface area is required. Kligman concluded that the most notable finding was that the total quantity of allergen is not important when the surface and vehicle concentrations are constant.

Table 3: The quantity of allergen / area was held constant by adjusting the volume applied to the specific area (Kligman, 1966).

Allergen	Sensitization Incidence		
	0.25 inch patch (0.36 cm ²)	1.5 inch patch (14 cm ²)	3.0 inch patch (56 cm ²)
Ammoniated mercury	6/22	12/24	12/23
Monobenzyl ether of hydroquinone	10/22	20/24	21/23
Nickel sulfate	2/22	4/24	5/23
Neomycin sulfate	0/22	5/24	8/23

He also showed that the sensitization rates were similar whether the subject was sensitized with four 1.5 inch (56 cm²) patches versus one 3 inch square patch (56 cm²) on the arm (Table 4).

Table 4: Human skin sensitization: Dose per unit area and patch number (Kligman, 1966).

Allergen	Sensitization Incidence	
	4-1.5 inch patch (56 cm ²)	1-3.0 inch patch (56 cm ²)
Ammoniated mercury	12/23	10/25
Monobenzyl ether of hydroquinone	21/23	23/25
Nickel sulfate	5/23	7/25
Neomycin sulfate	8/23	5/25

Kligman determined that two 1.5 inch patches on separate forearms were not more effective than one such patch on a single arm when using 4 different allergens. Thus, suggesting that doubling of area is of small significance even when involving more than one node. He concluded that sensitization on different extremities (involvement of more than one node) did not materially affect the sensitization rate though did have some impact on intensity of response observed at challenge. These results demonstrate the importance of dose/unit area when stimulating more than one lymph node for induction of skin sensitization are detailed in Table 5.

Table 5: Human skin sensitization: dose per unit area and number of extremities patched (Kligman, 1966).

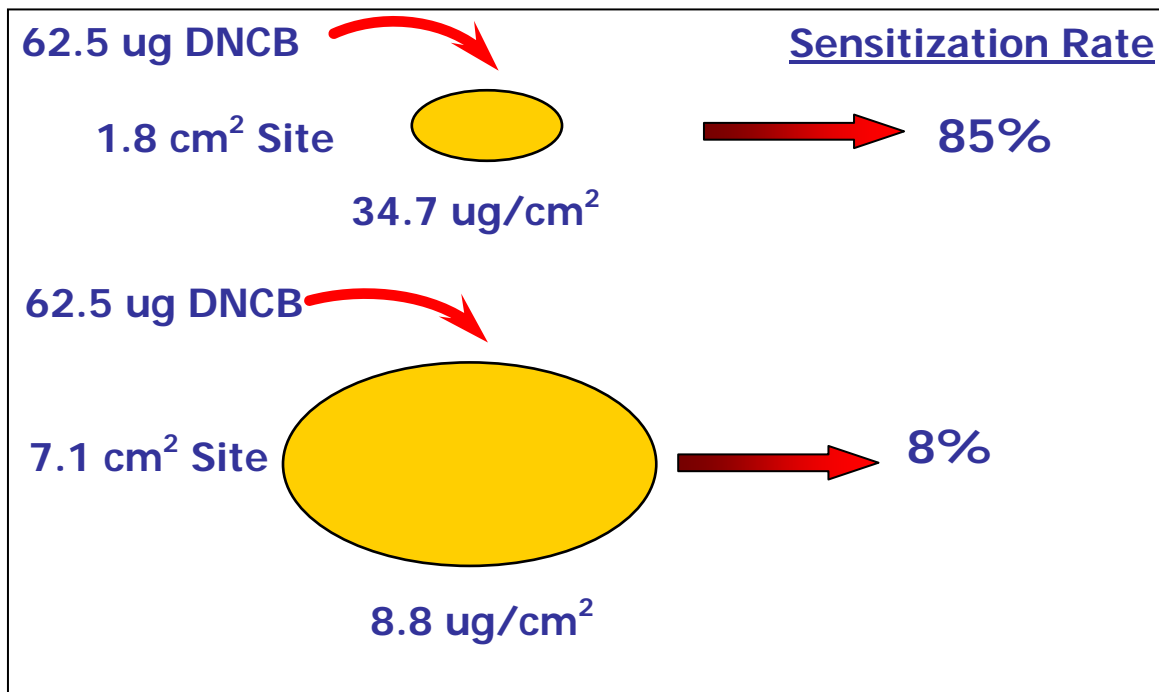
Allergen	Sensitization Incidence	
	1 Arm (14 cm ²)	2 Arms (28 cm ²)
Ammoniated mercury	12/24	15/23
Monobenzyl ether of hydroquinone	20/24	19/23
Nickel sulfate	4/24	7/23
Neomycin sulfate	5/24	7/23

In another study reported in Kligman 1966, different exposure conditions of hydroquinone were applied to one forearm versus over the entire body. The total dose applied in these two scenarios was similar (although not identical). The difference between these very different exposure scenarios (forearm vs. entire body) is that the dose per unit area was much greater on the forearm than for the entire body exposure and the skin sensitization rate was much greater for the forearm. These data are detailed in Table 6.

Table 6: Sensitization rate for forearm vs. whole body exposure to hydroquinone (Kligman, 1966).

	Forearm	Whole Body
Exposure Conditions	3 g of 20% hydroquinone 2X day/4 weeks	45 g of 1% hydroquinone 2X day/4 weeks
Estimated Dose/Area	1140 $\mu\text{g}/\text{cm}^2$	49 $\mu\text{g}/\text{cm}^2$
Total Dose Applied	1.2 g	0.9 g
Sensitization Rate	6/46	0/43

Work conducted by Friedmann and his colleagues in the 1980's (Friedmann *et al.*, 1983) clearly demonstrated that the total dose of allergen per area of skin (e.g. $\mu\text{g}/\text{cm}^2$) is the critical exposure determinant for the induction of contact sensitization, not the percent concentration. Human subjects were exposed to varying amounts of the potent contact allergen, dinitrochlorobenzene (DNCB) applied to a constant area. The amount of DNCB used ranged from 62.5 μg to 1000 μg applied to a 3 cm diameter circular area (7.1 cm^2). Only 8% of the subjects exposed to 62.5 μg (a dose/area of $8.8 \mu\text{g}/\text{cm}^2$) became sensitized while allergy was induced in 83% of the subjects exposed to 250 μg ($35.4 \mu\text{g}/\text{cm}^2$). 93% to 100% of the subjects exposed to 500 μg or more ($\geq 70.4 \mu\text{g}/\text{cm}^2$) were sensitized (see Figure 3).



Abstracted and amended from Upadhye and Maibach, 1992.

Figure 3: Influence of area of application of allergen on sensitization testing.

In additional studies conducted by White *et al.*, 1986, the amount of DNCB applied was held constant while the area of application was varied. Two groups of subjects were exposed to $35.4 \mu\text{g}$ DNCB/ cm^2 ; one group received a total dose

of 250 µg applied to a 7.1 cm² area while the other received 62.5 µg DNCB over 1.8 cm². The sensitization rates observed for the groups, 83% and 86% respectively, were essentially the same (see Table 7).

Table 7: Human skin sensitization: applied dose and area vary but dose/unit area constant (White *et al.*, 1986).

Applied Dose (µg)	Application Area (cm ²)	Dose/Unit Area (µg/cm ²)	Sensitization Incidence
58	3.5	16.4	12/22 (55%)
116	7.1	16.4	17/34 (50%)
232	14.2	16.4	10/15 (66%)
250	7.1	35.4	25/30 (83%)
62.5	1.8	35.4	6/7 (85%)

Rees *et al.*, 1990 looked at the relationship between dose per area and sensitization rate. They induced two groups of subjects with the same dose /unit area (38 µg/cm²) by applying 30 µg of DNCB to an area of 0.8 cm² to one group or 3 µg of DNCB to an area of 0.08 cm² in the second group. One month later, sensitivity was determined by measurement of responses to a graded series of challenge doses applied simultaneously: subjects were challenged with three doses of DNCB (8.8, 12.5, 17.5 µg) on a 0.8 cm² site (resulting in challenge doses of 11.0, 15.6 and 21.9 µg/cm²). The results indicate that at very small areas, under 0.1 cm², the dose response is significantly diminished. This would suggest there is a minimum area of contact required to induce contact allergy.

Magnusson and Kligman (1970) conducted guinea pig experiments that were designed to examine the importance of dose per unit area versus total dose. Increasing the area by ten and one hundred times but keeping the dose per unit area constant had no impact on the sensitization rates when using three different chemical allergens [DNCB, p-nitroso-dimethylaniline (NDMA), p-phenylenediamine (PPDA)]. However, as was observed in humans, when a small area was used (25 mm²) there was a reduction in the rate of sensitization with PPDA suggesting that a minimum area is required for optimal sensitization. The authors concluded from these guinea pig studies that the concentration of allergen per unit area of surface was most important and not the size of the area exposed or the total quantity of allergen applied (Table 8). The results verify earlier observations of Schnitzer (1942) where he showed similarly that with an identical surface concentration of DNCB the sensitization rates were the same whether the allergen was applied to half the total integument or to a 2.5 cm circle.

Table 8: Guinea pig experiments showing the importance of dose/unit area vs. total dose (Magnusson and Kligman, 1970).

Allergen, Concentration	Volume (mL)	Area (mm ²)	Dose/Area (µg/mm ²)	Number of Positive/Total
DNCB, 0.1%	0.01	25	0.4	5/25
	0.1	250	0.4	9/25
	1.0	2500	0.4	9/24
NDMA, 0.1%	0.01	25	0.4	18/27
	0.1	250	0.4	16/25
	1.0	2500	0.4	13/25
PPDA, 0.1%	0.01	25	0.4	15/25
	0.1	250	0.4	22/26
	1.0	2500	0.4	25/26

3.3.2. Consumer Exposure Level (CEL)

Consumer exposure level (CEL) is an essential element of QRA. As such it is important to understand how consumers will be exposed to fragrance ingredients from use of the consumer products. Exposure levels occurring under intended and foreseeable conditions of use, but not abuse need to be addressed. The calculation of consumer exposure must include parameters such as frequency, use practices (e.g. how a consumer actually uses the product), duration of use and amount of product used per application/use. It should be noted that the CEL defined within this technical dossier addresses consumer products that are bought for personal use. Occupational/professional exposure is not included at this time because comprehensive exposure data are not available. It will be important to address occupational/professional exposure in the QRA approach when these exposure data become available. This is explored further in the Expert Group's recommendations for refinement of the QRA approach.

The CEL is expressed as dose/unit area/day. This takes into account that frequency of product use may be more than once a day and may, depending upon the physical chemical properties of the material, lead to accumulation of material on the same skin site. It is recognized that this is a conservative approach, but is considered to be appropriate for the dermal sensitization endpoint. For frequency of use less than once per day, the conservative default of once per day was used with the exception of nail care products. When it is known that products are used in a regimen, such cumulative exposure should be taken into account. Although it is desirable to use aggregate exposure, there are insufficient data to allow this to occur at this time. This is identified as an area of refinement for a QRA approach. On this basis it is important to have reliable habits and practices data. It is equally important to have accurate human parameters data such as the body surface area over which the product is used. Skin penetration is not specifically addressed in measuring consumer exposure since the dose metric is unit weight applied per unit area of skin. As such, using a conservative approach, the applied dose is taken to be the delivered dose. Differences in skin penetration due to different product matrices is accounted for in the final risk assessment by use of the matrix uncertainty factor as previously discussed.

Using these criteria, the data sources listed below were used in the calculation of CEL. A hierarchy was established for how to use the data based on robustness, scope and whether data were measured or reported. The data sources listed below are listed according to this hierarchical approach. When measured data for the same product type were available from more than one source, then the most conservative value (i.e. the highest value) was used unless there was a sound scientific rationale to use data from another source (e.g. 1) Cano & Rich hydroalcoholic data were used over the CTFA hydroalcoholic data because the former reported distributions of amount, frequency and surface area in the same study while CTFA reported a distribution only of amounts in their study, 2) the Colipa, 2005 exposure study data were used over the CTFA data published in Loretz *et al.*, 2005 on the basis the Colipa study participants used their own products rather than products supplied by the study investigator as in the CTFA study).

- Habits and Practices Data – there are several sources of data:
 1. Cano & Rich, 2001; Tozer *et al.*, 2004 and Cano, 2006 data on hydroalcoholic products - measured distribution of amount, frequency and surface area.
Status: data available from company report and reported in presentations, but unpublished in full
 - 2.A. Colipa data - measured frequency, duration and amount for seven different consumer products, analysis based on a probabilistic basis.
Status: data available, but unpublished.
 - 2.B. Colipa data – measured frequency, duration and amount for different consumer products, based on a collated Colipa member companies.
Status: It should be noted that these additional exposure on was requested of Colipa by the SCCP in October 2005. These data were provided to the SCCP in December 2005. (Colipa, 2005)
 - 2.C. CTFA data – measured frequency, duration and amount for twelve different consumer products (CTFA, 2002; CTFA, 2002a; CTFA, 2004; CTFA, 2004a; CTFA, 2004b; Loretz *et al.*, 2005; CTFA, 2005; CTFA, 2005a; CTFA, 2005b)
Status: data available, both published and unpublished
 - 2.D. AISE/HERA, 2002 and Technical Guidance Document, 2003
Status: data available on HERA website
 - 2.E. FMA data – (Selim *et al.*, 2005)
Status: data available, but unpublished

3. RIFM data - measured frequency, duration and amount for different consumer product types, based on RIFM member company data.
Status: data available, but unpublished (RIFM, 2005; 2006)
- 4.A. EC data (EC, 1996. Technical guidance document in support of Commission Directive 93/67/EEC on risk assessment for new notified substances and Commission Regulation (EC) No 1488/94 on risk assessment for existing substances.)
Status: published data and used only when measured data are not available
- 4.B. SCCNFP Guidelines, 2003 – reports frequency, duration and amount (SCCNFP, 2003)
Status: published data and used only when measured data are not available

All of the above sources of exposure data are based on information of varying detail and completeness. This means that the robustness of the exposure data can also be different. For these reasons when evaluating a distribution of exposure data, the same percentile data point cannot be selected for each set of exposure data. For example, the 90th percentile was chosen from the Colipa exposure study to define the most appropriate exposure level. This was based on the incorporation of two very conservative assumptions in the exposure model used in this study. These were 1) it was assumed that all products purchased were used and 2) the scaling factor used to expand the data to a Europe-wide distribution was the highest factor possible derived from data on the amount of product used per application. On the other hand, whilst the study conducted by Cano & Rich, 2001, Tozer *et al.*, 2004 and Cano, 2006 measured distribution of amount, frequency of use and surface area it did not include the same conservatisms as the Colipa study. On this basis it was more appropriate to choose a higher percentile from this study and therefore the 95th percentile was chosen.

- Human Parameters Data – there are three sources of data. Again using a hierarchal approach, when data were available from all sources, a conservative approach was employed by using the smaller surface area. This resulted in a higher CEL.
 1. EPA (EPA, 1997) – body surface area estimates based on direct measurement. For the purposes of this technical dossier, 50th percentile surface areas were chosen consistent with the approach used by SCCNFP in their Notes of Guidance (SCCNFP, 2003).
 2. RIVM (Bremmer *et al.*, 2003) – body surface area estimates based on a computer program, CONSEXPO. CONSEXPO is used to calculate exposure in consumer products, using mathematical

models. Default models and default values have been determined per product category for use in the assessment of consumer exposure to compounds in cosmetics.

3. Individual published data for oral care (Collins *et al.*, 1987) – the surface area of the adult human mouth was measured in 10 adults/sex and expressed as the mean total surface area.
4. Individual published data for lip (Ferrario *et al.*, 2000) - the surface area of the adult female human lip was measured in 96 women and expressed as the mean total surface area.

Within these data sources, the individual references used to define the consumer exposure to different product types are detailed in Table 9.

Table 9: Summary of available habits and practices and human parameters data used in the calculation of consumer exposure to different product types (exposures used in the QRA method are bolded and highlighted).

Product Type	Surface Area cm ²	Surface Area Reference	Retention Factor ¹	EC or SCCNFP ¹			CTFA ²		Cano & Rich, 2001; Tozer <i>et al.</i> , 2004; Cano, 2006 ³	Colipa			HERA ⁴	FMA ⁵	RIFM ⁶	
				mg/ application	applications/ day	mg/cm ² / day	90 th Percentile			95 th Percentile	90 th Percentile					Dec. 2005
							mg/day	mg/cm ² / day			mg/cm ² / day	mg/day				
Deo/AP - Type not specified	100	Bremmer, 2003, per axillae	1	500	1	2.50										
Deo/AP Spray	100	Bremmer, 2003, per axillae	1							6100*	30.5*					
Deo/AP Non-Spray	100	Bremmer, 2003, per axillae	1							1500	7.5					
Deo/AP All Over Body	100	Bremmer, 2003, per axillae	1							6500*	32.5*					
Solid AP	100	Bremmer, 2003, per axillae	1				1700	8.50**								
Shaving Cream/ Depilatory ^{7,8}	305	Bremmer, 2003 (1/4 area head, male)	0.01	2000	1	0.07										
Lip Products	4.8	Ferrario <i>et al.</i> , 2000	1	10	4	8.33	55	11.46		56	11.67					
Eye Products ⁹	24	Bremmer, 2003	1	10	2	0.83	52	2.17				2.5				
Body Cream/Lotion	12895	EPA, 1997 (area body - head and 1/2 trunk, female) ¹⁰	1	8000	0.5	0.31	14400	1.12		7800	0.60					
Men's Facial Cream	775	Bremmer, 2003 (1/4 area head + 1/2 area hands, male)	1	800	2	2.06										
Toothpaste	216.8	Collins <i>et al.</i> , 1988; Ferrario <i>et al.</i> , 2000 (buccal + lips)	0.1 ¹¹	1400	2	1.29				2700	1.25		1.0 ¹¹			
Mouthwash	216.8	Collins <i>et al.</i> , 1988; Ferrario <i>et al.</i> , 2000 (buccal + lips)	0.01 ¹¹	10000	3	1.38						1.38	1.0 ¹¹			

Product Type	Surface Area cm ²	Surface Area Reference	Retention Factor ¹	EC or SCCNFP ¹			CTFA ²		Cano & Rich, 2001; Tozer et al., 2004; Cano, 2006 ³	Colipa			HERA ⁴	FMA ⁵	RIFM ⁶	
				mg/ application	applications/ day	mg/cm ² / day	90 th Percentile			95 th Percentile	90 th Percentile					Dec. 2005
							mg/day	mg/cm ² / day			mg/day	mg/cm ² / day				
Hydroalcoholic Products for Shaved Skin ¹²	775	Bremmer, 2003 (1/4 area head + 1/2 area hands, male)	1						2.21							
Hydroalcoholic Products for Unshaved Skin	100	Bremmer, 2003, perfume spray	1				1770	17.70	2.21							
Women's Facial Cream	555	EPA, 1997 (1/2 area head, female)	1	800	2	2.88	3500	6.31		1500	2.70					
Women's Facial Liquid Make-up	555	EPA ³ (1/2 area head, female)	1				1760	3.17			1.08					
Hair Sprays – type not specified	555	EPA, 1997(1/2 area head, female)	0.1	2700	2	0.97										
Hair Sprays – Aerosol ¹²	555	EPA, 1997(1/2 area head, female)	0.1				7730	1.39			0.45					
Hair Sprays - Pump Spray ¹²	555	EPA, 1997(1/2 area head, female)	0.1				12220	2.20***								
Hair Styling Aids	1010	Bremmer, 2003 & EPA, 1997 (1/2 area hands +1/2 head)	0.1	5000	2	0.99						0.15 (mousse) 0.5 (gel)				
Shampoo	1430	EPA, 1997 (area hands + 1/2 head)	0.01	8000	1	0.056	23630	0.17		10500	0.07					
Conditioners, Rinse-off	1430	EPA, 1997 (area hands +1/2 head)	0.01	14000	1	0.098	28200	0.20								
Make-up Remover	555	EPA, 1997 (1/2 area head, female)	0.1	2500	2	0.90						0.3				
Nail care	11	RIVM ²	0.1	250	0.43	0.97										
Bar Soaps	840	EPA, 1997 (area hands)	0.01	800	6	0.057						0.05				

Product Type	Surface Area cm ²	Surface Area Reference	Retention Factor ¹	EC or SCCNFP ¹			CTFA ²		Cano & Rich, 2001; Tozer et al., 2004; Cano, 2006 ³	Colipa			HERA ⁴	FMA ⁵	RIFM ⁶	
				mg/ application	applications/ day	mg/cm ² / day	90 th Percentile			95 th Percentile	90 th Percentile					Dec. 2005
							mg/day	mg/cm ² / day			mg/day	mg/cm ² / day				
Liquid Soap	840	EPA, 1997 (area hands)	0.01									0.2				
Hand Cream	840	EPA, 1997 (area hands)	1									4.2				
Face Washes, Gels, Scrubs	555	EPA, 1997 (1/2 area head, female)	0.01	800	2	0.03	8300	0.15								
Body Wash Gels, Foams, Mousses	16900	EPA, 1997 (body area, female)	0.01	5000	2	0.006	25500	0.015				0.009				
Bath Foams, Gels, Mousses ⁸	16900	EPA, 1997 (body area, female)	0.01	17000	1	0.010										
Feminine Hygiene - Tampons															2.9	
Feminine Hygiene -Pads															0.14	
Feminine Hygiene -Liners															0.14	
Baby Diapers															0.0006	
Baby Wipes															4.0	
Intimate Wipes															4.4	
Aerosol Air Freshener	3425	EPA, 1997 (1/2 area head + upper extremities, female)	1												0.025	
Handwash Laundry													0.1			
Laundry Tablets & Powder													Insignificant			
Hand Dishwashing													0.01			
Fabric Clothing													Insignificant			

Product Type	Surface Area cm ²	Surface Area Reference	Retention Factor ¹	EC or SCCNFP ¹			CTFA ²		Cano & Rich, 2001; Tozer <i>et al.</i> , 2004; Cano, 2006 ³	Colipa			HERA ⁴	FMA ⁵	RIFM ⁶	
				mg/ application	applications/ day	mg/cm ² / day	90 th Percentile			95 th Percentile	90 th Percentile					Dec. 2005
							mg/day	mg/cm ² / day			mg/cm ² / day	mg/day				
Hard Surface Cleaner													0.12			
Candles														0.00033		

*These data should not be used due to logistical difficulties with determination of the actual amount of product delivered on skin (Colipa,2005).

**This exposure value is used in the QRA for fragrance ingredients for all types of deodorants and antiperspirants.

***This exposure value is used in the QRA for fragrance ingredients for all types of hair sprays.

Note: Products that contain sunscreen are not addressed separately but are included in the major product type (e.g. lip creams with sunscreen are included in lip product category).

- 1) EC, 1996 or SCCNFP, 2003 Guidelines
- 2) CTFA, 2002; CTFA, 2002a; CTFA, 2004; CTFA, 2004a, CTFA, 2004b; Loretz *et al.*, 2005; CTFA, 2005; CTFA, 2005a²; CTFA, 2005b
- 3) Cano and Rich, 2001; Tozer *et al.*, 2004; Cano, 2006
- 4) HERA, Technical Guidance Document, 2003
- 5) Selim, 2005
- 6) RIFM, 2005, AM Api, Internal memo December 12, 2005, on dermal exposure to pressurized aerosol air fresheners. RIFM, 2006, Memo to AM Api from RIFM Member Company, May 2006 on exposure to feminine hygiene products and baby wipes.
- 7) Shaving cream/ depilatory cream products – the amount used was derived from the EC, 1996 Technical Guidance Document in support of Commission Directive 93/67/EEC on risk assessment for new notified substances and Commission Regulation (EC) No 1488/94 on risk assessment for existing substances. This reference did not distinguish between shaving the face or shaving the leg. As such, the dose/unit area for shaving the face was calculated and the same value was applied to shaving or depilating the legs. In the absence of more robust data, this was assumed to be a reasonable and conservative approach.
- 8) For frequency of use less than once per day, the default of once per day was used with the exception of nail care products.
- 9) Eye products – This is based on the CTFA measured data for all types of eye shadows from a specifically designed exposure study for eye products. The SCCNFP, 2003 exposure data on mascara product types were not used for the eye product category because there is little if any skin contact from this product type.
- 10) Body cream/lotion – The surface area comprises the total body surface area for a female minus the area of the head and half the trunk. This is based on habits and practices data for adults that indicate that body lotion is not applied to the head or the back.
- 11) These are product dilution factors. Different dilution factors are used for mouthwashes and toothpastes. The dilution factor used for mouthwashes is 1% or 0.01 and that used for toothpastes is 10% or 0.1. These values are different from the values used in the SCCNFP 2003 Guidelines, but considered to be more relevant since it takes into account the amount remaining in the oral cavity and perioral area rather than that ingested. It also takes into account salivation and distribution across the oral cavity surface (Muhlemann and Rudolf, 1975; Zero *et al.*, 1988; Issa and Toumba, 2004). The difference in the dilution factors used for mouthwashes and toothpastes is based on the fact that while very different volumes of each product are applied (i.e., 30 g/day of mouthwash vs. 2.7 g of toothpaste), it is reasonable to expect that similar amounts of product would be in contact with the mouth (buccal cavity and lips) at any one time since the same surface area is involved. The exposure to oral care products (toothpastes and mouthwashes) is impacted by salivation, product dilution and distribution across the oral surfaces and the focus for sensitization reactions is the perioral area. As such, in order to benchmark against the exposure approach used here, a worst case exposure scenario was evaluated using the principles of HERA. In HERA, it was assumed that a 0.01 cm film thickness was left on the skin (Vermeire *et al.*, 1993) from a 10% aqueous product solution. This would result in a worst case exposures of 1mg/cm², assuming 100% retention of the fragrance ingredient from the product solution. This is consistent with the value identified by the primary exposure approach.
- 12) Hair Spray – exposure for the pump spray is recommended for all hair sprays since this figure was the most conservative (e.g. highest) value.

3.4. Risk characterization

Once the NESIL, SAF and CEL for fragrance ingredients in the different product types are defined, it is possible to proceed to risk characterization using the principles of exposure-based quantitative risk assessment. There are two key elements involved in risk characterization in the recommended approach. These are the Acceptable Exposure Level (AEL) (see section 3.4.1 for a definition) and the comparison of that AEL to the CEL (AEL/CEL) (see section 3.4.2 for a definition). The practical application of risk characterization to the identification of product categories is detailed in section 3.4.3.

3.4.1. Acceptable Exposure Level (AEL)

The Acceptable Exposure Level (AEL) is determined by dividing the WoE NESIL by the product type SAF (WoE NESIL/SAF).

$$\text{AEL} = \frac{\text{WoE NESIL}}{\text{SAF}}$$

The AEL is expressed in terms of dose/unit area per diem. The definition of this AEL identifies exposures to fragrance ingredients that are acceptable (below the AEL) and unacceptable (above the AEL).

This is demonstrated below in Table 10 for a hypothetical fragrance ingredient (X) in a deodorant product and a hydroalcoholic product for unshaved skin.

Table 10: Risk characterization: Calculation of AEL for a hypothetical fragrance ingredient (X) in a deodorant product and hydroalcoholic product for unshaved skin.

Fragrance Ingredient X	Deodorant	Hydroalcoholic Product For Unshaved Skin
WoE NESIL	500 ug/cm ²	500 ug/cm ²
SAF	300	100
AEL= WoE NESIL/SAF	= 500/300	= 500/100
AEL	1.7 ug/cm ²	5.0 ug/cm ²

3.4.2. AEL/CEL ratio

The ratio of the AEL to the CEL is determined by dividing the AEL by the CEL (AEL/CEL). This defines the acceptability of the CEL relative to the identified AEL. The percent concentration of the fragrance ingredient in a product type is acceptable if the AEL exceeds the CEL. The converse, where the CEL exceeds the AEL, would require re-evaluation of the risk management and may lead to a decrease in the concentration of fragrance ingredient in that product type.

This is demonstrated below in Table 11 for the same hypothetical fragrance ingredient (X), which is being used at 0.1% in a deodorant product and in a hydroalcoholic product for unshaved skin. For the purposes of these practical examples, for an acceptable risk assessment, the AEL has to be greater than or equal to the CEL (i.e. $AEL \geq CEL$).

Table 11: Risk characterization: Determination of acceptability for 0.1% of fragrance ingredient X in a deodorant product and in a hydroalcoholic product for unshaved skin.

Fragrance Ingredient X	Deodorant	Hydroalcoholic Product For Unshaved Skin
WoE NESIL	500 ug/cm ²	500 ug/cm ²
SAF	300	100
AEL	1.7 ug/cm ²	5 ug/cm ²
Product Exposure ¹	8.5 mg/cm ² /day	2.2 mg/cm ² /day
Concentration of Fragrance X in the product	0.1%	0.1%
CEL	= 0.1%*8.5 mg/cm ² * 1000 µg/mg = 8.5 ug/cm ²	= 0.1%*2.2 mg/cm ² * 1000 µg/mg = 2.2 ug/cm ²
Risk Assessment	Unacceptable (AEL < CEL)	Acceptable (AEL ≥ CEL)

¹Product exposure selected for this example is the 90thile data from the CTFA Habits and Practices study on solid antiperspirants; and the 95thile data from the Tozer *et al.*, 2004 study for hydroalcoholic products for unshaved skin.

3.4.3. Product categories

A practical application of the recommended risk assessment approach for fragrance ingredients is to use product categories for the implementation of IFRA Standards. This will be achieved by grouping product types according to similar SAFs and exposure which lead to similar acceptable use levels of a fragrance ingredient. This activity will incorporate and integrate all relevant exposure data.

4. QRA implementation for selected fragrance ingredients

Several fragrance ingredients have been chosen to demonstrate the practical applications of the principles of quantitative risk assessment as defined and detailed in this technical dossier. There are several reasons for the choice of these fragrance ingredients. These include the availability of robust animal sensitization data, confirmatory human sensitization data as well as diagnostic patch test studies. Since product categories have not yet been defined (see section 3.4.3), the practical examples are provided for the product types included in Table 2, column 1.

For each example, three tables and two figures are provided.

- The first provides the WoE NESIL and a summary of the data used to generate that endpoint. This first table for each fragrance ingredient is a repeat of information provided in Table 1. They are made available again here for convenience of the reader.
- The second table provides all the summary data used in risk characterization for the use of each fragrance ingredient in two arbitrarily chosen product types. This second table for each fragrance ingredient also demonstrates how the data are used to determine acceptable levels of use in the two product types. A comparison to the IFRA Standard is provided when appropriate. In the absence of an IFRA Standard: 1) for hydroalcoholics, a comparison is made to the average maximum dermal concentration as provided by IFRA and 2) for solid antiperspirants, an acceptable level based on the AEL:CEL ratio is selected simply as an example.
- The third table lists a summary overview of acceptable levels for each fragrance ingredient in various product types defined in Table 2, column 1 using the approach given in the second table.
- For each fragrance ingredient, the examples from the second table are illustrated in a figure to demonstrate how the CEL and AEL relate to one another and ultimately the acceptability or unacceptability of the CEL.

The acceptable concentration of each fragrance ingredient in the final product should be identical in both the second and third tables. It should be noted that there may be very small discrepancies between these values in the second and third tables. This is simply due to a rounding up or down in the calculations.

A very important consideration to note in the third table for each fragrance ingredient example is that the acceptable concentrations provided are strictly the result of the calculation using the numbers derived for AEL and CEL in this QRA method. As such for some products, these calculated acceptable concentrations may be unrealistically high because the calculated consumer exposure levels in Table 9 for certain product types are very low. Practical management reasons demand setting a default maximum level of the fragrance ingredients identified as dermal sensitizers for these product types. This pragmatic level will be defined as that “not exceeding the usual concentration of the fragrance compound in the finished product”. In Tables 14, 17, 20, 23 and 26 these levels are indicated in the column identified as “Maximum Pragmatic Level”. If the AEL derived from the QRA for a fragrance ingredient in a specific product type is less than the concentration identified as the “Maximum Pragmatic Level”, the AEL must take precedence and be applied.

Appendices 1 through 5 contain a detailed summary of all the sensitization data considered in determination of the WoE NESIL for each fragrance ingredient. These Appendices are not attached to this technical report due to their large size, but are available from RIFM. Full study reports are on file with RIFM.

4.1. Cinnamic aldehyde

Table 12: Identification of WoE NESIL cinnamic aldehyde

Perfume Raw Material	CAS No.	IFRA Standard Limit (skin contact products)	LLNA weighted mean EC3 values ($\mu\text{g}/\text{cm}^2$) [no. studies]	Human Data			Potency Classification ¹	WoE NESIL [REXPAN NOEL]
				NOEL – HRIPT (induction) ($\mu\text{g}/\text{cm}^2$)	NOEL – MAX (induction) ($\mu\text{g}/\text{cm}^2$)	LOEL (induction) ($\mu\text{g}/\text{cm}^2$)		
Cinnamic Aldehyde	104-55-2	0.05%	262 [23]	591	NA	775	Moderate	590 [591]

NA= Not Available

¹Gerberick *et al.*, 2001

Table 13: Application of QRA to use of cinnamic aldehyde in hydroalcoholic products for unshaved skin and in solid antiperspirant product types.

Cinnamic Aldehyde	Hydroalcoholic Product For Unshaved Skin	Solid Antiperspirant
WoE NESIL (from Tables 1 and 13)	590 $\mu\text{g}/\text{cm}^2$	590 $\mu\text{g}/\text{cm}^2$
SAF (from Table 2)	100 (10 X 3 X 3)	300 (10 X 3 X 10)
AEL	5.9 $\mu\text{g}/\text{cm}^2$	2.0 $\mu\text{g}/\text{cm}^2$
CEL Product	2.2 $\text{mg}/\text{cm}^2/\text{day}$ ¹	8.5 $\text{mg}/\text{cm}^2/\text{day}$ ²
AEL/CEL	AEL/CEL (5.9 $\mu\text{g}/\text{cm}^2$ X 0.001 $\text{mg}/\mu\text{g}$) \div 2.2 $\text{mg}/\text{cm}^2/\text{day}$ = 0.0027	AEL/CEL (2.0 $\mu\text{g}/\text{cm}^2$ X 0.001 $\text{mg}/\mu\text{g}$) \div 8.5 $\text{mg}/\text{cm}^2/\text{day}$ = 0.0002
Concentration of Cinnamic Aldehyde in the product based on AEL \geq CEL	\leq 0.27%	\leq 0.02%
Risk Assessment	Acceptable if Cinnamic Aldehyde level is less than 0.27%	Acceptable if Cinnamic Aldehyde level is less than 0.02%
IFRA Standard	0.05% or 1.1 $\mu\text{g}/\text{cm}^2/\text{day}$	0.05% or 5.8 $\mu\text{g}/\text{cm}^2/\text{day}$
Applying QRA to IFRA Standard	IFRA Limit too restrictive	IFRA Limit not restrictive enough

¹Cano and Rich, 2001 and Tozer *et al.*, 2004, 95th percentile

²CTFA 95th percentile (CTFA, 2004), surface area of 100 cm^2 per axillae (Bremmer,2003).

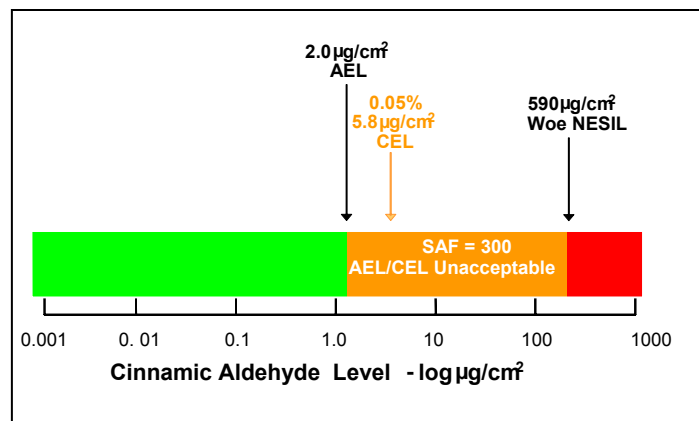
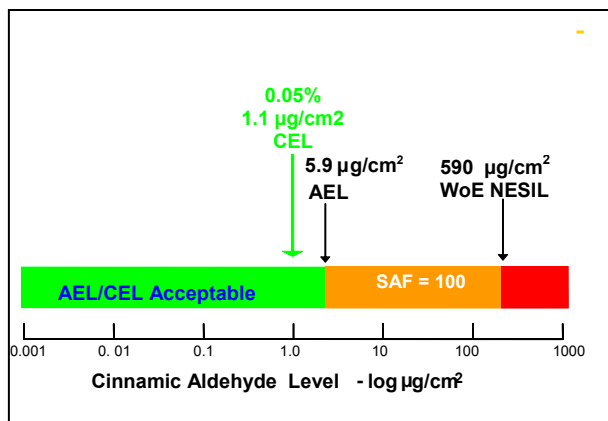


Figure 4: Illustration of AEL/CEL ratio for the current IFRA Standard for cinnamic aldehyde (0.05% skin contact) in a hydroalcoholic product for unshaved skin (left) and a solid antiperspirant (right).

Table 14: Acceptable levels of cinnamic aldehyde in various product types based on QRA.

Product Type	SAF	Exp.	Cinn. Ald.	Maximum Pragmatic Level ¹
Deo/Ap Non-Spray	300	Colipa	0.03%	
Solid Ap	300	CTFA	0.02%	
Lip Products	300	Colipa	0.02%	
Eye Shadow	300	CTFA	0.09%	
Men's Facial Cream	300	SCCP	0.09%	
Hydroalcoholics, Shaved Skin	300	C&R	0.09%	
Tampons	200	RIFM	0.1%	
Hydroalcoholics, Unshaved Skin	100	C&R	0.3%	
Hair Spray	100	CTFA	0.3%	
Body Cream/Lotion	300	Colipa	0.3%	
Women's Facial Cream	100	Colipa	0.2%	
Women's Facial Liquid Make-up	100	CTFA	0.2%	
Hand Cream	100	Colipa	0.1%	
Mouthwash	100	SCCP	0.4%	
Toothpaste	100	Colipa	0.5%	
Baby Wipes	300	RIFM	0.05%	
Intimate Wipes	300	RIFM	0.04%	
Hair Styling Aids	100	SCCP	0.6%	
Make-up Remover	100	SCCP	0.7%	
Nail Care	100	SCCP	0.6%	
Feminine Hygiene Pads	100	RIFM	4.2%	
Feminine Hygiene Liners	100	RIFM	4.2%	
Shampoo	100	CTFA	3.5%	
Liquid Soap	100	Colipa	3%	
Conditioners, Rinse-off	100	CTFA	3.0%	
Face Wash	100	CTFA	3.9%	
Shaving Cream	300	SCCP	2.8%	
Aerosol Air Freshener	100	RIFM	23.6%	5% The maximum concentration will not exceed 5% and may be lower if determined by the QRA.
Bar Soaps	100	SCCP	11.8%	
Body Wash/Shower Gel	100	CTFA	39.3%	
Bath Foams, Gels, Mousses	100	SCCP	59.0%	
Handwash Laundry	100	HERA	5.9%	
Hand Dishwashing	100	HERA	59.0%	2.5% The maximum concentration will not exceed 2.5% and may be lower if determined by the QRA.
Hard Surface Cleaner	100	HERA	4.9%	
Baby Diapers	100	RIFM	100%	
Candles	10	FMA	100%	Due to negligible skin contact, the concentration of fragrance ingredient should not exceed the usual concentration of the fragrance compound in the finished product

¹Practical management reasons demand setting a default maximum level of the fragrance ingredients identified as dermal sensitizers for certain product types. This pragmatic level will be defined as that "not exceeding the usual concentration of the fragrance compound in the finished product". If the AEL derived from the QRA for a fragrance ingredient in a specific product type is less than the concentration identified as the "Maximum Pragmatic Level", the AEL must take precedence and be applied.

4.2 Citral

Table 15: Identification of WoE NESIL citral.

Perfume Raw Material	CAS No.	IFRA Standard Limit (skin contact products)	LLNA weighted mean EC3 values ($\mu\text{g}/\text{cm}^2$) [no. studies]	Human Data			Potency Classification ¹	WoE NESIL [REXPAN NOEL]
				NOEL – HRIPT (induction) ($\mu\text{g}/\text{cm}^2$)	NOEL – MAX (induction) ($\mu\text{g}/\text{cm}^2$)	LOEL (induction) ($\mu\text{g}/\text{cm}^2$)		
Citral	5392-40-5	NA	1414 [11]	1400	NA	3876	Weak	1400 [1400]

NA= Not Available

¹Gerberick *et al.*, 2001

Table 16: Application of QRA to use of citral in hydroalcoholic products for unshaved skin and in solid antiperspirant product types.

Citral	Hydroalcoholic Product For Unshaved Skin	Solid Antiperspirant
WoE NESIL (from Tables 1 and 16)	1400 $\mu\text{g}/\text{cm}^2$	1400 $\mu\text{g}/\text{cm}^2$
SAF (from Table 2)	100 (10 X 3 X 3)	300 (10 X 3 X 10)
AEL	14.0 $\mu\text{g}/\text{cm}^2$	4.7 $\mu\text{g}/\text{cm}^2$
CEL Product	2.2 $\text{mg}/\text{cm}^2/\text{day}$ ¹	8.5 $\text{mg}/\text{cm}^2/\text{day}$ ²
AEL/CEL	AEL/CEL (14.0 $\mu\text{g}/\text{cm}^2 \times 0.001 \text{ mg}/\mu\text{g}$) \div 2.2 $\text{mg}/\text{cm}^2/\text{day}$ = 0.0064	AEL/CEL (4.7 $\mu\text{g}/\text{cm}^2 \times 0.001 \text{ mg}/\mu\text{g}$) \div 8.5 $\text{mg}/\text{cm}^2/\text{day}$ = 0.0005
Concentration of citral in the product based on AEL \geq CEL	$\leq 0.64\%$	$\leq 0.05\%$
Risk Assessment	Acceptable if citral level is less than 0.64%	Acceptable if citral level is less than 0.05%
IFRA Standard	No limit (quenching Standard)	No limit (quenching Standard)
Current Average Maximum Use Level	1.7% ³ or 37.4 $\mu\text{g}/\text{cm}^2/\text{day}$	No data

¹Cano and Rich, 2001 and Tozer *et al.*, 2004, 95th percentile

²CTFA 95th percentile (CTFA, 2004), surface area of 100 cm^2 per axillae (Bremmer,2003).

³IFRA, 2001

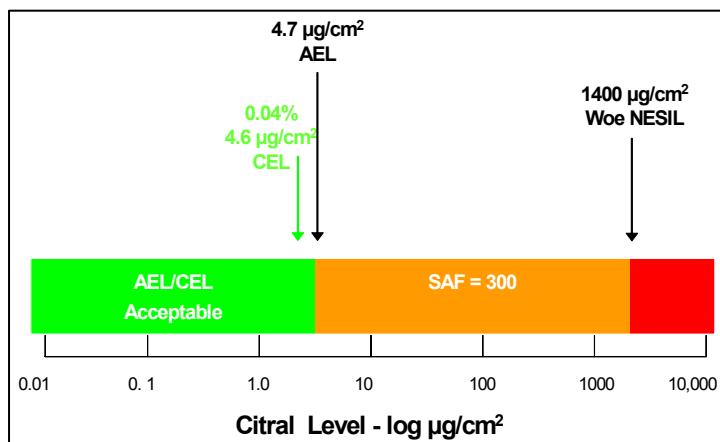
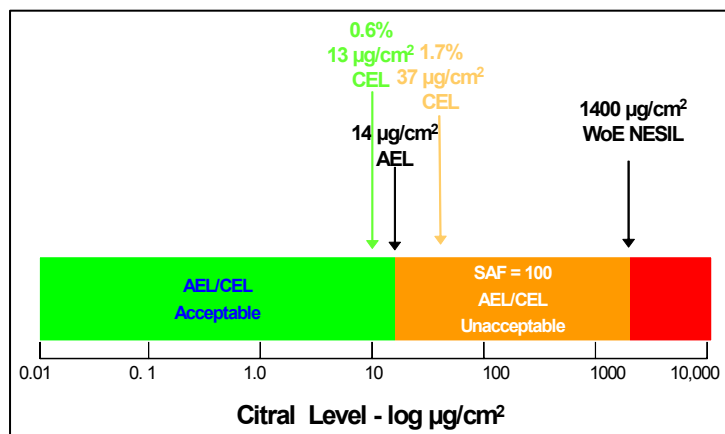


Figure 5: Illustration of AEL/CEL ratio for the current average maximum dermal use level for citral (1.7%; IFRA, 2001) in a hydroalcoholic product for unshaved skin (left) and an acceptable use level for citral in a solid antiperspirant (right).

Table 17: Acceptable levels of citral in various product types based on QRA.

Product Type	SAF	Exp.	Citral	Maximum Pragmatic Level ¹
Deo/Ap Non-Spray	300	Colipa	0.06%	
Solid Ap	300	CTFA	0.05%	
Lip Products	300	Colipa	0.04%	
Eye Shadow	300	CTFA	0.2%	
Men's Facial Cream	300	SCCP	0.2%	
Hydroalcoholics, Shaved Skin	300	C&R	0.2%	
Tampons	200	RIFM	0.2%	
Hydroalcoholics, Unshaved Skin	100	C&R	0.6%	
Hair Spray	100	CTFA	0.6%	
Body Cream/Lotion	300	Colipa	0.8%	
Women's Facial Cream	100	Colipa	0.5%	
Women's Facial Liquid Make-up	100	CTFA	0.4%	
Hand Cream	100	Colipa	0.3%	
Mouthwash	100	SCCP	1.0%	
Toothpaste	100	Colipa	1.1%	
Baby Wipes	300	RIFM	0.1%	
Intimate Wipes	300	RIFM	0.1%	
Hair Styling Aids	100	SCCP	1.4%	
Make-up Remover	100	SCCP	1.6%	
Nail Care	100	SCCP	1.4%	
Feminine Hygiene Pads	100	RIFM	10.0%	2% The maximum concentration will not exceed 2% and may be lower if determined by the QRA.
Feminine Hygiene Liners	100	RIFM	10.0%	
Shampoo	100	CTFA	8.2%	5% The maximum concentration will not exceed 5% and may be lower if determined by the QRA.
Liquid Soap	100	Colipa	7%	
Conditioners, Rinse-off	100	CTFA	7.0%	
Face Wash	100	CTFA	9.3%	
Shaving Cream	300	SCCP	6.7%	
Aerosol Air Freshener	100	RIFM	56.0%	
Bar Soaps	100	SCCP	28.0%	
Body Wash/Shower Gel	100	CTFA	93.3%	
Bath Foams, Gels, Mousses	100	SCCP	100%	
Handwash Laundry	100	HERA	14.0%	
Hand Dishwashing	100	HERA	100%	
Hard Surface Cleaner	100	HERA	11.7%	
Baby Diapers	100	RIFM	100%	
Candles	10	FMA	100%	Due to negligible skin contact, the concentration of fragrance ingredient should not exceed the usual concentration of the fragrance compound in the finished product

¹Practical management reasons demand setting a default maximum level of the fragrance ingredients identified as dermal sensitizers for certain product types. This pragmatic level will be defined as that "not exceeding the usual concentration of the fragrance compound in the finished product". If the AEL derived from the QRA for a fragrance ingredient in a specific product type is less than the concentration identified as the "Maximum Pragmatic Level", the AEL must take precedence and be applied.

4.3 Isoeugenol

Table 18: Identification of WoE NESIL isoeugenol.

Perfume Raw Material	CAS No.	IFRA Standard Limit (skin contact products)	LLNA weighted mean EC3 values ($\mu\text{g}/\text{cm}^2$) [no. studies]	Human Data			Potency Classification ¹	WoE NESIL [REXPAN NOEL]
				NOEL – HRIPT (induction) ($\mu\text{g}/\text{cm}^2$)	NOEL – MAX (induction) ($\mu\text{g}/\text{cm}^2$)	LOEL (induction) ($\mu\text{g}/\text{cm}^2$)		
Isoeugenol	97-54-1	0.02%	498 [18]	250	NA	775	Moderate	250 [250]

NA= Not Available

¹Gerberick *et al.*, 2001

Table 19: Application of QRA to use of isoeugenol in hydroalcoholic products for unshaved skin and in solid antiperspirant product types.

Isoeugenol	Hydroalcoholic Product For Unshaved Skin	Solid Antiperspirant
WoE NESIL (from Tables 1 and 19)	250 $\mu\text{g}/\text{cm}^2$	250 $\mu\text{g}/\text{cm}^2$
SAF (from Table 2)	100 (10 X 3 X 3)	300 (10 X 3 X 10)
AEL	2.5 $\mu\text{g}/\text{cm}^2$	0.83 $\mu\text{g}/\text{cm}^2$
CEL Product	2.2 $\text{mg}/\text{cm}^2/\text{day}^1$	8.5 $\text{mg}/\text{cm}^2/\text{day}^2$
AEL/CEL	AEL/CEL (2.5 $\mu\text{g}/\text{cm}^2$ X 0.001 $\text{mg}/\mu\text{g}$) \div 2.2 $\text{mg}/\text{cm}^2/\text{day}$ = 0.0011	AEL/CEL (0.83 $\mu\text{g}/\text{cm}^2$ X 0.001 $\text{mg}/\mu\text{g}$) \div 8.5 $\text{mg}/\text{cm}^2/\text{day}$ = 0.00098
Concentration of Isoeugenol in the product based on AEL \geq CEL	$\leq 0.11\%$	$\leq 0.01\%$
Risk Assessment	Acceptable if isoeugenol level is less than 0.11%	Acceptable if isoeugenol level is less than 0.01%
IFRA Standard	0.02% or 0.44 $\mu\text{g}/\text{cm}^2/\text{day}$	0.02% or 2.3 $\mu\text{g}/\text{cm}^2/\text{day}$
Applying QRA to IFRA Standard	IFRA Limit too restrictive	IFRA Limit not restrictive enough

¹Cano and Rich, 2001 and Tozer *et al.*, 2004, 95th percentile

²CTFA 95th percentile (CTFA, 2004), surface area of 100 cm^2 per axillae (Bremmer,2003).

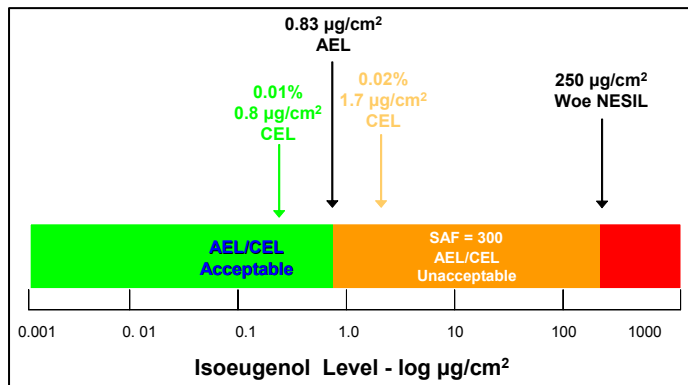
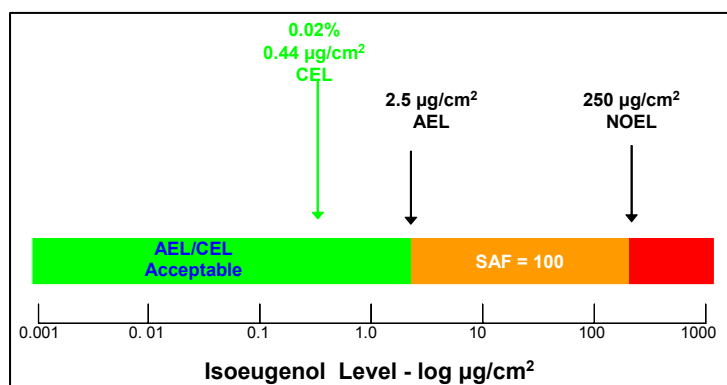


Figure 6: Illustration of AEL/CEL ratio for the current IFRA Standard for isoeugenol (0.02% skin contact) in a hydroalcoholic product for unshaved skin (left) and an acceptable use level for isoeugenol in a solid antiperspirant (right).

Table 20: Acceptable levels of isoeugenol in various product types based on QRA.

Product Type	SAF	Exp.	Isoeugenol	Maximum Pragmatic Level ¹
Deo/Ap Non-Spray	300	Colipa	0.01%	
Solid Ap	300	CTFA	0.01%	
Lip Products	300	Colipa	0.01%	
Eye Shadow	300	CTFA	0.04%	
Men's Facial Cream	300	SCCP	0.04%	
Hydroalcoholics, Shaved Skin	300	C&R	0.04%	
Tampons	200	RIFM	0.04%	
Hydroalcoholics, Unshaved Skin	100	C&R	0.1%	
Hair Spray	100	CTFA	0.1%	
Body Cream/Lotion	300	Colipa	0.1%	
Women's Facial Cream	100	Colipa	0.1%	
Women's Facial Liquid Make-up	100	CTFA	0.1%	
Hand Cream	100	Colipa	0.1%	
Mouthwash	100	SCCP	0.2%	
Toothpaste	100	Colipa	0.2%	
Baby Wipes	300	RIFM	0.02%	
Intimate Wipes	300	RIFM	0.02%	
Hair Styling Aids	100	SCCP	0.3%	
Make-up Remover	100	SCCP	0.3%	
Nail Care	100	SCCP	0.3%	
Feminine Hygiene Pads	100	RIFM	1.8%	
Feminine Hygiene Liners	100	RIFM	1.8%	
Shampoo	100	CTFA	1.5%	
Liquid Soap	100	Colipa	1%	
Conditioners, Rinse-off	100	CTFA	1.3%	
Face Wash	100	CTFA	1.7%	
Shaving Cream	300	SCCP	1.2%	
Aerosol Air Freshener	100	RIFM	10.0%	5% The maximum concentration will not exceed 5% and may be lower if determined by the QRA.
Bar Soaps	100	SCCP	5.0%	
Body Wash/Shower Gel	100	CTFA	16.7%	5% The maximum concentration will not exceed 5% and may be lower if determined by the QRA.
Bath Foams, Gels, Mousses	100	SCCP	25.0%	
Handwash Laundry	100	HERA	2.5%	
Hand Dishwashing	100	HERA	25.0%	2.5% The maximum concentration will not exceed 2.5% and may be lower if determined by the QRA.
Baby Diapers	100	RIFM	100%	
Hard Surface Cleaner	100	HERA	2.1%	
Candles	10	FMA	100%	Due to negligible skin contact, the concentration of fragrance ingredient should not exceed the usual concentration of the fragrance compound in the finished product

¹Practical management reasons demand setting a default maximum level of the fragrance ingredients identified as dermal sensitizers for certain product types. This pragmatic level will be defined as that "not exceeding the usual concentration of the fragrance compound in the finished product". If the AEL derived from the QRA for a fragrance ingredient in a specific product type is less than the concentration identified as the "Maximum Pragmatic Level", the AEL must take precedence and be applied.

4.4 Eugenol

Table 21: Identification of WoE NESIL eugenol.

Perfume Raw Material	CAS No.	IFRA Standard Limit (skin contact products)	LLNA weighted mean EC3 values ($\mu\text{g}/\text{cm}^2$) [no. studies]	Human Data			Potency Classification ¹	WoE NESIL [REXPAN NOEL]
				NOEL – HRIPT (induction) ($\mu\text{g}/\text{cm}^2$)	NOEL – MAX (induction) ($\mu\text{g}/\text{cm}^2$)	LOEL (induction) ($\mu\text{g}/\text{cm}^2$)		
Eugenol	97-53-0	0.5%	2703 [6]	5906	NA	NA	Weak	5,900 [5900]

NA= Not Available

¹Gerberick *et al.*, 2001

Table 22: Application of QRA to use of eugenol in hydroalcoholic products for unshaved skin and in solid antiperspirant product types.

Eugenol	Hydroalcoholic Product For Unshaved Skin	Solid Antiperspirant
WoE NESIL (from Tables 1 and 22)	5900 $\mu\text{g}/\text{cm}^2$	5900 $\mu\text{g}/\text{cm}^2$
SAF (from Table 2)	100 (10 X 3 X 3)	300 (10 X 3 X 10)
AEL	59.0 $\mu\text{g}/\text{cm}^2$	19.7 $\mu\text{g}/\text{cm}^2$
CEL Product	2.2 $\text{mg}/\text{cm}^2/\text{day}$ ¹	8.5 $\text{mg}/\text{cm}^2/\text{day}$ ²
AEL/CEL	AEL/CEL (59.0 $\mu\text{g}/\text{cm}^2$ X 0.001 $\text{mg}/\mu\text{g}$) \div 2.2 $\text{mg}/\text{cm}^2/\text{day}$ = 0.027	AEL/CEL (19.7 $\mu\text{g}/\text{cm}^2$ X 0.001 $\text{mg}/\mu\text{g}$) \div 8.5 $\text{mg}/\text{cm}^2/\text{day}$ = 0.0023
Concentration of Eugenol in the product based on AEL \geq CEL	\leq 2.7%	\leq 0.2%
Risk Assessment	Acceptable if eugenol level is less than 2.7%	Acceptable if eugenol level is less than 0.2%
IFRA Standard	0.5% or 11 $\mu\text{g}/\text{cm}^2/\text{day}$	0.5% or 58 $\mu\text{g}/\text{cm}^2/\text{day}$
Applying QRA to IFRA Standard	IFRA Limit too restrictive	IFRA Limit not restrictive enough

¹Cano and Rich, 2001 and Tozer *et al.*, 2004, 95th percentile

²CTFA 95th percentile (CTFA, 2004), surface area of 100 cm^2 per axillae (Bremmer,2003).

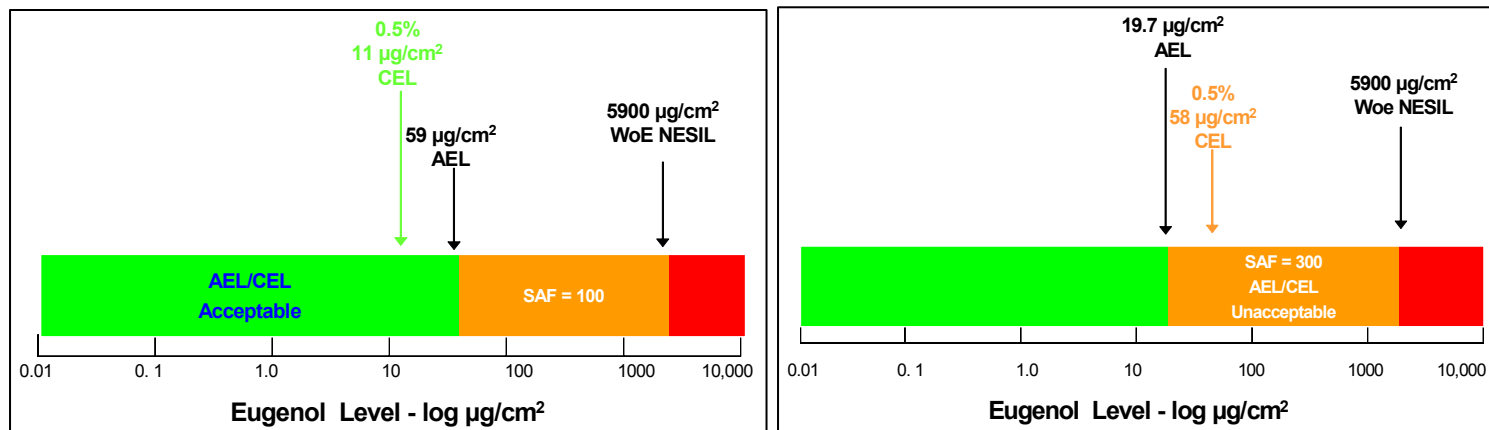


Figure 7: Illustration of AEL/CEL ratio for the current IFRA Standard for eugenol (0.5 % skin contact) in a hydroalcoholic product for unshaved skin (left) and an acceptable use level for eugenol in a solid antiperspirant (right).

Table 23: Acceptable levels of eugenol in various product types based on QRA.

Product Type	SAF	Exp.	Eugenol	Maximum Pragmatic Level ¹
Deo/Ap Non-Spray	300	Colipa	0.3%	
Solid Ap	300	CTFA	0.2%	
Lip Products	300	Colipa	0.2%	
Eye Shadow	300	CTFA	0.9%	
Men's Facial Cream	300	SCCP	0.9%	
Hydroalcoholics, Shaved Skin	300	C&R	0.9%	
Tampons	200	RIFM	1.0%	
Hydroalcoholics, Unshaved Skin	100	C&R	2.7%	
Hair Spray	100	CTFA	2.7%	
Body Cream/Lotion	300	Colipa	3.3%	
Women's Facial Cream	100	Colipa	2.2%	
Women's Facial Liquid Make-up	100	CTFA	1.9%	
Hand Cream	100	Colipa	1.4%	
Mouthwash	100	SCCP	4.3%	
Toothpaste	100	Colipa	4.7%	
Baby Wipes	300	RIFM	0.5%	
Intimate Wipes	300	RIFM	0.4%	
Hair Styling Aids	100	SCCP	5.9%	2% The maximum concentration will not exceed 2% and may be lower if determined by the QRA.
Make-up Remover	100	SCCP	6.6%	
Nail Care	100	SCCP	6.1%	
Feminine Hygiene Pads	100	RIFM	42.1%	
Feminine Hygiene Liners	100	RIFM	42.1%	5% The maximum concentration will not exceed 5% and may be lower if determined by the QRA.
Shampoo	100	CTFA	34.7%	
Liquid Soap	100	Colipa	30%	
Conditioners, Rinse-off	100	CTFA	29.5%	
Face Wash	100	CTFA	39.3%	
Shaving Cream	300	SCCP	28.1%	
Aerosol Air Freshener	100	RIFM	100%	
Bar Soaps	100	SCCP	100%	
Body Wash/Shower Gel	100	CTFA	100%	
Bath Foams, Gels, Mousses	100	SCCP	100%	
Handwash Laundry	100	HERA	59.0%	2.5% The maximum concentration will not exceed 2.5% and may be lower if determined by the QRA.
Hand Dishwashing	100	HERA	100%	
Hard Surface Cleaner	100	HERA	49.2%	
Baby Diapers	100	RIFM	100%	Due to negligible skin contact, the concentration of fragrance ingredient should not exceed the usual concentration of the fragrance compound in the finished product
Candles	10	FMA	100%	

¹Practical management reasons demand setting a default maximum level of the fragrance ingredients identified as dermal sensitizers for certain product types. This pragmatic level will be defined as that "not exceeding the usual concentration of the fragrance compound in the finished product". If the AEL derived from the QRA for a fragrance ingredient in a specific product type is less than the concentration identified as the "Maximum Pragmatic Level", the AEL must take precedence and be applied.

4.5 Phenylacetaldehyde

Table 24: Identification of WoE NESIL phenylacetaldehyde.

Perfume Raw Material	CAS No.	IFRA Standard Limit (skin contact products)	LLNA weighted mean EC3 values ($\mu\text{g}/\text{cm}^2$) [no. studies]	Human Data			Potency Classification ¹	WoE NESIL [REXPAN NOEL]
				NOEL – HRIPT (induction) ($\mu\text{g}/\text{cm}^2$)	NOEL – MAX (induction) ($\mu\text{g}/\text{cm}^2$)	LOEL (induction) ($\mu\text{g}/\text{cm}^2$)		
Phenyl acetaldehyde	122-78-1	NA	962 [2]	591	NA	1181	Moderate	590 [590]

NA= Not Available

¹Gerberick *et al.*, 2001

Table 25: Application of QRA to use of phenylacetaldehyde in hydroalcoholic products for unshaved skin and in solid antiperspirant product types.

Phenylacetaldehyde	Hydroalcoholic Product For Unshaved Skin	Solid Antiperspirant
WoE NESIL (from Tables 1 and 25)	590 $\mu\text{g}/\text{cm}^2$	590 $\mu\text{g}/\text{cm}^2$
SAF (from Table 2)	100 (10 X 3 X 3)	300 (10 X 3 X 10)
AEL	5.9 $\mu\text{g}/\text{cm}^2$	2.0 $\mu\text{g}/\text{cm}^2$
CEL Product	2.2 $\text{mg}/\text{cm}^2/\text{day}$ ¹	8.5 $\text{mg}/\text{cm}^2/\text{day}$ ²
AEL/CEL	AEL/CEL (5.9 $\mu\text{g}/\text{cm}^2$ X 0.001 $\text{mg}/\mu\text{g}$) \div 2.2 $\text{mg}/\text{cm}^2/\text{day}$ = 0.0027	AEL/CEL (2.0 $\mu\text{g}/\text{cm}^2$ X 0.001 $\text{mg}/\mu\text{g}$) \div 8.5 $\text{mg}/\text{cm}^2/\text{day}$ = 0.00024
Concentration of phenylacetaldehyde in the product based on AEL \geq CEL	$\leq 0.27\%$	$\leq 0.02\%$
Risk Assessment	Acceptable if phenylacetaldehyde level is less than 0.27%	Acceptable if phenylacetaldehyde level is less than 0.02%
IFRA Standard	No limit (quenching Standard)	No limit (quenching Standard)
Current Average Maximum Use Level	0.1% ³ or 2.2 $\mu\text{g}/\text{cm}^2/\text{day}$	NA

¹Cano and Rich, 2001 and Tozer *et al.*, 2004, 95th percentile

²CTFA 95th percentile (CTFA, 2004), surface area of 100 cm^2 per axillae (Bremmer,2003).

³IFRA, 2001

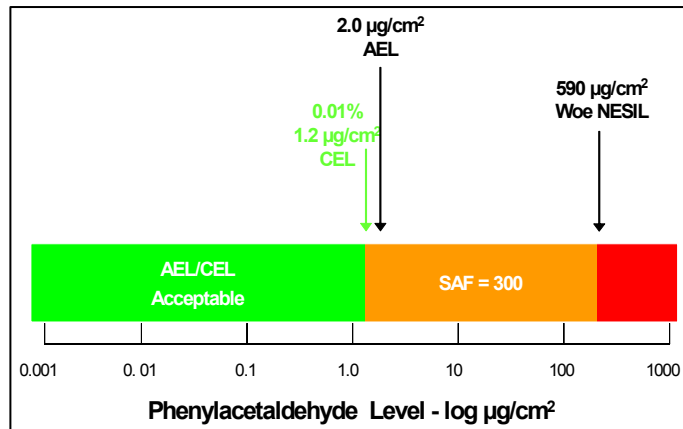
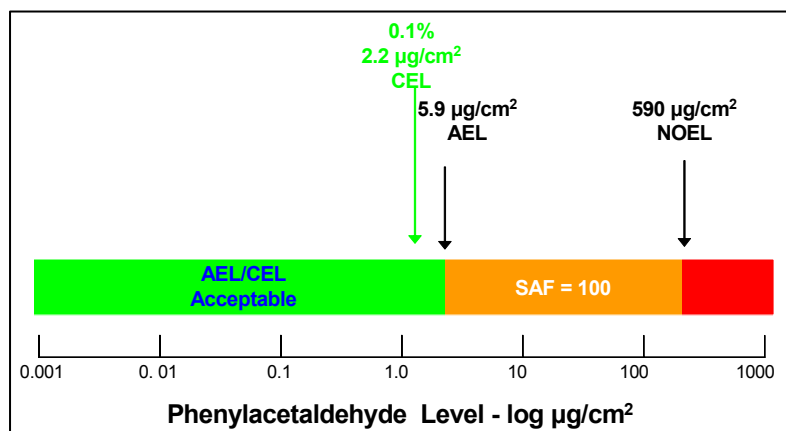


Figure 8: Illustration of AEL/CEL ratio for the current average maximum dermal use level for phenylacetaldehyde (0.1%; IFRA, 2001) in a hydroalcoholic product for unshaved skin (left) and an acceptable use level for phenylacetaldehyde in a solid antiperspirant (right).

Table 26: Acceptable levels of phenylacetaldehyde in various product types based on QRA.

Product Type	SAF	Exp.	Phenyl-acetaldehyde.	Maximum Pragmatic Level ¹
Deo/Ap Non-Spray	300	Colipa	0.03%	
Solid Ap	300	CTFA	0.02%	
Lip Products	300	Colipa	0.02%	
Eye Shadow	300	CTFA	0.09%	
Men's Facial Cream	300	SCCP	0.09%	
Hydroalcoholics, Shaved Skin	300	C&R	0.09%	
Tampons	200	RIFM	0.1%	
Hydroalcoholics, Unshaved Skin	100	C&R	0.3%	
Hair Spray	100	CTFA	0.3%	
Body Cream/Lotion	300	Colipa	0.3%	
Women's Facial Cream	100	Colipa	0.2%	
Women's Facial Liquid Make-up	100	CTFA	0.2%	
Hand Cream	100	Colipa	0.1%	
Mouthwash	100	SCCP	0.4%	
Toothpaste	100	Colipa	0.5%	
Baby Wipes	300	RIFM	0.05%	
Intimate Wipes	300	RIFM	0.04%	
Hair Styling Aids	100	SCCP	0.6%	
Make-up Remover	100	SCCP	0.7%	
Nail Care	100	SCCP	0.6%	
Feminine Hygiene Pads	100	RIFM	4.2%	
Feminine Hygiene Liners	100	RIFM	4.2%	
Shampoo	100	CTFA	3.5%	
Liquid Soap	100	Colipa	3%	
Conditioners, Rinse-off	100	CTFA	3.0%	
Face Wash	100	CTFA	3.9%	
Shaving Cream	300	SCCP	2.8%	
Aerosol Air Freshener	100	RIFM	23.6%	5% The maximum concentration will not exceed 5% and may be lower if determined by the QRA.
Bar Soaps	100	SCCP	11.8%	
Body Wash/Shower Gel	100	CTFA	39.3%	
Bath Foams, Gels, Mousses	100	SCCP	59.0%	2.5% The maximum concentration will not exceed 2.5% and may be lower if determined by the QRA.
Handwash Laundry	100	HERA	5.9%	
Hand Dishwashing	100	HERA	59.0%	
Hard Surface Cleaner	100	HERA	4.9%	
Baby Diapers	100	RIFM	100%	
Candles	10	FMA	100%	Due to negligible skin contact, the concentration of fragrance ingredient should not exceed the usual concentration of the fragrance compound in the finished product

¹Practical management reasons demand setting a default maximum level of the fragrance ingredients identified as dermal sensitizers for certain product types. This pragmatic level will be defined as that "not exceeding the usual concentration of the fragrance compound in the finished product". If the AEL derived from the QRA for a fragrance ingredient in a specific product type is less than the concentration identified as the "Maximum Pragmatic Level", the AEL must take precedence and be applied.

5. Confirmation of predicted use levels for fragrance ingredients

Risk assessments for products containing fragrance ingredients are conducted prior to product launch. An essential element of product risk management is to be able to determine that the risk assessment was appropriate or needs further refinement. This can be achieved through monitoring the market place after product launch. Typically this is accomplished for fragrance ingredients through individual company post-market surveillance systems and through the dermatology community monitoring incidence rates of relevant positive patch tests to fragrance ingredients.

In the context of the QRA activity, RIFM sponsored a survey of the patch test database at the Contact Allergy Unit, University Hospital Leuven, Belgium. The survey commissioned for the QRA activity for fragrance ingredients focused on three areas: 1) identification of the product types that contained specific fragrance ingredients identified in section 4 (cinnamic aldehyde, citral and isoeugenol); and 2) the number of positive clinical patch test reactions to these fragrance ingredients in the different product types covering the period 2000-2005.

A total of 3323 subjects were investigated by the Contact Allergy Unit. 9.1% of these patients were found to have a positive patch test reaction to the fragrance-mix; 6.7% to balsam of Peru; 4.8 % to colophony. Some of these patients showed positive reactions to multiple fragrance ingredients. Of the patients who reacted positively to the fragrance mix, 133 exhibited positive patch tests to their own cosmetic products. Of these 133 patients, 66 involved fragrance-related contact-allergic reactions and served as the basis for the results detailed in Table 27.

Table 27: Identification of the type of product type and positive patch test reactions to cinnamic aldehyde, isoeugenol and citral from the patch test database (Contact Allergy Unit, University Hospital Leuven, Belgium).

Fragrance Ingredient	Product Type	Positive Patch Test Reactions to Product Type
Cinnamic aldehyde	Deodorant products	2
Isoeugenol	Aftershave product	1
	Deodorant product	1
	Hair dye product	1
	Toilet water/perfume products	5
Citral	Toilet water/perfume products	6

At this time it is difficult to fully interpret these patch test database survey results in the context of the QRA for cinnamic aldehyde, citral and isoeugenol since final risk assessments, based on the most appropriate exposure data are not yet

available. However it is reasonable to make some preliminary conclusions for each of these fragrance ingredients. The results indicate products which were reported to cause elicitation of skin sensitization. It must be noted that it is difficult to relate to the product that caused the induction of skin sensitization. Therefore use of such surveys is of limited use regarding induction of skin sensitization, but do identify fragrance ingredients for which there may be an incidence of patch test positive patients in the dermatology clinics.

Cinnamic aldehyde: The average maximum dermal use level in hydroalcoholics was reported to be 1% (IFRA, 1999). In 2004, IFRA revised the Standard on Cinnamic aldehyde, which limited the use of the material to 0.05% for all skin contact products. Clinical reports (Johansen and Menne, 1995; Buckley et al., 2000) have shown a significant reduction in the frequency of positive reactions to cinnamic aldehyde from 1980-1996. This is supported from the patch test database survey results listed above. The reduction in the frequency of positive reactions may be due to decrease in the use of cinnamic aldehyde due to marketing trends. The current IFRA Standard (issued in 2004) which further reduces the use of cinnamic aldehyde (by an additional 0.5%) should result in a continuation of this downward trend. It is important to continue to monitor the clinical situation to confirm this. The 90th percentile QRA for cinnamic aldehyde would have predicted that the use of 1% cinnamic aldehyde was not restrictive enough and that the current IFRA limit of 0.05% is too restrictive for hydroalcoholic products for unshaved skin. On the other hand, the risk assessment would have predicted that the IFRA limit of 0.05% for some other product types is not restrictive enough (e.g. deodorant, lip products).

Isoeugenol: From 1980 to 1992, the IFRA Standard limited the use of isoeugenol to 0.2% in all skin contact products. In 1998, the IFRA Standard further limited the use to 0.02%. Schnuch *et al.*, 2004 reported that with the exception of a peak in 1999, isoeugenol exhibited the same positive patch test incidence after that time through 2002. Although there are some products containing isoeugenol identified in the patch test database survey, the number is consistent with the Schnuch *et al.*, 2004 data. It is clear from the published literature and the patch test database survey that active monitoring of diagnostic patch data to isoeugenol is needed. In terms of the 90th percentile QRA for isoeugenol, the current IFRA limit of 0.02% is too restrictive for hydroalcoholics for unshaved skin. On the other hand, the risk assessment would have predicted that the IFRA limit of 0.02% for some other product types is not restrictive enough (e.g. deodorant, lip products).

Citral: It is not possible at this time to interpret the patch test database survey relative to the 90th percentile QRA for citral. This is on the basis that current use levels in products other than hydroalcoholics are not known. These data are currently being generated by IFRA. The patch test database survey indicate, at least for toilet water/perfume product, a limit for citral should be established. It is recommended that this limit is established on the basis of a QRA for this fragrance ingredient.

It is the intention to re-address the patch test database survey relative to QRA once final risk assessments are available for these fragrance ingredients.

6. Conclusions

Key conclusions on this QRA approach for fragrance ingredients, which are listed below, can be drawn from the detailed technical information within this dossier. It is important to refer to the details within each section of this dossier to put the key conclusions into context.

- General principles of risk assessment principles to contact allergy can be applied as induction of skin sensitization is a threshold phenomenon. However, these general principles require tailoring to take into account unique elements of skin sensitization as a toxicity endpoint.
- The principles of exposure-based QRA are appropriate for the evaluation of contact allergens for fragrance ingredients.
- Following the identification of a fragrance ingredient as a potential skin sensitizer, a weight of evidence approach is used to determine a NESIL, which introduces a more robust approach to allergen potency evaluation for use in risk assessment.
- Sensitization assessment factors (referred to as uncertainty factors in the remit) within the exposure-based quantitative risk assessment process are based on published peer-reviewed scientific data. It can be concluded that they are based on sound science.
- SAFs can and have been predefined for certain product types. Product categories can be further defined according to similar combinations of SAFs and exposures which will lead to similar acceptable use levels of a fragrance ingredient.
- One critical element of QRA for contact allergy is the dose metric of dose per unit area for induction of contact allergy. It is essential to express the NESIL and CEL in dose per unit area.
- These tools can be used to estimate safe exposure levels and for fragrance ingredients not yet in marketed products, QRA can be used prospectively to identify acceptable levels in a range of different products.
- For fragrance ingredients already in marketed products, QRA could be used both prospectively and retrospectively. Prospective use of QRA in this context would address identifying acceptable levels in products for which IFRA Standards do not exist. Retrospective use of QRA could help to determine the acceptability or unacceptability of current IFRA Standards. Caution needs to be exercised when applying QRA retrospectively. This is on the basis that several data sources may exist and all data need to be taken into account to ensure the most appropriate acceptable exposure levels are selected.

- The use of QRA for fragrance ingredients will facilitate the establishment of IFRA Standards through better definition of product categories versus the current system of only two product categories (skin contact and non-skin contact).
- QRA can be used in combination with the clinical results from the dermatology community and company post-market surveillance data to confirm the effectiveness of fragrance ingredient use limits.
- QRA represents an important step forward in skin sensitization risk assessment, but it should be recognized that there may be refinements to the method in the future.

7. Recommendations for refinement

The QRA methodology for fragrance ingredients described in this dossier is ready for implementation.

This does not mean that the method cannot be further refined. In fact further refinement is encouraged as new information becomes available. Throughout this dossier, several recommendations for refinement have been identified. These recommendations on this QRA approach for fragrance ingredients, which are listed below, can be drawn from the detailed technical information within this dossier. It is important to refer to the details to put the key recommendations into context.

- Exposure is an integral part of any risk assessment and the use of the best available exposure data is essential. Improved exposure data would be very worthwhile (i.e. habits and practices, human parameter data) to further refine CEL.
- As more exposure data become available, the QRA for fragrance ingredients and product categories may need to be re-evaluated.
- As more experience is gained with use of LLNA EC3 values as an indicator of human allergenic potency, its influence in WoE NESIL determinations for use in risk assessments to calculate safe product levels of fragrance ingredients may be refined.
- As a conservative approach was taken in establishing SAFs for fragrance ingredients different product types, additional data (e.g. the influence of evaporation, of retention factors) may lead to their refinement.
- Although it is desirable to use aggregate exposure, there are insufficient data to allow this to occur at this time. This is an area where more evaluation is needed and more data may need to be generated.

- Occupational/professional exposure is not included at this time because comprehensive exposure data are not available. It will be important to address occupational/professional exposure in the QRA approach when these exposure data become available.

8. References

The full references are not included with the technical dossier, but are available at RIFM. The numbers that appear after the reference are the unique location identifiers.

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