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Dermal sensitization quantitative risk assessment (QRA) for fragrance ingredients

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Abstract

Based on chemical, cellular, and molecular understanding of dermal sensitization, an exposure-based quantitative risk assessment (QRA) can be conducted to determine safe use levels of fragrance ingredients in different consumer product types. The key steps are: (1) determination of benchmarks (no expected sensitization induction level (NESIL)); (2) application of sensitization assessment factors (SAF); and (3) consumer exposure (CEL) calculation through product use. Using these parameters, an acceptable exposure level (AEL) can be calculated and compared with the CEL. The ratio of AEL to CEL must be favorable to support safe use of the potential skin sensitizer. This ratio must be calculated for the fragrance ingredient in each product type. Based on the Research Institute for Fragrance Materials, Inc. (RIFM) Expert Panel's recommendation, RIFM and the International Fragrance Association (IFRA) have adopted the dermal sensitization QRA approach described in this review for fragrance ingredients identified as potential dermal sensitizers. This now forms the fragrance industry's core strategy for primary prevention of dermal sensitization to these materials in consumer products. This methodology is used to determine global fragrance industry product management practices (IFRA Standards) for fragrance ingredients that are potential dermal sensitizers. This paper describes the principles of the recommended approach, provides detailed review of all the information used in the dermal sensitization QRA approach for fragrance ingredients and presents key conclusions for its use now and refinement in the future.

Keywords: Quantitative risk assessment; Dermal sensitization; Fragrance ingredients; NESIL; SAF; AEL; CEL

1. Introduction

Although some substances in common use today may have the potential to cause dermal sensitization, they can be formulated into consumer products at safe levels. This is also the case for fragrance ingredients.

IFRA provides the fragrance industry with risk management strategies on the use of fragrance ingredients includ-

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ing those ingredients identified as contact allergens. Historically they achieved this through the establishment of Standards based on no-effect concentrations and translated these as maximum limits that were applied equally to all types of skin contact products with different limits only for non-contact products.

More recently, significant developments have been incorporated in the way dermal sensitization risk assessments are conducted for fragrance ingredients (Gerberick et al., 2001). The general toxicological principles of quantitative risk assessment can be applied here, since it is known that the induction of dermal sensitization is also a threshold based phenomenon (Kimber et al., 1999; Robinson et al., 2000). With this and based on an understanding of the chemical, cellular, and molecular principles of dermal sensitization, it is possible to conduct an exposure-based quantitative risk assessment (QRA) to determine safe use levels of fragrance ingredients in a variety of consumer product types.

This paper describes the principles of the approach for fragrance ingredients in consumer products and provides detailed review of all the areas and information used. There will be other publications that demonstrate the implementation by providing practical examples for individual fragrance ingredients.

1.1. Review of dermal sensitization risk assessment methodologies for recommendation of the QRA approach for fragrance ingredients

The safety assessment of 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).

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 sensitization. Several papers (Farage et al., 2003; Felter et al., 2002, 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.

For the purpose of this review, two key methods were considered in detail (Gerberick et al., 2001; 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 that were used as a starting point to define the refined risk assessment methodology for fragrance ingredients based on the induction of dermal sensitization.

The key refinements that have been introduced in this paper are the establishment of known benchmarks [weight of evidence no expected sensitization induction level (NESIL)] and the determination of uncertainty factors (sensitization assessment factors). As with any risk assessment, exposure is an essential element of the risk assessment process. Elements addressed here are the appropriate dose metric and how to prioritize exposure data from different sources. All of these refinements are described in detail in this review and clear guidance is provided on their use within this dermal sensitization risk assessment approach.

1.1.1. QRA methodology for fragrance ingredients

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

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. Confirmatory human assays such as the Human Repeat Insult Patch Test (HRIPT) may also be subsequently conducted to provide substantiation of the NOEL. Relative skin permeability and integrity are also considered in this section.

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. These terms have been adopted to take into account unique elements of quantitative risk assessment for skin sensitization.

1.2. Hazard identification

1.2.1. Animal data

Historically, there are several animal models that have been used to determine the potential for a fragrance ingre-

dient to induce sensitization. Guinea pig tests (adjuvant and non-adjuvant) have been used for many years to assess the inherent contact sensitization 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 can be used both to determine the potential of a material to induce contact sensitization and to estimate the relevant sensitizing potency of contact allergens by using the EC3 value: the concentration required to induce a threshold positive response (Basketter et al., 1999). 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, 1999; Gerberick et al., 2001,a, 2004; Griem et al., 2003; Schneider and Akkan, 2004).

1.3. Dose–response or hazard quantification

1.3.1. No Expected Sensitizing Induction Level (NESIL)

The NESIL is a benchmark that is derived from animal (see above) and human data (see below) through application of weight of evidence approach to all the relevant data. The NESIL is expressed as a dose per unit area (e.g., μg/cm^2) value. In contact allergy, there is now overwhelming empirical support for using quantity per unit area rather than other dose metrics such as concentration applied to the skin (Kligman, 1966; Magnusson and Kligman, 1970; Friedmann and Moss, 1985; White et al., 1986; Rees et al., 1990; Upadhye and Maibach, 1992). An in-depth review of the published studies including those mentioned above that support the use of dose per unit area in risk assessments for induction of dermal sensitization is provided in the publication by Kimber et al. (2008).

1.3.2. Human data

A human sensitization test is used 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.

The test most typically conducted is the human repeat insult patch test (HRIPT) (McNamee et al., 2008). Dose for dose, this test exaggerates exposure from normal use of consumer products. Such tests must meet current ethical and methodological criteria.

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 described by Politano and Api (2008).

Diagnostic patch test data from dermatology clinics are not used in the determination of the NESIL. This is because these data are a measure of elicitation of allergic contact dermatitis, not induction of dermal sensitization. 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.

1.3.3. 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. To this end, weight of evidence (WoE) guidelines (see Fig. 1) have been developed.

These guidelines have been developed specifically for fragrance ingredients and are intended only to be applied to fragrance ingredients. These guidelines may also address some unusual situations for which discrepancies between data generated in non-adjuvant guinea pig tests, LLNA and human data (HRIPT), human maximization test (HMT) need to be resolved.

In the previous risk assessment approach for dermal sensitization, the RIFM Expert Panel (REXPAN) has been the advisory body responsible for determination of no-effect levels used to establish limits of use described in the IFRA Standards. REXPAN will continue to have this responsibility, but will determine the NESIL rather than the no-effect levels for a fragrance ingredient. They have adopted the guidelines outlined below for establishing WoE NESILs for fragrance ingredients. Scientific judgment will prevail when establishing WoE NESILs for fragrance ingredients.

1.3.3.1. WoE NESILs for selected fragrance ingredients identified as potential dermal sensitizers.

Animal (guinea pig and mouse), human (maximization, 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.

1.3.4. 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.,

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. μg/cm²), is considered as the most appropriate dose metric for skin sensitization. This is 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

GUIDELINE #2.

A NOEL from a well run HRIPT, will be given precedence over NOELs from other repeated exposure clinical tests that were conducted in human subjects 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 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 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 (Guinea Pig Maximization Test (GPMT), Freund's Complete Adjuvant Test (FCAT), Mouse Ear Swelling Test (MEST), etc.) and non-adjuvant tests in guinea pigs (e.g. Buehler Test, Open Epicutaneous Test (OET), Closed Epicutaneous Test (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. Exceptionally, (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. 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. Any 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.

Fig. 1. Guidelines for applying weight of evidence (WoE) approach for use of induction sensitization data on fragrance ingredients for derivation of NESILs.

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) which takes account of three parameters—inter-individual variability (the same as in general toxicology), vehicle/product matrix effects, and use considerations (specific for dermal sensitization). The concept of and the parameters affecting the SAF for fragrance ingredients were originally proposed by Gerberick et al. (2001) and expanded by Felter et al. (2002). The SAFs recommended in this paper draw and build from the previous publications.

Key SAF areas to be addressed are given in the forthcoming sections.

1.3.4.1. Inter-individual variability. The SAF for inter-individual variability allows for possible variations in the sensitivity of individuals within the human population due to different parameters such as genetic effects, sensitive subpopulations, inherent barrier function, age, gender, and ethnicity. Genetic factors are not totally understood, but are clearly instrumental in determining individual susceptibility (Felter et al., 2002; Smith and Hotchkiss, 2001). There are several studies that address the importance of subpopulations, such as those with multiple allergies who may be more susceptible (Felter et al., 2002; Friedmann

Table 1
No expected sensitization induction level (NESIL) for fragrance ingredients derived by application of weight of evidence guidelines

Fragrance ingredient	CAS No.	LLNA weighted mean EC3 values (μg/cm^2) [no. of studies]	Potency classification based on animal data[b]	Human data			WoE NESIL[c] (μg/cm^2)
				NOEL HRIPT (induction) (μg/cm^2)	NOEL HMT (induction) (μg/cm^2)	LOEL[a] (induction) (μg/cm^2)	
α-Amylcinnamaldehyde	122-40-7	2420 [4]	Weak	23,622[d]	NA	NA	23,600
α-Amylcinnamyl alcohol	101-85-9	>6250 [1][e]	Weak	3543[d]	NA	NA	3500
Anisyl alcohol	105-13-5	1475 [1][e]	Moderate	NA	3448[d]	NA	1500
Benzyl alcohol	100-51-6	>12,500 [1][e]	Weak	5906	6897	8858	5900
Benzyl benzoate	120-51-4	>12,500 [1][e]	Weak	59,050[d]	20,690[d]	NA	59,000
Benzyl cinnamate	103-41-3	4600 [1][e]	Weak	4720[d]	5517[d]	NA	4700
Benzyl salicylate	118-58-1	725 [1][e]	Moderate	17,717[d]	20,690[d]	NA	17,700
p-t-Butyl-α-methylhydro-cinnamic aldehyde (BMHCA)	80-54-6	2372 [6]	Weak	4125	NA	29,528	4100[f]
Cinnamyl alcohol	104-54-1	5250[1][e]	Weak	3000	2759	4724	3000
Cinnamaldehyde	104-55-2	262 [23]	Moderate	591	NA	775	590
Citral	5392-40-5	1414 [11]	Moderate	1400	NA	3876	1400
DL-Citronellol	106-22-9	10,875 [1][e]	Weak	29,528[d]	4138	NA	29,500[g]
Coumarin	91-64-5	>6250 [1][e]	Weak	3543	5517	8858	3500
Eugenol	97-53-0	2703 [6]	Weak	5906	NA	NA	5900
Farnesol	4602-84-0	1200 [2]	Moderate	2755	NA	6897[h]	2700
Geraniol	106-24-1	3525 [5]	Weak	11,811	NA	NA	11,800
α-Hexyl-cinnamaldehyde	101-86-0	2372 [>5]	Weak	23,622[d]	NA	NA	23,600
Hydroxycitronellal	107-75-5	5612 [9]	Weak	5000	NA	5906	5000
3 & 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde (HMPCC)	31906-04-4	4275 [1][e]	Weak	4000	NA	NA	4000
Isoeugenol	97-54-1	498 [18]	Moderate	250	NA	775	250
D-Limonene[i]	5989-27-5	10,075 [5]	Weak	10,000[d]	5517[d]	NA	10,000
Linalool[i]	78-70-6	12,650 [2]	Weak	15,000[d]	13,793[d]	NA	15,000
Methyl 2-octynoate (Methyl heptine carbonate)	111-12-6	<125 [1][e]	Strong	118	NA	194	120
Methyl 2-nonynoate (Methyl octine carbonate)	111-80-8	<1250 Estimated 625 [1][e]	Moderate	24	NA	118	24
α-iso-Methylionone	127-51-5	5450 [1][e]	Weak	70,866[d]	NA	NA	71,000
Phenylacetaldehyde	122-78-1	962 [2]	Moderate	591	NA	1181	590
Oakmoss	90028-68-5	970 [1][e]	Moderate	700	NA	NA	700[j]
Treemoss	90028-67-4	2163 [2]	Moderate	700	NA	NA	700[k]
trans-2-Hexenal	6728-26-3	1012 [2]	Moderate	24	NA	236	24
Isocyclogeraniol	68527-77-5	>6250 [1][e]	Weak	3898	NA	7752	3900
Cinnamyl nitrile	1885-38-7	>2500 [1][e]	Weak	581	NA	1250	580[l]

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; HMT = human maximization test; LOEL = lowest observed effect level; NA = not available.

[a] Data derived from HRIPT or HMT.

[b] Based on animal data using classification defined in ECETOC, Technical Report No. 87, 2003.

[c] WoE NESIL limited to three significant figures.

[d] MT-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.

[e] EC3 value from one LLNA, not the mean.

[f] BMHCA—HRIPT LOEL data suggest that the NOEL is likely to be in the region of 29,000 μg/cm^2. On this basis, the IFRA Joint Advisory Group (JAG) was asked to supply any sensitization data on final products containing BMHCA.

[g] DL-Citronellol—IFRA Joint Advisory Group was asked to supply any sensitization data on final products containing DL-Citronellol.

[h] LOEL from human maximization test, not a human repeated insult patch test.

[i] 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.

[j] Oakmoss—Pending LLNA and a confirmatory HRIPT on new qualities of oakmoss, which contain significantly lower levels of atranol and choloratranol. All data presented are on qualities of oakmoss containing typical levels of atranol and chloroatranaol.

[k] Treemoss—Pending LLNA and a confirmatory HRIPT on new qualities of treemoss, which contain significantly lower levels of atranol and choloratranol. All data presented are on qualities of treemoss containing typical levels of atranol and chloroatranaol.

[l] RIFM sponsored HRIPT with 1000 μg/cm^2 cinnamyl nitrile is in progress.

and Moss, 1985; Moss et al., 1985). Inherent barrier function for inter-individual susceptibility is an important to consider because its function can be compromised and could lead to greater susceptibility for induction of contact allergy. Age, gender, and ethnicity may have an effect on inherent barrier function in healthy skin.

Skin barrier function is very similar from infancy to adulthood (Cunico et al., 1977; Cassimos et al., 1980; West et al., 1981; Holbrook, 1982; McCormack et al., 1982; Wester and Maibach, 1982; Fairley and Rasmussen, 1983; Harpin and Rutter, 1983). Decreases in the skin barrier function can occur at either end of the age spectrum—pre-term infant (Kalia et al., 1998) and geriatric under certain conditions (Leveque et al., 1984; Ghadially et al., 1995). Pre-term infants were not included in this review since they would be under medical care.

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 (Robinson, 1999; Felter et al., 2002). Weight of evidence indicates individuals of different ethnic origins are not substantially more susceptible to induction of contact allergy (Kligman, 1966; Weigand et al., 1974).

Genetic effects, sensitive subpopulations, and inherent barrier function are known to be generally more influential than age, gender, and ethnicity (Robinson, 1999; Felter et al., 2002).

1.3.4.2. 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.

- *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 possibly by influencing the magnitude of response or by influencing other steps in the induction of allergy (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).
- *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.

1.3.4.3. 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.

Regional differences in dermal absorption can be substantial. Table 2 provides a comprehensive list of references that describe important considerations for application to different sites of contact. Variations in barrier integrity can be influenced by consumer practices. 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 and Friedlander, 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).

Occlusion of the skin increases the hydration of the stratum corneum, skin temperature, microbial count, pH, and dermal irritation (Zhai and Maibach, 2001) which can influence dermal penetration. The human data used to define NESILs are obtained under semi- or fully-occlusive experimental patch conditions. Under most circumstances

Table 2
Derivation of SAFs for fragrance ingredients in different product types using RIFM data: rationale and the literature references

Product type	Inter-individual[a,b,c,d,e,f,g,h,i,j,k,l,m,n,o,p,q,r,s,t,u,v,w,x,y,z,aa] SAF	Matrix SAF	Matrix SAF rationale[f,z,ab,ac,ad,ae,af] (experimental versus real life exposure)	Use SAF	Use SAF rationale (experimental versus 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[ag]; the skin is easily irritated[ah], highly follicular[ag] and an area that is shaved [ai]. Type of occlusion is similar to that of the experimental test conditions[aj].	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[ag]; the skin is easily irritated[ah], highly follicular[ag] and an area that is shaved[ai]. Type of occlusion is similar to that of the experimental test conditions[aj].	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[ag]; the skin is easily irritated[ah], highly follicular[ag] and an area that is shaved[ai]. Type of occlusion is similar to that of the experimental test conditions[aj].	300
Roll-on deodorant	10	3*	Matrix for the product not the same as the experimental conditions.	10	The area is the underarm[ag]; the skin is easily irritated[ah], highly follicular[ag] and an area that is shaved[ai]. Type of occlusion is similar to that of the experimental test conditions[aj].	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[ag]; the skin is easily irritated[ah], highly follicular[ag] and an area that is shaved[ai]. Type of occlusion is similar to that of the experimental test conditions[aj].	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[ag]; the skin is easily irritated[ah], highly follicular[ag] and an area that is shaved[ai]. Type of occlusion is similar to that of the experimental test conditions[aj].	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[ag]; the skin is easily irritated[ah], highly follicular[ag] and an area that is shaved[ai]. Type of occlusion is similar to that of the experimental test conditions[aj].	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[ag] and mucous membranes [ak]; the skin is easily irritated[ah], highly follicular[ag] and an area that is shaved[ai]. Type of occlusion is similar to that of the experimental test conditions[aj].	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[ag]	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[ag], highly follicular[ag] and possible abrasion from shaving[ai].	300
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[ag], highly follicular[ag] and possible abrasion from shaving[ai].	300

(*continued on next page*)

Table 2 (*continued*)

Product type	Inter-individual[a,b,c,d,e,f,g,h,i,j,k,l,m,n,o,p,q,r,s,t,u,v,w,x,y,z,aa] SAF	Matrix SAF	Matrix SAF rationale[f,z,ab,ac,ad,ae,af] (experimental versus real life exposure)	Use SAF	Use SAF rationale (experimental versus real life exposure)	SAF
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[al].	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[ag] which may include, dry skin[am], abraded skin[ai] (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[am], there may be compromised skin due to dermatitis[ah], 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[ag].	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[ag].	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[aw]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[ag]. Type of occlusion is similar to that of the experimental test conditions[aj].	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[ag] and highly follicular[ag] and possible abrasion from shaving[ai].	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[ag].	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[ag] which may include, dry skin[am], abraded skin[ai] (e.g., underarms, legs) and possible exposure to mucous membranes [ak,an, ao,ap,aq,ar,as,at,au,av] .	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[ag] and the scalp which is more permeable[ag,aw].	100
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[ag] and the scalp which is more permeable[ag,aw].	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[ag] and the scalp which is more permeable[ag,aw].	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[ag] and the scalp which is more permeable[ag,aw].	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[ag] which may include, dry skin[am], abraded skin[ai] (e.g., underarms, legs), there may be compromised skin due to dermatitis[ah] and possible exposure to mucous membranes[ak,an,ao,ap,aq,ar,as,at,au,av]	100

Table 2 (*continued*)

Product type	Inter-individual[a,b,c,d,e,f,g,h,i,j,k,l,m,n,o,p,q,r,s,t,u,v,w,x,y,z,aa] SAF	Matrix SAF	Matrix SAF rationale[f,z,ab,ac,ad,ae,af] (experimental versus real life exposure)	Use SAF	Use SAF rationale (experimental versus real life exposure)	SAF
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[am], there may be compromised skin due to dermatitis[ah].	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[ag].	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[ag] which may include, dry skin[am], abraded skin[ai] (e.g., underarms, legs) and possible exposure to mucous membranes [ak,an,ao,ap,aq,ar,as,at,au,av]. 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[ag].	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)[ak,ax,ay,az,ba,bb,bc,bd,be]. Data suggest the peri-oral skin (a site of concern) is highly permeable[bf] 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[ak].	100
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)[ak,ax,ay,az,ba,bb,bc,bd,be]. Data suggest the peri-oral skin (a site of concern) is highly permeable[bf] 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[ak].	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[bg] but there may be compromised skin due to dermatitis[ah].	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[bh].	10
Closed air fresheners	10	1	Enclosed product; limited contact with fragrance.	1	Closed product, only rare accidental contact may occur.	10

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Table 2 (*continued*)

Product type	Inter-individual[a,b,c,d,e,f,g,h,i,j,k,l,m,n,o,p,q,r,s,t,u,v,w,x,y,z,aa] SAF	Matrix SAF	Matrix SAF rationale[f,z,ab,ac,ad,ae,af] (experimental versus real life exposure)	Use SAF	Use SAF rationale (experimental versus real life exposure)	SAF
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[ak,an,ao,ap,aq,ar,as,at,au,av]; Type of occlusion is similar to that of the experimental test conditions[aj].	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[ak,an,ao,ap,aq,ar,as,at,au,av] and outer labia, which are highly follicular[ag]. Type of occlusion, due to under clothing, is similar to that of the experimental test conditions[aj].	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[ak]	The area is vaginal mucous membrane[ak,an,ao,ap,aq,ar,as,at,au,av] includes non-keratinized mucous membrane-increased permeability[az,bf,bi]. The nature of occlusion is different, but effect is expected to be similar to that of the experimental test conditions[aj].	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)[bj] and involve mucous membrane exposure[ak,an,ao,ap,aq,ar,as,at,au,av]. There is occlusion through diaper use[bj].	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)[bj] and involve mucous membrane exposure[ak,an,ao,ap,aq,ar,as,at,au,av]. There may be occlusion through diaper use[bj].	300
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)[ag] or possibly whole body[ag] and mucous membrane exposure (body wash)[ak,an,ao,ap,aq,ar,as,at,au,av].	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[ag] and the skin integrity may be compromised (diaper rash)[bj] and mucous membrane exposure (body wash)[ak,an,ao,ap,aq,ar,as,at,au,av].	100
Baby cream	10	3*	Matrix for the product is designed to enhance penetration.	10	The area is possibly whole body[ag] or head (scalp more permeable)[ag] and the skin integrity may be compromised (diaper rash)[bj] and mucous membrane exposure (body wash)[ak,an,ao,ap,aq,ar,as,at,au,av]. There may be occlusion through diaper use[bj]	300
Baby oil	10	3*	Matrix for the product is designed to enhance penetration.	10	The area is possibly whole body[ag] or head (scalp more permeable)[ag] and the skin integrity may be compromised (diaper rash)[bj] and mucous membrane exposure (body wash)[ak,an,ao,ap,aq,ar,as,at,au,av]. There may be occlusion through diaper use[bj].	300

Table 2 (*continued*)

Product type	Inter-individual[a,b,c,d,e,f,g,h,i,j,k,l,m,n,o,p,q,r,s,t,u,v,w,x,y,z,aa] SAF	Matrix SAF	Matrix SAF rationale[f,z,ab,ac,ad,ae,af] (experimental versus real life exposure)	Use SAF	Use SAF rationale (experimental versus real life exposure)	SAF
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[ag] and the skin integrity may be compromised (diaper rash)[bi] and mucous membrane exposure[ak,an,ao,ap,aq,ar,as,at,au,av]. There may be occlusion through diaper use[bj].	100
Tights with moisturizers	10	3*	Matrix for the product not the same as the experimental conditions.	10	The area is the lower extremities which may include, dry skin[am], abraded skin[ai] (e.g., shaved legs)$^{-}$ and semi- occlusion.	300
Insect Repellents (intended to be applied to the skin)	10	3*	Matrix for the product not the same as the experimental conditions. May contain irritating ingredients.	10	The area is the exposed skin (25% of their average total body surface area [bk]) which may include, hands, head, forearms, legs, dry skin[am], abraded skin[ai] (e.g., legs)$^{-}$.	300
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[ag]. May involve skin sites with dermatitis[ah].	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[ag]. May involve skin sites with dermatitis[ah].	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[ag]. May involve skin sites with dermatitis[ah].	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[ag]. May involve skin sites with dermatitis[ah].	100

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).

[a] Travis and White (1988).
[aa] Weigand et al. (1974).
[ab] Robinson et al. (2000).
[ac] Smith et al. (2000).
[ad] Cumberbatch et al. (1993).
[ae] Scheuplein and Ross (1970).
[af] Schaefer and Redelmeier (1996).
[ag] Feldmann and Maibach (1967).
[ah] Benfeldt et al. (1999).
[ai] Edman (1994).
[aj] Bucks et al. (1989).
[ak] Farage et al. (2003).
[al] Nuutinen et al. (2003).
[am] Matts and Rawlings (2005).
[an] Britz and Maibach (1979).
[ao] Britz and Maibach (1979a).
[ap] Elsner and Maibach (1990).
[aq] Elsner et al. (1990).
[ar] Elsner et al. (1990a).
[as] Elsner et al. (1990b).
[at] Elsner et al. (1990c).
[au] Elsner et al. (1991).
[av] Farage and Maibach (2004).
[aw] Zhai et al. (2004).
[ax] Kobayashi and Tagami (2004).
[ay] de Vries et al. (1991).
[az] Harris and Robinson (1992).

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Table 2 (*continued*)

[b] Chappell and Mordenti (1991).
[ba] Lesch et al. (1989).
[bb] Squier and Hall (1985).
[bc] Squier (1986).
[bd] Squier (1991).
[be] Sayani and Chien (1996).
[bf] Kobayashi and Tagami (2004a).
[bg] American Beauty Association (2002).
[bh] Selim (2005).
[bi] Thompson et al. (2001).
[bj] Odio and Friedlander (2000).
[bk] EPA (2001b).
[c] Renwick and Lazarus (1998).
[d] Burin and Saunders (1999).
[e] Aldridge et al. (2003).
[f] Felter et al. (2002).
[g] Robinson (1999).
[h] Smith and Hotchkiss (2001).
[i] Dupuis (1979).
[j] Friedmann and Moss (1985).
[k] Moss et al. (1985).
[l] Cassimos et al. (1980).
[m] Cunico et al. (1977).
[n] West et al. (1981).
[o] Holbrook (1982).
[p] McCormack et al. (1982).
[q] Wester and Maibach (1982).
[r] Fairley and Rasmussen (1983).
[s] Harpin and Rutter (1983).
[t] Kalia et al. (1998).
[u] Leveque et al. (1984).
[v] Ghadially et al. (1995).
[w] Jordan and King (1977).
[x] Rees et al. (1989).
[y] Young et al. (1988).
[z] Kligman (1966).
[*] For practical purposes the number 3 is the practical representation of 3.16 (half log of 10)].

consumers are exposed to products under less than full occlusive conditions (examples of exceptions are diapers and axillary products). For those products where occlusion in the consumer exposure scenario is greater than that of the experimental situation, the SAF is 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 SAF to reflect the large differences between the experimental situation and real life scenarios here.

1.3.4.4. 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. In general, the more impactful 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. (For purposes practical in this paper the value of 3.16 is represented simply as the number 3.) 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). 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 for use considerations 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. Fig. 2 illustrates the approach to assign SAFs.

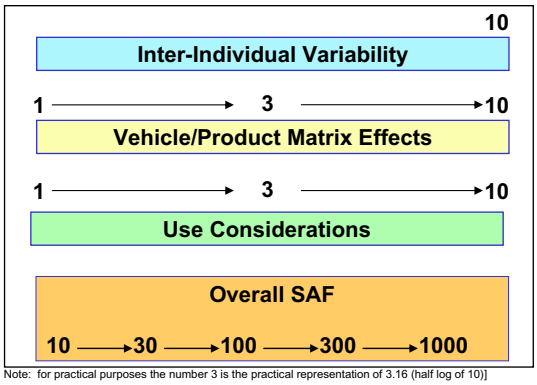

Fig. 2. Sensitization assessment factor (SAF). (SAF is calculated by multiplying sub-factors for inter-individual variability, vehicle/matrix effects, and use considerations.)

1.3.4.5. 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. Since 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, 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 are based on the use of a vehicle containing ethanol. Key factors in determining this 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 based on RIFM data, the SAFs for fragrance ingredients are based on the use of confirmatory human data which were 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 h per patch application. Key factors in determining this SAF are primarily site of contact and personal practices that impact barrier function.

Table 2 details the numbers assigned to each of the components of the SAF for fragrance ingredients in different types of products. 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.

1.3.4.6. Choice of consumer product types. 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 and is based on those products listed in the SCCNFP Notes of Guidance (SCCNFP, 2003), on products surveyed by CTFA and Colipa, on products specified in the IFRA Standards and the experience of the authors. This list is not intended to be all inclusive.

1.4. Exposure

1.4.1. Dose metric

As indicated above, the dose metric recommended for use in dermal sensitization risk assessments for fragrance ingredients is dose/area (μg/cm^2). Support for this position is based on an understanding of the immunological principles of induction of dermal sensitization and from clinical and pre-clinical data.

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 and to migrate out of the skin to the nearest lymph node 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 Fig. 3.

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

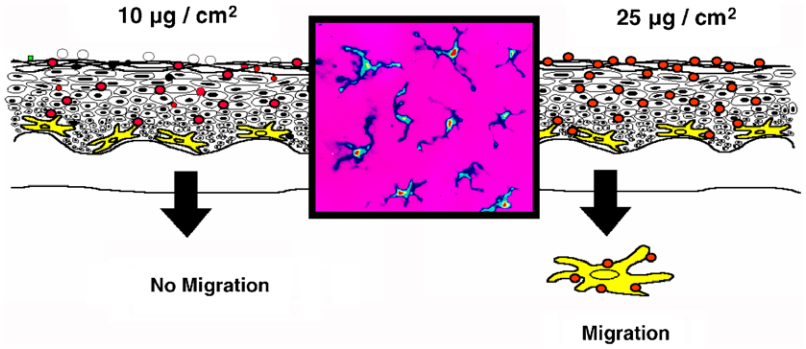

Fig. 3. Importance of dose/unit area for the induction of skin sensitization.

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).

One of the most important pieces of work in this area was conducted by Kligman in 1966 in which he investigated sensitizing areas of exposure, stimulation of more than one lymph node using various sites, use of a number of smaller patches versus one larger patch and different exposure conditions. Work conducted by Friedmann and his colleagues in the 1980s (Friedmann et al., 1983) clearly demonstrated that the total dose of allergen per area of skin (e.g., µg/cm^2) is the critical exposure determinant for the induction of contact sensitization. This was confirmed by White et al. in 1986. Moreover, the work of Rees et al. (1990) identified that for very small areas, under 0.1 cm^2, the dose–response is significantly diminished. This suggested there is a minimum area of contact required to induce contact allergy.

The animal data are consistent with the human clinical data. Magnusson and Kligman (1970) guinea pig data showed that the concentration of allergen per unit area was most important.

A comprehensive in-depth review of the published studies including those mentioned above that support the use of dose per unit area in risk assessments for induction of dermal sensitization is provided in the publication by Kimber et al. (2008).

The effectiveness with which a material can cause dermal sensitization depends on a number of factors. Of key importance is the skin penetration of the material, i.e., the topical dose versus the dose delivered to the viable epidermis in the skin. In addition to skin penetration, other factors, such as evaporation, metabolism (either inactivation of activation), sequestration in the stratum corneum, binding to protein or cells in the epidermis, and uptake and presentation by antigen-presenting cells, determine if and how strong an immune response is triggered. Typically there is very little information available about the bioavailability of the material in either the experimental situation or real life exposure scenario. The application of the SAF account for this area of uncertainty. Consequently, for QRA, topical doses, expressed as dose/unit area, can be used in the definition of NESIL and CEL.

1.4.2. Consumer exposure level (CEL)

Consumer exposure level (CEL) is an essential element of QRA. As such a prerequisite for risk characterization is to understand how consumers will be exposed to fragrance ingredients from use of the consumer products. The CEL (expressed as dose/unit area/day) is a measure of exposure under intended and foreseeable conditions of use (but not abuse) and takes account of the frequency of use, habits, and practices (e.g., how consumers use the product), duration of use and amount of product used per application/use.

It should be noted that the CEL defined within this paper addresses consumer products that are bought for personal use. Occupational/professional exposure is not addressed in this paper because comprehensive exposure data are not available.

If the frequency of product use may be more than once a day, material accumulation on the same skin site should be considered, (depending upon the physical chemical properties of the material). 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. It is important to have reliable habits and practices and accurate human parameters data. Skin penetration is not specifically addressed in measuring consumer exposure since the dose metric is unit weight applied per unit area to the outer surface of the skin. As such, using a conservative approach, the topical dose is taken to be the delivered dose. Differences in skin penetration due to different product matrices are accounted for in the final risk assessment by use of the matrix SAF as previously discussed.

Using these criteria, the data sources given in Table 3 were used in the calculation of CEL. A hierarchy was established for how to use the data based on robustness and scope. When measured data for the same product type were available from more than one source, the most conservative value (i.e., the highest value) was used unless there was a sound scientific rationale to use data from

Table 3
Calculation of consumer exposure levels (CEL) from available habits and practices and human parameters data for different product types (exposures used in the QRA method are bold and italicized)

Product Type	Surface area, cm^2	Surface area reference	Retention factor[a]	EC or SCCNFP[a]			CTFA[b]		Cano and Rich (2001); Tozer et al. (2004); Cano (2006)[c]	Colipa[d]			HERA[e] (mg/cm^2/day)	FMA[f] (mg/cm^2/day)	RIFM[g] (mg/cm^2/day)
				mg/application	applications/day	mg/cm^2/day	90th Percentile		95th Percentile (mg/cm^2/day)	90th Percentile		Dec. 2005 (mg/cm^2/day)			
							mg/day	mg/cm^2/day		mg/day	mg/cm^2/day				
Deo/AP-type not specified	100	Bremmer et al. (2003), per axillae	1	500	1	2.50									
Deo/AP spray	100	Bremmer et al. (2003), per axillae	1							6100*	30.5*				
Deo/AP non-spray	100	Bremmer et al. (2003), per axillae	1							1500	7.5				
Deo/AP all over body	100	Bremmer et al. (2003), per axillae	1							6500*	32.5*				
Solid AP	100	Bremmer et al. (2003), per axillae	1				1700	8.50							9.1**
Shaving cream/depilatory[g,h]	305	Bremmer et al. (2003) (1/4 area head, male)	0.01	2000	1	0.07									
Lip products	4.8	Ferrario et al. (2000)	1	10	4	8.33	55	11.46		56	11.67				
Eye products[i]	24	Bremmer et al. (2003)	1	10	2	0.83	52	2.17				2.5			
Body cream/lotion	12,895	EPA (1997) (area body – head and 1/2 trunk, female)[j]	1	8000	0.5	0.31	14,400	1.12		7800	0.60				
Men's facial cream	775	Bremmer et al. (2003) (1/4 area head + 1/2 area hands, male)	1	800	2	2.06									
Toothpaste	216.8	Collins and Dawes (1987); Ferrario et al. (2000) (buccal + lips)	0.1[k]	1400	2	1.29				2700	1.25		1.0[k]		
Mouthwash	216.8	Collins and Dawes (1987); Ferrario et al. (2000) (buccal + lips)	0.01[k]	10,000	3	1.38						1.38	1.0[k]		
Hydroalcoholic products for shaved skin[l]	775	Bremmer et al. (2003) (1/4 area head + 1/2 area hands, male)	1						***2.21***						
Hydroalcoholic products for unshaved skin	100	Bremmer et al. (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[c] (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[l]	555	EPA (1997) (1/2 area head, female)	0.1				7730	1.39			0.45				
Hair sprays—pump spray[l]	555	EPA (1997) (1/2 area head, female)	0.1				12220	***2.20******							
Hair styling aids	1010	Bremmer et al. (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[b]	0.1	250	0.43	***0.97***									
Bar soaps	840	EPA (1997) (area hands)	0.01	800	6	***0.057***						0.05			
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	16,900	EPA (1997) (body area, female)	0.01	5000	2	0.006	25500	***0.015***				0.009			
Bath foams, gels, mousses[h]	16,900	EPA (1997) (body area, female)	0.01	17000	1	***0.010***									

(*continued on next page*)

Table 3 (*continued*)

Product Type	Surface area, cm^2	Surface area reference	Retention factor[a]	EC or SCCNFP[a]			CTFA[b]		Cano and Rich (2001); Tozer et al. (2004); Cano (2006)[c]	Colipa[d]			HERA[e] (mg/cm^2/day)	FMA[f] (mg/cm^2/day)	RIFM[g] (mg/cm^2/day)
				mg/application	applications/day	mg/cm^2/day	90th Percentile		95th Percentile (mg/cm^2/day)	90th Percentile		Dec. 2005 (mg/cm^2/day)			
							mg/day	mg/cm^2/day		mg/day	mg/cm^2/day				
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
Insect repellent (intended to be applied to the skin)	4225	EPA (2001b) (25% body area, female—head, hands, forearms, legs)													***3.02***
Handwash laundry													***0.1***		
Laundry tablets & Powder													***Insignificant***		
Hand dishwashing													***0.01***		
Fabric clothing													***Insignificant***		
Tights with moisturizers	6570	EPA (1997) (lower extremities, female)	1												***0.00005***
Hard surface cleaner													***0.12***		
Candles														***0.00033***	

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).
Hair spray—exposure for the pump spray is recommended for all hair sprays since this figure was the most conservative (e.g., highest) value.

[a] EC (1996) or SCCNFP (2003) Guidelines.

[b] Loretz et al. (2005, 2006); CTFA (2005,a,b).

[c] Cano and Rich (2001); Tozer et al. (2004); Cano (2006).

[d] Colipa (2005).

[e] AISE/HERA (2002).

[f] Api et al. (2007).

[g] 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, diapers, intimate wipes and baby wipes; RIFM (2007), Memo to AM Api from RIFM Member Company, January 2007 on exposure to tights with moisturizers, RIFM (2007), Memo to AM Api from RIFM Member Company, January 2007 on exposure to insect repellents intended to be applied on the skin, Cowan-Ellsberry et al. (2008), exposure on axilla surface area.

[h] 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.

[i] For frequency of use less than once per day, the default of once per day was used with the exception of nail care products.

[j] 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.

[k] 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.

[l] 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 versus 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 1 mg/cm^2, assuming 100% retention of the fragrance ingredient from the product solution. This is consistent with the value identified by the primary exposure approach.

[*] 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.

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, (3) Cowan-Ellsberry et al. (2008) deodorant/antiperspirant data were used over CTFA and Colipa data because Cowan-Ellsberry et al. (2008) used measured 90th percentile exposure (amount) and surface area data and integrated it into a per diem exposure).

All of these 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 given the conservatisms in the model (Colipa, 2005). On the other hand, whilst the study conducted by Cano and 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.

Several authoritative sources of human parameters data (i.e., skin site surface areas) (Collins and Dawes, 1987; EPA, 1997; Ferrario et al., 2000; Bremmer et al., 2003; Cowan-Ellsberry et al., 2008) were used and a hierarchal approach applied. Preference was given conservatively to the smaller surface area (i.e., 50th percentile in combination with the measured CTFA and Colipa exposure data). The exceptions to this are studies in which exposure and surface area data are integrated (i.e., Cano and Rich, 2001; Tozer et al., 2004; Cano, 2006; Cowan-Ellsberry et al., 2008). Within these human parameters data sources, the individual references used to support the consumer exposure to different product types are detailed in Table 3.

1.5. Risk characterization

There are two key elements involved in risk characterization in the recommended approach. These are the Acceptable Exposure Level (AEL) and the comparison of that AEL to the CEL. The practical application of risk characterization to the identification of product categories is detailed below.

1.5.1. Acceptable exposure level (AEL)

The AEL is determined by dividing the WoE NESIL by the product type SAF.

Table 4
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 μg/cm^2	500 μg/cm^2
SAF	300	100
AEL = WoE NESIL/SAF	=500/300	=500/100
AEL	1.7 μg/cm^2	5.0 μg/cm^2

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

The AEL is expressed in terms of dose/unit area/day. The definition of this AEL allows identification of exposures to fragrance ingredients that are acceptable (below the AEL) or unacceptable (above the AEL).

This is demonstrated below in Table 4 for a hypothetical fragrance ingredient (X) in a deodorant product and a hydroalcoholic product for unshaved skin.

1.5.2. AEL/CEL ratio

To establish the acceptability of consumer exposure to a fragrance ingredient in a given product, the ratio of the AEL to the CEL is determined by dividing the AEL by the CEL (AEL/CEL). 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 5 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 $\geqslant$ CEL).

1.5.3. Product categories

A practical application of the recommended risk assessment approach for fragrance ingredients is to form product categories for the implementation of IFRA Standards. The process to define product categories and the use of this approach to establish IFRA Standards is described in separate publications (Api and Vey, submitted for publication).

2. Conclusions

QRA represents a very important step forward in skin sensitization risk assessment. Implementation by the fragrance industry of the QRA approach for fragrance ingredients described in this review has now begun.

Principles of general toxicology risk assessment can be applied to induction of skin sensitization since this

Table 5
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 μg/cm^2	500 μg/cm^2
SAF	300	100
AEL	1.7 μg/cm^2	5 μg/cm^2
Product exposure[a]	9.1 mg/cm^2/day	2.2 mg/cm^2/day
Concentration of fragrance *X* in the product	0.1%	0.1%
CEL	=0.1% * 9.1 mg/cm^2 * 1000 μg/mg =9.1 μg/cm^2	=0.1% * 2.2 mg/cm^2 * 1000 μg/mg =2.2 μg/cm^2
Risk assessment	Unacceptable (AEL < CEL)	Acceptable (AEL ⩾ CEL)

[a] Product exposure selected for this example is the data from Cowan-Ellsberry et al. (2008) for antiperspirants; and the 95 percentile data from Tozer et al. (2004) study for hydroalcoholic products for unshaved skin.

is also a threshold phenomenon. However, these general principles require tailoring to take into account unique elements of dermal sensitization as a toxicity endpoint. Following identification of a fragrance ingredient as a potential dermal sensitizer, a weight of evidence approach is used to determine its NESIL. This introduces a better approach to allergen potency evaluation for use in risk assessment. SAFs within the dermal sensitization QRA approach are based on published peer-reviewed scientific data and have been predefined for certain product types. As with all risk assessment, exposure is a critical element and in this approach the CEL is calculated using the best available habits and practices and human parameters data. The NESIL, CEL, and AEL are expressed in quantity of allergen per unit area in keeping with empirical evidence.

The dermal sensitization QRA approach can be used to estimate safe exposure levels for fragrance ingredients. In this way, it can be used as a basis for risk management. For fragrance ingredients 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.

With the 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 HIRPT protocol are available from Politano and Api (2008). Diagnostic patch test data from dermatology clinics are not used in the determination of the NESIL. This is because these data are a measure of elicitation of allergic contact dermatitis, not induction of dermal sensitization. To date there are insufficient data to discern any quantitative relationship between induction and elicitation. Clinical results from the dermatology community and company post-market surveillance data should be used to confirm the effectiveness of QRA-based risk management procedures.

There may be refinements to this dermal sensitization QRA approach for fragrance ingredients in the future as new information becomes available. Some key areas for potential refinement are (1) improved exposure data (i.e., habits and practices, human parameter data) to further refine CEL and extend it to include occupational/professional exposure to consumer products; (2) the influence of LLNA EC3 values on the WoE NESIL determinations, may be re-evaluated as more experience is gained with its use as a indicator of human allergenic potency; and (3) SAFs, where additional data (e.g., the influence of evaporation, of retention factors) may lead to refinement.

Conflict of Interest

Anne Marie Api is an employee of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances.

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