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Introduction

Special issue on QRA

Significant developments have recently been incorporated in the way dermal sensitization risk assessments are conducted for fragrance ingredients. The details about the methodology used in dermal sensitization quantitative risk assessment (QRA) for fragrance ingredients are explained in Api et al. (2008). This QRA methodology is a major improvement over the former approach because it is (1) quantitative versus qualitative, (2) exposure-based, (3) consistent with modern principles of toxicological risk assessment, (4) can be applied to dermal sensitization since this is a threshold phenomenon, and (5) addresses factors that are specific to dermal sensitization, such as those related to different products types and use patterns.

The Research Institute for Fragrance Materials, Inc. (RIFM) is the international scientific authority for the safe use of fragrance materials. The scientific program at RIFM is guided by a panel of independent and international experts, the RIFM Expert Panel (REXPAN). This panel reviewed the new approach to conducting dermal sensitization risk assessments for fragrance ingredients as a basis for establishment of risk management measures by the fragrance industry. The panel concluded that the QRA process is scientifically defensible being based on sound principles of general toxicology and contact allergy and the latest in-depth technical knowledge of exposure scenarios and appropriately conservative assessment factors. The panel also recommended continuing refinement of this methodology as detailed in Api et al. (2008).

Following a recommendation from REXPAN, this QRA methodology was formally adopted as the core strategy for primary prevention of dermal sensitization to fragrance materials in consumer products. This methodology is now being used to determine global fragrance industry product management practices for potentially sensitizing fragrance ingredients on an ongoing basis and is being implemented through the International Fragrance Association (IFRA) Standards.

Key steps of the QRA process are determination of known safe benchmarks, application of sensitization assessment factors, and calculation of consumer exposure through normal product use. With these parameters, an acceptable exposure level (AEL) can be calculated and compared with the consumer exposure level (CEL). The ratio of the AEL–CEL must be favorable to support the safe use of the skin sensitizer. This ratio must be calculated and compared with the consumer exposure level that was identified as a no effect level (NOEL) in an animal model or derived as a likely NOEL from quantitative structure-activity relationships is a valuable part of the weight of evidence approach to risk assessment for consumer protection. This value is based on the fact that while the LLNA is good predictor of the NOEL in humans, it is not perfect. The paper by McNamee et al. (2008) is a review of human repeat insult patch test (HRRIPT) methodology. It describes the key factors that are critical to the conduct and interpretation of the test methodology and provides general guidelines for evaluation of skin responses. With implementation of the QRA approach, IFRA/RIFM are recommending the use of the RIFM standard HRRIPT protocol for generation of confirmatory human data for use in QRA. The article by Politano and Api (2008) details the standard confirmatory HRRIPT protocol used by RIFM for over 20 years.

Another critical element of QRA for dermal sensitization is the dose metric of dose per unit area for induction of contact allergy, which is considered the most appropriate dose metric for dermal sensitization. A detailed review of the evidence that supports dose per unit area in this context and the mechanistic basis for this relationship is provided in the article by Kimber et al. (2008).

Although the QRA methodology for fragrance ingredients has been implemented as described above, it is generally recognized that the method should be further refined as new information becomes available. The last paper in this special issue, by Cowan–Ellsberry et al. (2008), describes the results from a study measuring the axilla surface area. This is a refinement of the previously available data for this body site, which were modeled data. Combining these new surface area data with measured use data also reported in the Cowan–Ellsberry paper along with other studies enabled calculations of consumer exposure to solid antiperspirant/deodorant products.

To implement the dermal sensitization QRA for fragrance ingredients, a practical approach was needed to convert the QRA on a fragrance ingredient in specific product types to an effective risk management system. Consumer exposure to fragrances can arise
from the use of a multitude of different types of cosmetic and household products. A realistic application of the recommended QRA approach is achieved by grouping consumer product types according to key parameters identified within the QRA approach. For a detailed description of the implementation guidelines and product categories, see Api and Vey (2008). This article also provides a practical example of how this QRA methodology is implemented for the fragrance ingredient citral. A summary of the dermal sensitization data on citral can be found in the paper by Lalko and Api (2008).

References


Anne Marie Api*

Research Institute for Fragrance Materials, Inc.,
50 Tice Boulevard, Floor 3,
Woodcliff Lake, NJ 07677, USA
Fax: +1 201 689 8090.
E-mail address: amapi@rifm.org

Matthias Vey

International Fragrance Association,
Brussels, Belgium
Dermal sensitization quantitative risk assessment (QRA) for fragrance ingredients

Anne Marie Api a,*, David A. Basketter b,1, Peter A. Cadby c, Marie-France Cano d,2, Graham Ellis e, G. Frank Gerberick f, Peter Griem g, Pauline M. McNamee h, Cindy A. Ryan f, Robert Safford b

a Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ, USA
b Unilever SEAC, Colworth House, Sharnbrook, Bedford MK44 1LQ, United Kingdom
c Firmenich SA, Corporate Product Safety & Regulatory Affairs, Case Postale 239, 1, Route des Jeunes/de la Jonction, Geneva 8 CH-1211, Switzerland
d LVMH, Fragrance Safety and Regulatory Affairs, 185 Avenue de Verdun, Saint Jean de Braye Cedex F-45804, France
e Givaudan Suisse SA, 5 chemin de la parfumerie, Vernier CH 1214, Switzerland
f The Procter & Gamble Company, Miami Valley Laboratories, 11810 East Miami River Road, Cincinnati, OH 45252, USA
g Clariant Produkte (Deutschland) GmbH, Corporate Product Safety, Am Unisys-Park 1, 65843 Sulzbach, Germany
h The Procter & Gamble Technical Centres Ltd, Whitehall Lane, Egham Surrey TW20 9NW, United Kingdom

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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.

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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-
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-
rient 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., 2001a, 2004; Griem et al., 2003; Schneider and Akkan, 2004).

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/cm²) 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 (HRPT) (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 HRPT protocol for generation of confirmatory human data for use in QRA. Details of this standard HRPT 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 (HRPT), 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.

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 sub-populations, 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 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.

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

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

<table>
<thead>
<tr>
<th>Fragrance ingredient</th>
<th>CAS No.</th>
<th>LLNA weighted mean IC3 values (µg/cm²)</th>
<th>Potency classification based on animal dataa</th>
<th>Human data</th>
<th>WoE NESILb (µg/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>[no. of studies]</td>
<td></td>
<td>NOEL HRIPT (induction) (µg/cm²)</td>
<td>NOEL HMT (induction) (µg/cm²)</td>
</tr>
<tr>
<td>α-Amylcinnamyl alcohol</td>
<td>101-85-9</td>
<td>&gt;6250 [1]f</td>
<td>Weak</td>
<td>3543e</td>
<td>NA</td>
</tr>
<tr>
<td>Amyl alcohol</td>
<td>105-13-5</td>
<td>1475 [1]f</td>
<td>Moderate</td>
<td>3484d</td>
<td>NA</td>
</tr>
<tr>
<td>Benzyl alcohol</td>
<td>103-51-6</td>
<td>&gt;12,500 [1]f</td>
<td>Weak</td>
<td>9506</td>
<td>6897</td>
</tr>
<tr>
<td>Benzyl benzoate</td>
<td>122-27-6</td>
<td>&gt;6250 [1]f</td>
<td>Weak</td>
<td>59,050d</td>
<td>20,690d</td>
</tr>
<tr>
<td>Benzyl cinnamate</td>
<td>103-41-3</td>
<td>&gt;4000 [1]f</td>
<td>Weak</td>
<td>4720f</td>
<td>5517f</td>
</tr>
<tr>
<td>Benzyl salicylate</td>
<td>118-38-1</td>
<td>725 [1]f</td>
<td>Moderate</td>
<td>17,714d</td>
<td>20,690d</td>
</tr>
<tr>
<td>p-Butyl-α-methylhydro-cinnamic aldehyde (BMHCA)</td>
<td>80-54-6</td>
<td>2372 [6]f</td>
<td>Weak</td>
<td>4125</td>
<td>NA</td>
</tr>
<tr>
<td>Cinnamaldehyde</td>
<td>104-54-1</td>
<td>5250[1]f</td>
<td>Weak</td>
<td>3543</td>
<td>5517</td>
</tr>
<tr>
<td>Citral</td>
<td>104-55-2</td>
<td>262 [23]</td>
<td>Moderate</td>
<td>591</td>
<td>NA</td>
</tr>
<tr>
<td>Citral</td>
<td>106-22-9</td>
<td>10,875 [1]f</td>
<td>Weak</td>
<td>29,528d</td>
<td>4138</td>
</tr>
<tr>
<td>Coumarin</td>
<td>91-64-5</td>
<td>&gt;6250 [1]f</td>
<td>Weak</td>
<td>5906</td>
<td>NA</td>
</tr>
<tr>
<td>Eugenol</td>
<td>97-53-0</td>
<td>2703 [6]f</td>
<td>Weak</td>
<td>1181</td>
<td>NA</td>
</tr>
<tr>
<td>Farnesol</td>
<td>4602-84-0</td>
<td>1200 [2]f</td>
<td>Moderate</td>
<td>2755</td>
<td>6897h</td>
</tr>
<tr>
<td>Geraniol</td>
<td>106-24-1</td>
<td>3525 [5]c</td>
<td>Weak</td>
<td>11,811</td>
<td>NA</td>
</tr>
<tr>
<td>α-Hexyl-cinnamaldehyde</td>
<td>101-86-0</td>
<td>2372 [-5]</td>
<td>Weak</td>
<td>23,622d</td>
<td>NA</td>
</tr>
<tr>
<td>Hydroxycitronellal</td>
<td>107-73-5</td>
<td>5612 [9]f</td>
<td>Weak</td>
<td>5000</td>
<td>NA</td>
</tr>
<tr>
<td>3 &amp; 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyd (HMPCC)</td>
<td>31906-04-4</td>
<td>4275 [1]f</td>
<td>Weak</td>
<td>4000</td>
<td>NA</td>
</tr>
</tbody>
</table>

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.


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/cm². 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 chloroatranaol. 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 chloroatranaol. All data presented are on qualities of treemoss containing typical levels of atranol and chloroatranaol.

l RIFM sponsored HRIPT with 1000 µg/cm² cinnamyl nitrile is in progress.
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 (Cunicko 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 (Kalila 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**

<table>
<thead>
<tr>
<th>Product type</th>
<th>Inter-individual</th>
<th>Matrix SAF</th>
<th>Matrix SAF rationale (experimental versus real life exposure)</th>
<th>Use SAF</th>
<th>Use SAF rationale (experimental versus real life exposure)</th>
<th>SAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerosol deodorant</td>
<td>10</td>
<td>3*</td>
<td>Matrix for the product not the same as the experimental conditions.</td>
<td>10</td>
<td>The area is the underarm; the skin is easily irritated, highly follicular and an area that is shaved. Type of occlusion is similar to that of the experimental test conditions.</td>
<td>300</td>
</tr>
<tr>
<td>Aerosol antiperspirant</td>
<td>10</td>
<td>3*</td>
<td>Matrix for the product not the same as the experimental conditions and may contain irritating active ingredients.</td>
<td>10</td>
<td>The area is the underarm; the skin is easily irritated, highly follicular and an area that is shaved. Type of occlusion is similar to that of the experimental test conditions.</td>
<td>300</td>
</tr>
<tr>
<td>Stick deodorant/antiperspirant</td>
<td>10</td>
<td>3*</td>
<td>Matrix for the product not the same as the experimental conditions and may contain irritating active ingredients.</td>
<td>10</td>
<td>The area is the underarm; the skin is easily irritated, highly follicular and an area that is shaved. Type of occlusion is similar to that of the experimental test conditions.</td>
<td>300</td>
</tr>
<tr>
<td>Roll-on deodorant</td>
<td>10</td>
<td>3*</td>
<td>Matrix for the product not the same as the experimental conditions.</td>
<td>10</td>
<td>The area is the underarm; the skin is easily irritated, highly follicular and an area that is shaved. Type of occlusion is similar to that of the experimental test conditions.</td>
<td>300</td>
</tr>
<tr>
<td>Roll-on antiperspirant</td>
<td>10</td>
<td>3*</td>
<td>Matrix for the product not the same as the experimental conditions and may contain irritating active ingredients.</td>
<td>10</td>
<td>The area is the underarm; the skin is easily irritated, highly follicular and an area that is shaved. Type of occlusion is similar to that of the experimental test conditions.</td>
<td>300</td>
</tr>
<tr>
<td>Cream deodorant/antiperspirant</td>
<td>10</td>
<td>3*</td>
<td>Matrix for the product not the same as the experimental conditions and may be designed to enhance penetration. May contain irritating active ingredients.</td>
<td>10</td>
<td>The area is the underarm; the skin is easily irritated, highly follicular and an area that is shaved. Type of occlusion is similar to that of the experimental test conditions.</td>
<td>300</td>
</tr>
<tr>
<td>Gel deodorant/antiperspirant</td>
<td>10</td>
<td>3*</td>
<td>Matrix for the product not the same as the experimental conditions and may contain irritating active ingredients.</td>
<td>10</td>
<td>The area is the underarm; the skin is easily irritated, highly follicular and an area that is shaved. Type of occlusion is similar to that of the experimental test conditions.</td>
<td>300</td>
</tr>
<tr>
<td>Deodorant cologne (body sprays)</td>
<td>10</td>
<td>3*</td>
<td>Matrix for the product not the same as the experimental conditions.</td>
<td>10</td>
<td>The area is whole body including underarm and mucous membranes, the skin is easily irritated, highly follicular and an area that is shaved. Type of occlusion is similar to that of the experimental test conditions.</td>
<td>300</td>
</tr>
<tr>
<td>Hydroalcoholic products applied to unshaved skin</td>
<td>10</td>
<td>3*</td>
<td>Matrix for the product not the same as the experimental conditions.</td>
<td>3</td>
<td>The area is the neck, wrists, antecubital fossa that may have increased permeability.</td>
<td>100</td>
</tr>
<tr>
<td>Hydroalcoholic products applied to recently shaved skin</td>
<td>10</td>
<td>3*</td>
<td>Matrix for the product not the same as the experimental conditions.</td>
<td>10</td>
<td>The area is the face with increased permeability, highly follicular and possible abrasion from shaving.</td>
<td>300</td>
</tr>
<tr>
<td>Men’s facial cream and balms</td>
<td>10</td>
<td>3*</td>
<td>Matrix for the product not the same as the experimental conditions and may be designed to enhance penetration. May contain irritating ingredients.</td>
<td>10</td>
<td>The area is the face with increased permeability, highly follicular and possible abrasion from shaving.</td>
<td>300</td>
</tr>
</tbody>
</table>

(continued on next page)
<table>
<thead>
<tr>
<th>Product type</th>
<th>Inter-individual SAF Matrix SAF rationale</th>
<th>Use SAF</th>
<th>Use SAF rationale (experimental versus real life exposure)</th>
<th>SAF</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Eye products (includes: eye shadow, mascara, eyeliner, eye make-up)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body creams, lotions</td>
<td>Matrix for the product not the same as the experimental conditions, but not expected to be more irritating.</td>
<td>10</td>
<td>The area is the eye area with increased permeability and easily irritated.</td>
<td>300</td>
</tr>
<tr>
<td>Hand cream</td>
<td>Matrix for the product not the same as the experimental conditions and may be designed to enhance penetration.</td>
<td>3</td>
<td>The area is the entire body which may include, dry skin, abraded skin (e.g., underarms, legs) and semi-occlusion, due to clothing occurs.</td>
<td>300</td>
</tr>
<tr>
<td>Women’s facial cream/ facial make-up</td>
<td>Matrix for the product not the same as the experimental conditions.</td>
<td>3</td>
<td>The area is mainly the hands, which may include dry skin, there may be compromised skin due to dermatitis, but occlusion does not occur.</td>
<td>100</td>
</tr>
<tr>
<td>Make-up remover</td>
<td>Matrix for the product not the same as the experimental conditions and may be designed to enhance penetration. May contain irritating ingredients.</td>
<td>3</td>
<td>The area is the face with increased permeability.</td>
<td>100</td>
</tr>
<tr>
<td>Lip products</td>
<td>Matrix is very different from the experimental test conditions, however, it is not expected to be more irritating.</td>
<td>10</td>
<td>The site is highly vascular and there is exposure to mucous membranes and possible exposure to dry or chapped lips.</td>
<td>300</td>
</tr>
<tr>
<td>Foot care products</td>
<td>Matrix for the product not the same as the experimental conditions and may be designed to enhance penetration.</td>
<td>3</td>
<td>The area is the feet, which are less permeable. Type of occlusion is similar to that of the experimental test conditions.</td>
<td>300</td>
</tr>
<tr>
<td>Shaving creams</td>
<td>Matrix for the product not the same as the experimental conditions and may be designed to enhance penetration. May contain irritating ingredients.</td>
<td>10</td>
<td>The area is the face with increased permeability and highly follicular and highly possible abrasion from shaving.</td>
<td>100</td>
</tr>
<tr>
<td>Depilatory</td>
<td>Matrix is very different from the experimental test conditions and contains highly irritating ingredients.</td>
<td>3</td>
<td>The area is the underarm, upper part of the leg and lower part of the leg.</td>
<td>300</td>
</tr>
<tr>
<td>Body wash/shower gels</td>
<td>Matrix for the product not the same as the experimental conditions and may be designed to enhance penetration. May contain irritating ingredients.</td>
<td>3</td>
<td>The area is the entire body which may include, dry skin, abraded skin (e.g., underarms, legs) and possible exposure to mucous membranes.</td>
<td>100</td>
</tr>
<tr>
<td>Hair styling aids (mousse, gels, leave in conditioners)</td>
<td>Matrix is very different from the experimental test conditions and may contain ingredients that are irritating.</td>
<td>3</td>
<td>The area is the head which is highly follicular and the scalp which is more permeable.</td>
<td>100</td>
</tr>
<tr>
<td>Hair sprays</td>
<td>Matrix for the product not the same as the experimental conditions.</td>
<td>3</td>
<td>The area is the head which is highly follicular and the scalp which is more permeable.</td>
<td>100</td>
</tr>
<tr>
<td>Shampoo</td>
<td>Matrix for the product is very different from experimental conditions and may contain irritating ingredients.</td>
<td>3</td>
<td>The area is the head which is highly follicular and the scalp which is more permeable.</td>
<td>100</td>
</tr>
<tr>
<td>Conditioner (rinse-off)</td>
<td>Matrix for the product not the same as the experimental conditions and may be designed to enhance penetration. May contain irritating ingredients.</td>
<td>3</td>
<td>The area is mainly the hands, but may include the entire body which may include, dry skin, abraded skin (e.g., underarms, legs), there may be compromised skin due to dermatitis, and possible exposure to mucous membranes.</td>
<td>100</td>
</tr>
<tr>
<td>Bar soap</td>
<td>Matrix for the product not the same as the experimental conditions and may be designed to enhance penetration. May contain irritating ingredients.</td>
<td>3</td>
<td>The area is mainly the hands, but may include the entire body which may include, dry skin, abraded skin (e.g., underarms, legs), there may be compromised skin due to dermatitis and possible exposure to mucous membranes.</td>
<td>100</td>
</tr>
<tr>
<td>Product type</td>
<td>Inter-individual</td>
<td>Matrix SAF</td>
<td>Matrix SAF rationale (experimental versus real life exposure)</td>
<td>Use SAF</td>
</tr>
<tr>
<td>------------------------------</td>
<td>------------------</td>
<td>------------</td>
<td>---------------------------------------------------------------</td>
<td>---------</td>
</tr>
<tr>
<td>Liquid soap</td>
<td>10</td>
<td>3&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Matrix for the product not the same as the experimental conditions and may be designed to enhance penetration. May contain irritating ingredients.</td>
<td>3&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>Face washes, gels, scrubs</td>
<td>10</td>
<td>3&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Matrix for the product not the same as the experimental conditions and may be designed to enhance penetration. May contain irritating ingredients.</td>
<td>3&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bath gels, foams, mousses</td>
<td>10</td>
<td>3&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Matrix for the product not the same as the experimental conditions and may be designed to enhance penetration. May contain irritating ingredients.</td>
<td>3&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>Aerosol air fresheners</td>
<td>10</td>
<td>3&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Matrix for the product not the same as the experimental conditions.</td>
<td>3&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>Toothpaste</td>
<td>10</td>
<td>3&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Matrix is different from the experimental test conditions and may contain irritating ingredients.</td>
<td>3&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mouthwash</td>
<td>10</td>
<td>3&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Matrix for the product not the same as the experimental conditions but, not expected to be more irritating than the experimental conditions.</td>
<td>3&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>Nail care</td>
<td>10</td>
<td>3&lt;sup&gt;+&lt;/sup&gt;</td>
<td>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.</td>
<td>3&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>Candle not in a jar</td>
<td>10</td>
<td>1</td>
<td>Fragrance is not freely available for release from the matrix, unlike experimental conditions.</td>
<td>1</td>
</tr>
<tr>
<td>Closed air fresheners</td>
<td>10</td>
<td>1</td>
<td>Enclosed product; limited contact with fragrance.</td>
<td>1</td>
</tr>
</tbody>
</table>

(continued on next page)
<table>
<thead>
<tr>
<th>Product type</th>
<th>Inter-individual SAF</th>
<th>Matrix SAF</th>
<th>Matrix SAF rationale (experimental versus real life exposure)</th>
<th>Use SAF</th>
<th>Use SAF rationale (experimental versus real life exposure)</th>
<th>SAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feminine hygiene conventional pads, liners, interlabial pads</td>
<td>10</td>
<td>1</td>
<td>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.</td>
<td>10</td>
<td>The area is vulval mucous membrane and outer labia, which are highly follicular. Type of occlusion is similar to that of the experimental test conditions.</td>
<td>100</td>
</tr>
<tr>
<td>Intimate wipes</td>
<td>10</td>
<td>3*</td>
<td>Matrix is different from the experimental test conditions, however, it is not expected to be more irritating.</td>
<td>10</td>
<td>The area is vulval mucous membrane and vaginal mucous membrane includes non-keratinized mucous membrane-increased permeability. The nature of occlusion is different, but effect is expected to be similar to that of the experimental test conditions.</td>
<td>300</td>
</tr>
<tr>
<td>Tampons</td>
<td>10</td>
<td>1</td>
<td>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.</td>
<td>20*</td>
<td>The area is the baby’s buttocks, groin, lower stomach and upper thighs and the skin integrity may be compromised (diaper rash) and involve mucous membrane exposure. There is occlusion through diaper use.</td>
<td>200</td>
</tr>
<tr>
<td>Baby diapers</td>
<td>10</td>
<td>1</td>
<td>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.</td>
<td>10</td>
<td>The area is primarily the baby’s buttocks, groin, lower stomach and upper thighs and the skin integrity may be compromised (diaper rash) and mucous membrane exposure. There may be occlusion through diaper use.</td>
<td>300</td>
</tr>
<tr>
<td>Baby wipes</td>
<td>10</td>
<td>3*</td>
<td>Matrix is different from the experimental test conditions, however, it is not expected to be more irritating.</td>
<td>10</td>
<td>The area is possibly whole body or head (scalp more permeable) or possibly whole body and mucous membrane exposure (body wash).</td>
<td>300</td>
</tr>
<tr>
<td>Baby shampoo</td>
<td>10</td>
<td>3*</td>
<td>Matrix for the product is very different from experimental conditions and may contain irritating ingredients.</td>
<td>3</td>
<td>The area is the head (scalp more permeable) or possibly whole body and mucous membrane exposure (body wash).</td>
<td>100</td>
</tr>
<tr>
<td>Baby wash, bath</td>
<td>10</td>
<td>3*</td>
<td>Matrix for the product is very different from experimental conditions and may contain irritating ingredients.</td>
<td>3</td>
<td>The area is possibly whole body and the skin integrity may be compromised (diaper rash) and mucous membrane exposure (body wash). There may be occlusion through diaper use.</td>
<td>100</td>
</tr>
<tr>
<td>Baby cream</td>
<td>10</td>
<td>3*</td>
<td>Matrix for the product is designed to enhance penetration.</td>
<td>10</td>
<td>The area is possibly whole body or head (scalp more permeable) or head and the skin integrity may be compromised (diaper rash) and mucous membrane exposure (body wash). There may be occlusion through diaper use.</td>
<td>300</td>
</tr>
<tr>
<td>Baby oil</td>
<td>10</td>
<td>3*</td>
<td>Matrix for the product is designed to enhance penetration.</td>
<td>10</td>
<td>The area is possibly whole body or head (scalp more permeable) or head and the skin integrity may be compromised (diaper rash) and mucous membrane exposure (body wash).</td>
<td>300</td>
</tr>
</tbody>
</table>
Table 2 (continued)

<table>
<thead>
<tr>
<th>Product type</th>
<th>Inter-individual</th>
<th>Matrix SAF</th>
<th>Matrix SAF rationale</th>
<th>Use SAF</th>
<th>Use SAF rationale (experimental versus real life exposure)</th>
<th>SAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baby powder</td>
<td>10</td>
<td>1</td>
<td>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.</td>
<td>10</td>
<td>The area is possibly whole body and the skin integrity may be compromised (diaper rash) and mucous membrane exposure. There may be occlusion through diaper use.</td>
<td>100</td>
</tr>
<tr>
<td>Tights with moisturizers</td>
<td>10</td>
<td>3°</td>
<td>Matrix for the product not the same as the experimental conditions.</td>
<td>10</td>
<td>The area is the lower extremities which may include, dry skin, abraded skin (e.g., shaved legs) and semi-occlusion.</td>
<td>300</td>
</tr>
<tr>
<td>Insect Repellents (intended to be applied to the skin)</td>
<td>10</td>
<td>3°</td>
<td>Matrix for the product not the same as the experimental conditions. May contain irritating ingredients.</td>
<td>10</td>
<td>The area is the exposed skin (25% of their average total body surface area) which may include, hands, head, forearms, legs, dry skin, abraded skin (e.g., legs).</td>
<td>300</td>
</tr>
<tr>
<td>Handwash laundry detergent</td>
<td>10</td>
<td>3°</td>
<td>Matrix for the product is very different from experimental conditions and may contain irritating ingredients.</td>
<td>3</td>
<td>Hands and lower arms. May involve skin sites with dermatitis.</td>
<td>100</td>
</tr>
<tr>
<td>Laundry pre-treatment</td>
<td>10</td>
<td>3°</td>
<td>Matrix for the product is very different from experimental conditions and may contain irritating ingredients.</td>
<td>3</td>
<td>Hands and lower arms. May involve skin sites with dermatitis.</td>
<td>100</td>
</tr>
<tr>
<td>Hand dishwashing detergent</td>
<td>10</td>
<td>3°</td>
<td>Matrix for the product is very different from experimental conditions and may contain irritating ingredients.</td>
<td>3</td>
<td>Hands and lower arms. May involve skin sites with dermatitis.</td>
<td>100</td>
</tr>
<tr>
<td>Hard surface cleaner</td>
<td>10</td>
<td>3°</td>
<td>Matrix for the product is different from experimental conditions and may contain solvents and other irritating ingredients.</td>
<td>3</td>
<td>Hands and lower arms. May involve skin sites with dermatitis.</td>
<td>100</td>
</tr>
</tbody>
</table>

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).
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 Mats and Rawlings (2005).
an Britz and Maibach (1979).
ao Britz and Maibach (1979a).
aq Elsner et al. (1990).
ar Elsner et al. (1990a).
as Elsner et al. (1990b).
at Elsner et al. (1990c).
av Farage and Maibach (2004).
aw Zhai et al. (2004).
az Harris and Robinson (1992).

(continued on next page)
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 scenarios here.

<table>
<thead>
<tr>
<th>component of the SAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAF number</td>
</tr>
<tr>
<td>for inter-individual</td>
</tr>
<tr>
<td>variability</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>for matrix effects</td>
</tr>
<tr>
<td>and use considerations</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>3 (half log of 10)</td>
</tr>
<tr>
<td>10</td>
</tr>
</tbody>
</table>

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

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/cm²). 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...
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/cm²) 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 cm², 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>
<thead>
<tr>
<th>Product Type</th>
<th>Surface area, cm²</th>
<th>Surface area reference</th>
<th>Retention factor</th>
<th>EC or SCCNFP *</th>
<th>CTFAd</th>
<th>Cano and Rich (2001); Tozer et al. (2004); Cano (2006)a</th>
<th>Colopab</th>
<th>HERA * (mg/cm²/day)</th>
<th>FMA * (mg/cm²/day)</th>
<th>RIFM * (mg/cm²/day)</th>
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<td>(mg/cm²/day)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>mg/</td>
<td>mg/cm²/day</td>
<td>mg/cm²/day</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>applications/day</td>
<td>mg/cm²/day</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>mg/day</td>
<td>mg/cm²/day</td>
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<tr>
<td>Deo/AP-type not specified</td>
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<td>Bremmer et al. (2003), per axillae</td>
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<td>2.50</td>
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<td>Bremmer et al. (2003), per axillae</td>
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</tr>
<tr>
<td>Deo/AP non-spray</td>
<td>100</td>
<td>Bremmer et al. (2003), per axillae</td>
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<td></td>
</tr>
<tr>
<td>Deo/AP all over body</td>
<td>100</td>
<td>Bremmer et al. (2003), per axillae</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Shaving cream/depilatory a,b</td>
<td>305</td>
<td>Bremmer et al. (2003) (1/4 area head, male)</td>
<td>0.01</td>
<td>2000</td>
<td>1</td>
<td>0.07</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Lip products</td>
<td>48</td>
<td>Ferrario et al. (2000)</td>
<td>1</td>
<td>10</td>
<td>4</td>
<td>8.33</td>
<td></td>
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<tr>
<td>Eye products</td>
<td>24</td>
<td>Ferrario et al. (2000)</td>
<td>1</td>
<td>10</td>
<td>2</td>
<td>0.83</td>
<td></td>
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</tr>
<tr>
<td>Body cream/lotion</td>
<td>12,895</td>
<td>EPA (1997) (area body – head and 1/2 trunk, female)</td>
<td>1</td>
<td>8000</td>
<td>0.5</td>
<td>0.31</td>
<td>14,400</td>
<td>1.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men’s facial cream</td>
<td>775</td>
<td>Bremmer et al. (2003) (1/4 area head + 1/2 area hands, male)</td>
<td>1</td>
<td>800</td>
<td>2</td>
<td>2.06</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Toothpaste</td>
<td>216.8</td>
<td>Collins and Dawes (1987); Ferrario et al. (2000) (buccal + lips)</td>
<td>0.1</td>
<td>1400</td>
<td>2</td>
<td>1.29</td>
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</tr>
<tr>
<td>Mouthwash</td>
<td>216.8</td>
<td>Collins and Dawes (1987); Ferrario et al. (2000) (buccal + lips)</td>
<td>0.01</td>
<td>10,000</td>
<td>3</td>
<td>1.38</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Hydroalcoholic products for shaved skin</td>
<td>775</td>
<td>Ferrario et al. (2000)</td>
<td>1</td>
<td>1770</td>
<td></td>
<td>2.21</td>
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<td></td>
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<tr>
<td>Hydroalcoholic products for unshaved skin</td>
<td>100</td>
<td>Bremmer et al. (2003), perfume spray</td>
<td>1</td>
<td>1770</td>
<td>17.70</td>
<td>2.21</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women’s facial cream</td>
<td>555</td>
<td>EPA (1997) (1/2 area head, female)</td>
<td>1</td>
<td>800</td>
<td>2</td>
<td>2.88</td>
<td>3500</td>
<td>6.31</td>
<td>1500</td>
<td>2.70</td>
</tr>
<tr>
<td>Women’s facial liquid make-up</td>
<td>555</td>
<td>EPA (1/2 area head, female)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>1760</td>
<td>3.17</td>
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<tr>
<td>Hair sprays—type not specified</td>
<td>555</td>
<td>EPA (1997) (1/2 area head, female)</td>
<td>0.1</td>
<td>2700</td>
<td>2</td>
<td>0.97</td>
<td></td>
<td></td>
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<tr>
<td>Hair sprays—aerosol i</td>
<td>555</td>
<td>EPA (1997) (1/2 area head, female)</td>
<td>0.1</td>
<td>7730</td>
<td>1.39</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Hair styling aids</td>
<td>1010</td>
<td>Bremmer et al. (2003) &amp; EPA (1997) (1/2 area head + 1/2 head)</td>
<td>0.1</td>
<td>5000</td>
<td>2</td>
<td>0.99</td>
<td></td>
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<tr>
<td>Shampoo</td>
<td>1430</td>
<td>EPA (1997) (area hands + 1/2 head)</td>
<td>0.01</td>
<td>8000</td>
<td>1</td>
<td>0.056</td>
<td>23630</td>
<td>0.17</td>
<td>10500</td>
<td>0.07</td>
</tr>
<tr>
<td>Conditioners, rinse-off</td>
<td>1430</td>
<td>EPA (1997) (area hands + 1/2 head)</td>
<td>0.01</td>
<td>14000</td>
<td>1</td>
<td>0.098</td>
<td>28200</td>
<td>0.20</td>
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<tr>
<td>Make-up remover</td>
<td>555</td>
<td>EPA (1997) (1/2 area head, female)</td>
<td>0.1</td>
<td>2500</td>
<td>2</td>
<td>0.90</td>
<td></td>
<td></td>
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<tr>
<td>Nail care</td>
<td>11</td>
<td>RIVM b</td>
<td>0.1</td>
<td>250</td>
<td>0.43</td>
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<tr>
<td>Bar soaps</td>
<td>840</td>
<td>EPA (1997) (area hands)</td>
<td>0.01</td>
<td>800</td>
<td>6</td>
<td>0.057</td>
<td></td>
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<tr>
<td>Liquid soap</td>
<td>840</td>
<td>EPA (1997) (area hands)</td>
<td>0.01</td>
<td></td>
<td></td>
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<td>Hand cream</td>
<td>840</td>
<td>EPA (1997) (area hands)</td>
<td>1</td>
<td>800</td>
<td>2</td>
<td>0.03</td>
<td>8300</td>
<td>0.15</td>
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<td>Face washes, gels, scrubs</td>
<td>555</td>
<td>EPA (1997) (1/2 area head, female)</td>
<td>0.01</td>
<td>5000</td>
<td>2</td>
<td>0.06</td>
<td>25500</td>
<td>0.015</td>
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<tr>
<td>Body wash gels, foams, mousses</td>
<td>16,900</td>
<td>EPA (1997) (body area, female)</td>
<td>0.01</td>
<td>17000</td>
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<td>0.010</td>
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(continued on next page)
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<tr>
<th>Product Type</th>
<th>Surface area reference</th>
<th>Surface area, cm²</th>
<th>Retention factor²</th>
<th>EC or SCCNFP³</th>
<th>CTFA⁴</th>
<th>Cano and Rich (2001); Tozer et al. (2004); Cano (2006); Colipa (2005)</th>
<th>HERA⁵ (mg/cm²/day) Dec. 2005</th>
<th>FMA⁶ (mg/cm²/day)</th>
<th>RIFM⁷ (mg/cm²/day)</th>
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<td>Feminine hygiene—tampons</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.9</td>
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<td>Feminine hygiene—pads</td>
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<td></td>
<td></td>
<td>0.14</td>
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<td>Feminine hygiene—liners</td>
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<td></td>
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<td>0.14</td>
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<td>4.0</td>
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<tr>
<td>Intimate wipes</td>
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<td></td>
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<td></td>
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<td>Aerosol air freshener</td>
<td>EPA (1997) (1/2 area head + upper extremities, female)</td>
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<td>3.02</td>
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<tr>
<td>Insect repellent</td>
<td>EPA (2001b) (25% body area, female—head, hands, forearms, legs)</td>
<td>4225</td>
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<td></td>
<td></td>
<td>Insignificant</td>
<td>0.01</td>
<td>Insignificant</td>
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<td></td>
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<td>Hand dishwashing</td>
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<td></td>
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<tr>
<td>Tights with moisturizers</td>
<td>EPA (1997) (lower extremities, female)</td>
<td>6570</td>
<td>1</td>
<td></td>
<td></td>
<td>Insignificant</td>
<td>0.000005</td>
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<tr>
<td>Hard surface cleaner</td>
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<td></td>
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<td></td>
<td></td>
<td>Insignificant</td>
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</tbody>
</table>

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.

⁴ Loretz et al. (2005, 2006); CTFA (2005a,b).
⁵ Cano and Rich (2001); Tozer et al. (2004); Cano (2006).
⁶ Colipa (2005).
⁷ AISE/HERA (2002).
⁸ Api et al. (2007).
¹¹ 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.
¹² For frequency of use less than once per day, the default of once per day was used with the exception of nail care products.
¹³ 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; Zerof 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 mouthwash) 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/cm², assuming 100% retention of the fragrance ingredient from the product solution. This is consistent with the value identified by the primary exposure approach.
¹⁴ These data should not be used due to logistical difficulties with determination of the actual amount of product delivered on skin (Colipa, 2005).
¹⁵ This expression value is used in the QRA for fragrance ingredients for all types of deodorants and antiperspirants.
¹⁶ This expression 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.

\[
AEL = \frac{\text{WoE NESIL}}{\text{SAF}}
\]

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 ≥ 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>
<thead>
<tr>
<th>Fragrance ingredient (X)</th>
<th>Deodorant</th>
<th>Hydroalcoholic product for unshaved skin</th>
</tr>
</thead>
<tbody>
<tr>
<td>WoE NESIL</td>
<td>500 µg/cm²</td>
<td>500 µg/cm²</td>
</tr>
<tr>
<td>SAF</td>
<td>300</td>
<td>100</td>
</tr>
<tr>
<td>AEL = WoE NESIL/SAF</td>
<td>1.7 µg/cm²</td>
<td>5.0 µg/cm²</td>
</tr>
</tbody>
</table>

\[
AEL = \frac{\text{WoE NESIL}}{\text{SAF}}
\]
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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A review of critical factors in the conduct and interpretation of the human repeat insult patch test

Pauline M. McNamee a,*, Anne Marie Api b, David A. Basketter c,1, G. Frank Gerberick d, Deborah A. Gilpin e, Barbara M. Hall f, Ian Jowsey c, Michael K. Robinson d

a The Procter & Gamble Company, Whitehall Lane, Egham, Surrey TW20 9NW, UK
b Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ, USA
c Unilever Safety and Environmental Assurance Centre, Sharnbrook, Bedford MK44 1LQ, UK
d The Procter & Gamble Company, Miami Valley Innovation Center, 11810 East Miami River Road, Cincinnati, OH 45252, USA
e The Procter & Gamble Company, F&HC Innovation Center, 5299 Spring Grove Avenue, Cincinnati, OH 45217, USA
f L’Oréal, 25-29 Quai Aulagnier, 92600 Asnières-sur-Seine, France

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Abstract

This paper reviews key factors that are critical to the conduct and interpretation of Human Repeat Insult Patch Tests (HRIPTs). A methodology for HRIPT testing is described and general guidelines for evaluation of responses indicative of induction and elicitation of skin sensitization and skin irritation are detailed. Understanding and applying these key factors is crucial to the design of such studies and reliability of the resulting data when used in the overall risk assessment process.

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Keywords: Skin; Contact allergy; HRIPT; Critical factors; Design; Evaluation

1. Introduction

Integral to the development of consumer products and their ingredients is the evaluation of their potential to cause skin sensitization and allergic contact dermatitis (ACD). This is done by risk assessment, a multi-step iterative process which has been reviewed elsewhere (Basketter et al., 1999; Felter et al., 2003; Gerberick and Robinson, 2000; Gerberick et al., 1993, 2001; Nusair et al., 1988; Robinson et al., 1989, 2000).

The skin sensitization potential of a material is established pre-clinically through (1) its analytical and structural characterization; (2) literature review and where appropriate (3) animal testing [e.g., murine local lymph node assay (LLNA) or guinea pig tests (GPT)]. The availability of data that confirm humans will not respond adversely remains an important element of the overall risk assessment process. In the absence of existing human data, it may be advantageous to perform Human Repeat Insult Patch Testing (HRIPT) either to confirm a No-Effect Level (NOEL) used as one of the data sources in the establishment of a No Expected Sensitization Induction Level (NESIL) as part of a recently described Quantitative Risk Assessment (QRA) framework (Api et al., 2008) or to demonstrate that humans will not respond adversely to a particular formulation. Other complementary but less reliable on their own sources of such human data may be clinical in-use testing and later monitoring/follow-up of consumer comments.

Human patch testing methodology has evolved over more than 50 years, since first proposed in 1944 by Schwartz and Peck (Schwartz and Peck, 1944) and has since been extensively reviewed (Griffith, 1969; Hardy, 1973; Kligman, 1966a; Marzulli and Maibach, 1976a, 1996;
In every method one or more 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, induction patch number, 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 skin sensitization is measured.

With cumulative experience from the consumer products industry has come an awareness of certain factors that are believed to be critical to a reliable test result. This paper discusses those key factors that can affect the design, conduct and interpretation of the results, and that are believed to provide greater assurance of a test result of high quality obtained within ethical guidelines for human volunteer testing.

2. Critical factors affecting the design and interpretation of HRIPTs

In 1966, Kligman followed his critique of standard methods (Kligman, 1966a) with a review of factors influencing the induction and measurement of ACD (Kligman, 1966b). Marzulli and Maibach stated in 1974 that it is important to take into account factors that could adversely affect the calculated margin of safety, such as frequency of application, contact area, permeability of skin site and occlusion when conducting threshold studies using the human Draize procedure (Marzulli and Maibach, 1974). More recently, Emmet et al. have suggested that factors such as heat, moisture, pressure, occlusion, duration of contact and irritation may affect sensitization dose–response relationships (Emmet et al., 1994). There are several factors that experience indicates are critical to consider before conduct of an HRIPT and for interpretation of the results (Fig. 1).

- Vehicle/Matrix Effects
- Test Material Concentration (Dose/Unit Area)
- Amount of Test Material Applied
- Occlusion
- Chemistry
- Target Population
- Allergen Potency

Fig. 1. Critical factors for HRIPT conduct and interpretation.

2.1. Vehicle/matrix effects

If it is appropriate and possible, the preferred method is to use a test material either undiluted or at the NOEL concentration (chosen based on other human and/or pre-clinical data). Where irritation or other considerations necessitate dilution or an undiluted test material represents highly unrealistic exposure (e.g., fragrance oil), then selection of a suitable vehicle (diluent) becomes necessary. Since the choice of vehicle can have a profound effect on the physicochemical properties of the test material and its bioavailability, it is essential to choose the appropriate vehicle with due regard to whether it is inherently irritating (Robinson et al., 1991), potentially sensitizing (Stotts and Ely, 1977), enhances penetration of materials across the skin (Robinson et al., 1991; Heylings et al., 1996), can interact with or alter the test material (Calvin, 1992) and is a suitable solvent (i.e., can solubilize or produce a stable suspension) for the test material (Marzulli and Maibach, 1976b).

A vehicle can be a simple single moiety (e.g., water), mixtures (acetone/water, ethanol/water) or a complex matrix presented in undiluted or diluted form. The effect of a complex matrix, as a vehicle, on the physicochemical parameters and bioavailability of a test material may be substantially different from that of a simple vehicle. The test material may preferentially partition into one phase of such a vehicle, resulting in a higher (possibility of inducing contact sensitization) or lower (possible false negative result) concentration than anticipated.

The skin sensitization potential of a test material can be affected by the vehicle (Stotts, 1980; Kligman, 1966b; Marzulli and Maibach, 1976b). For this reason, a vehicle relevant to a final formulation is the optimum choice. Additionally, in the event of follow-up (or re-challenge) work with a subject who may be sensitized, testing of an individual product ingredient may require a different vehicle than that relevant for a product formulation. As such, any alternative vehicle(s) may influence reactivity of a sensitized subject at re-challenge (Stotts, 1980). Ethanol (1:1 in water), for example, has been shown to be a skin sensitizer under certain exposure conditions (Stotts and Ely, 1977). The potential of ethanol to sensitize under occluded patches can be reduced by allowing evaporation for 10 to 20 min before the patch is applied or by using an alternative patching technique. Evaporation, however, has the disadvantage of altering the composition of the material placed on the patch (Stotts, 1980). Mineral oil (liquid petrolatum), as another example, has important advantages of being non-irritating, non-sensitizing and often permits the testing of a high sample concentration. However, it may not solubilize all test material components. One special precaution is that allergic reactions may be weaker in intensity and slower to develop with mineral oil as a vehicle rather than water or an organic solvent (Stotts, 1980). Acetone (1:1 in water), corn oil, and glycerin are other vehicles which may be useful under certain conditions (Stotts, 1980).
2.2. Test material concentration (dose/unit area)

The selection of an appropriate concentration is crucial to avoid false negative results or the unnecessary induction of skin sensitization. The proper concentration of material tested in an HRIPT is primarily determined by integrating information available from such sources as: prior skin sensitization results in animals (e.g., local lymph node assay (LLNA) data (Felter et al., 2003; Gerberick and Robinson, 2000; Gerberick et al., 2001); results from repeated application irritation patch studies in humans (e.g., a 3-application patch test (Robinson et al., 1989); the need/desire to exaggerate exposure under the test conditions relative to that anticipated from intended and foreseeable uses (if irritation considerations permit) and prior experience with either the ingredient and/or a product or similar materials.

The concentration used is expressed as a dose/unit area of skin as defined by the absolute amount of test material applied and the area of skin exposed. Historically, the exposure to a test material in an HRIPT has been expressed as weight/volume (%). This has led to the false assumption that across different patch test methods, the same % of test material would result in equal exposure and subsequently similar skin reactions. Instead, the expression of the exposure across patch test systems as dose/unit area demonstrates significantly different exposures even though the % of a test material remains constant (Table 1 (Robinson et al., 2000). It is well established for assessing skin sensitization potential that the dose of test material applied/unit area of skin is critical rather than the absolute amount of the material applied (Felter et al., 2003; Gerberick and Robinson, 2000; Gerberick et al., 2001). Several studies have demonstrated that for a constant area, higher dosages of an allergen increase the probability of skin sensitization, while increasing area with increasing doses of allergen results in a very similar incidence, and decreasing the area with a constant dose of allergen also increases the probability of skin sensitization (Friedmann et al., 1983; Rees et al, 1990; White et al., 1986; Upadhye and Maitland, 1992).

In the context of patch testing, it is especially important to express the test material exposure as dose/unit area to allow comparison when: (1) producing data under different patching conditions; (2) defining thresholds for induction or elicitation of reactivity; (3) changing the patch type from that used in previous testing; and (4) relating patch test data to consumer exposure.

Another consideration for test material concentration is based on test material irritant potential. It may be especially difficult to distinguish irritation, especially very strong irritant reactions, from allergy following challenge. The temptation to reduce the concentration at challenge may result in a false-negative interpretation if the level is below a subject’s reactivity threshold (Marzulli and Maitland, 1976b). Under these circumstances, it is often preferred that such test materials are tested at the highest concentration which is minimally irritant as determined in a pre-HRIPT human irritant screen. Inclusion of at least two, and preferably three, concentrations in the screen permits selection of one which is not excessively irritating and yet may produce some mild erythema. Such minimal dermal irritation during the induction phase is well tolerated by the subjects and is an additional way to exaggerate the exposure conditions of the HRIPT. Mild irritation observed during patch test of inherently irritant test materials (for example surfactants) which need be tested diluted, is reassuring as it confirms a correct selection of the concentration for the patch test. In this case the patch test conditions may be considered “optimal” for activation of the immune system by preserving the “danger” signal of the test material (McFadden and Baskett, 2000).

For non-irritant materials/products, irritant potential is not one of the considerations for selection of test material concentration. For these test materials an appropriate exaggeration over expected normal or anticipated use exposures, if possible, may be selected (Stotts, 1980).

2.3. Amount of test material applied

The amount of test material applied is one of the defining parameters for the calculation of concentration as dose/unit area and as such needs to remain consistent throughout the course of the study. The weight or volume will vary depending on the physical properties of the test material and the type and size of patch used. Liquids should fully saturate the patch pad without seeping out onto the adhesive when the patch is in place. For semi-solid or solid materials, a thin, uniform covering of the patch pad is sufficient. In the case of a solid material, the patch pad is moistened with water or saline to enhance hydration in the patch environment (Stotts, 1980). Use of an inappropriately low (incomplete patch coverage) or high (seepage beyond the patch) amount of test material can introduce uncertainty into the calculation of test material concentration (dose/unit area). In addition, use of an excessively high amount of test material may cross-contaminate and thereby compromise other patch sites in a multi-patch test. It is good practice when handling a novel test material or patch system to check and record, before the study, the appropriate quantity of the test material to be patched.

Table 1

<table>
<thead>
<tr>
<th>Patch type and dose/unit area: calculation of a 1% solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patch type</td>
</tr>
<tr>
<td>------------</td>
</tr>
<tr>
<td>8 mm Finn® (Epitest, Tuusula, Finland)</td>
</tr>
<tr>
<td>19 mm Hill Top Chamber® (Hill Top Research, Inc, Cincinnati, Ohio, USA)</td>
</tr>
<tr>
<td>2 x 2 cm Webril® (Kendall Healthcare, Mansfield, Massachusetts, USA)</td>
</tr>
</tbody>
</table>
2.4. Occlusion

The use of occlusion is one of the ways to exaggerate exposure under HRIP test conditions. In most situations, complete occlusion is the method of choice, since it provides the greatest exaggeration of exposure (Bird, 1981). Non-woven cotton pads [e.g., Webril® (Kendall Healthcare, Mansfield, Massachusetts, USA)] are covered by and held securely to the skin on all sides with an occlusive, hypoallergenic tape [e.g., Blenderm™ (3M Healthcare, St. Paul, Minnesota, USA)] or secured within a plastic chamber [e.g., Hill Top Chamber® (Hill Top Research, Inc., Cincinnati, Ohio, USA)]. Other occlusive patch test systems are also available. However, as the patch test system itself is known to potentially influence skin responses, it is important not to change it unless absolutely necessary in order to preserve continuity of the historical data.

In circumstances where the test material is irritating and cannot be diluted, a semi-occlusive patch may be used. This consists of a non-woven cotton pad (e.g., Webril®) covered by and held securely to the skin on a) all sides with a porous, hypoallergenic tape [e.g., Micropore™ (3M Healthcare, St. Paul, Minnesota, USA)] or b) two opposing sides with a hypoallergenic tape (Blenderm™).

For highly irritant materials, two other methods are possible: (1) a semi-open application consisting of a non-woven cotton pad (e.g., Webril®) held to the skin on two opposing sides by a strip of perforated hypoallergenic tape [e.g., Transpore™ (3M Healthcare, St. Paul, Minnesota, USA)] with holes over the pad (pad is open on two sides) or (2) an open application consisting of direct placement of the test material on the skin and covering the site with a loosely woven covering (i.e., cotton gauze) held in place with hypoallergenic tape (e.g., Micropore™) along two opposing edges.

For the semi-occlusive, semi-open, and open applications, there is a potential reduction in bioavailability of the test material compared to complete occlusion. The patch type for a given test substance will typically be used throughout all phases of the study unless significant (and unexpected) irritation occurs in the early part of the induction phase that necessitates moving to a less occlusive patch, e.g., semi-occlusive, for the remainder of the study.

2.5. Chemistry

The factors that create the skin sensitizing potential for a chemical, such as molecular weight, charge, electrophilic potential and lipophilicity, are unique to that chemical. Moreover, typically chemicals must cause sufficient local trauma to induce/augment cutaneous cytokine production. Such a chemical must be able to penetrate through the stratum corneum. Generally, such chemicals must be relatively low in molecular weight (<1000) (Belsito, 1989) and have appropriate physicochemical properties (e.g., lipophilicity) (Kimber and Dearman, 1996). Once in the viable epidermis, the chemical reacts with protein. To do this, the chemical must be inherently protein reactive or be converted (metabolized/degraded) into a reactive form (Barratt and Basketter, 1992).

It is also very important to know, not only for the logistics of choosing an appropriate patch test concentration, if a test material is a skin irritant. The enhancing effects of skin irritation on the skin sensitization response are well documented (Kligman, 1966c; Cumberbatch et al., 1993). Chemical inflammation, if not too severe, increases the opportunity for skin sensitization to occur through keratinocyte activation and cytokine release. This effect may be produced by the allergenic chemical itself, by some other component of the chemical delivery system, or by some form of physical insult (Robinson et al., 2000).

Analytical characterization also identifies any expected contaminants or reaction by-products with known skin sensitizing properties e.g., unsaturated or chlorinated sulphones which, as potent skin sensitizers, were contaminants of certain anionic surfactants and were the cause of a major outbreak of ACD in Scandinavia in the late 1960s (Magnusson and Gilje, 1973).

2.6. Target population

In general, it has been recommended that subjects of relevant age, gender and ethnicity are included in human volunteer studies (Walker et al., 1996). However, limitations of access to such diverse test subjects and the relatively small number of patch test contract facilities often means that study populations are generally disproportionately comprised of Caucasian, middle-aged females. This clearly raises the question of whether evaluation in one test population is sufficient to predict potential reactivity for other populations worldwide or could there be an underestimate of the risk of inducing ACD among other users of the products. Recent reviews by Robinson (Robinson, 1999, 2006, 2007) noted that susceptibility to skin sensitization tends to be more related to exposure than to any inherent differences in sensitivity, though some data did suggest a slightly increased sensitivity in females versus males (Jordan and King, 1977). On this basis, standard procedures for skin safety testing such as those encountered in the HR IPT in terms of study population are considered relatively conservative and appropriate. Human volunteer studies such as HRIPTs conducted in the past and that have incorporated ethnically diverse test populations have not shown any tendency for Caucasian subjects to be less responsive. As such, there is no reason to suspect that current testing of predominantly female Caucasians in any way under-predicts the allergic potential of chemical ingredients or products.

This does not negate the need to consider if a specific population (e.g., atopics, “sensitive skin”) should be included or excluded from an HRIPT or if there is a behavior or characteristic that the test subjects must possess. There may be important reasons to pursue a population-specific testing strategy in certain situations, perhaps due
to regional, gender-specific or age-specific marketing or to satisfy a regulatory need. In this circumstance, a clear rationale for this type of testing and flexibility in the approach are important and it is reasonable to consider conducting patch testing among panelists of the anticipated ethnicity and/or gender. Such testing would always be on a case by case basis. However there is, as yet, no specific scientific justification to mandate such a strategy based on current knowledge of skin responsiveness and how it compares across diverse populations. If so, including this information in the inclusion/exclusion criteria would aid in successful conduct of the study.

2.7. Allergen potency

An HRIPT is not designed to obtain information about the potency of a skin sensitizing material. This is established in the pre-clinical evaluation of the test material. It is possible, however, to compare levels, e.g., the highest levels of known skin sensitizers that did not result in skin sensitization. These data are useful to determine a threshold value that can be used in the context of risk assessment.

Ethically, it is very important to consider allergen potency when any material with allergenic potential is included in an HRIPT. Generally, materials with known skin sensitization potential are not tested in humans unless there is minimal to no risk of causing skin sensitization or the potential benefit of the material warrants the testing (e.g., transdermal drug) (Robinson et al., 1991). Allergen potency is one of the key factors that is included in the decision to test or not and at which concentration to ensure confirmation of the human safety of a product and/or its ingredients while minimizing the risks of inducing skin sensitization among the study subjects (Felter et al., 2003; Gerberick and Robinson, 2000; Gerberick et al., 2001; Robinson et al., 2000).

3. HRIPT methodology

3.1. Test design

One standard HRIPT methodology (Stotts, 1980) is described below. The HRIPT consists of a 3 week induction phase (Weeks 1–3), a rest period of 14–17 days (Weeks 4 and 5), and a 1 week challenge (elicitation) phase (Week 6). Such a test design is schematically represented in Fig. 2.

3.2. Induction phase

During the 3 week induction period test materials, under appropriate patches, are applied 3 times per week (each Monday, Wednesday, and Friday) to a single site (typically the extensor surface of the upper arm) for a total of 9 patches. Each patch is left in place for 24 h and then removed by the subject or by the test centre staff. The patch site is graded for skin responses such as erythema, edema, papules and vesicles 24 h after patch removal (48 h over a weekend) and immediately prior to the next patch application. The 24–48 h period when the patch is not in place allows any skin response from the previous patch application(s) to develop completely. An appropriate decision can then be made regarding subsequent patch placement to reduce strong reactions to a minimum. In the event of any significant response, the patch site is moved to a new “naïve” site adjacent to the original site of application.

3.3. Rest period

A “rest period” of 14–17 days occurs following the induction phase and prior to the challenge (elicitation) phase of the study. This rest period allows time for any immunological response to develop and also allows any previous reactions developing at the induction sites to subside prior to the re-application of the test substances at challenge. The inclusion of a rest period rather than uninterrupted patch applications also allows more accurate monitoring of reaction patterns during induction which aids in interpretation of the study data.

3.4. Challenge phase

Fourteen to seventeen days after the last induction application (Monday of week 6), challenge patches are applied to the original site of application and to a naïve alternate site on the opposite side of the body (typically the opposite arm) for 24 h. The sites are graded for skin responses 48 and 96 h after patch application, although 72 and 120 h (or longer) gradings are performed if circumstances warrant. This phase is used for the assessment of delayed cutaneous responses, which may be indicative of contact sensitization.

3.5. Re-challenge

When a response observed at challenge is inconclusive and/or more information about challenge responses is needed, a re-challenge can be performed between 4 and 12 weeks after the initial challenge phase. This delay before re-challenge allows for previous reactions to subside, and is the optimum time for confirmation of the presence or absence of skin sensitization. The re-challenge is conducted...
in the same manner as the challenge phase of the HRIPT. Patches are applied to the original site and/or a naïve alternate site on the opposite side of the body for 24 h. Alternatively, re-challenge patches can be applied at a totally new skin site, such as the upper back in the case of the upper arm HRIPT or vice versa. The sites are graded for skin responses 48 and 72 h or 48 and 96 h after patch application. The original test material is always evaluated in a re-challenge. In addition, purified test substance, product sub-mixtures and ingredients themselves may also be tested in the re-challenge phase at previously determined non-irritant concentrations. In conducting a re-challenge, it may be appropriate to include control subject(s) as well as the reactive subject(s) in order to establish a normal profile for skin irritation. The control subject(s) may be recruited from among the non-reactive subjects of the HRIPT. Whilst the emphasis must always be on avoiding the acquisition of skin sensitization during the induction phase, should a subject become sensitized, it is during the re-challenge phase that a dose–response study may be conducted to determine whether an elicitation threshold may be established.

The protocol described in this publication refers to a 24 h patch application time. Another commonly used protocol employs patches applied according to the above schedule but each patch is left in place for 48 h (72 h at the weekend). The patches are removed by the test centre staff and the patch sites are graded 30 min after patch removal. Otherwise, the methodology described above applies to these different HRIPT protocols regardless of the patch application time.

3.6. Inclusion/exclusion criteria

A standard list of criteria that are used to include or exclude subjects from participation in an HRIPT is normally listed in the study protocol. The type of material tested typically determines if additional inclusion/exclusion criteria need to be considered. In this case, listing these requirements in the inclusion/exclusion criteria will aid design of the most appropriate study. Subjects are excluded for the following reasons (note—partial listing without qualifying details): clinically significant active dermatitis; immunological disease; insulin-dependent diabetes; routine use of anti-inflammatory drugs; immunosuppressive drug therapy; bilateral mastectomy or mastectomy within the last year; recent participation in a patch test (e.g., within 30 days), unwillingness or inability to give informed consent or otherwise to comply with protocol requirement. Experience indicates that subjects who report dermatitis from or believe they are “allergic” to one or more consumer products are nearly always able to participate in an HRIPT without incident. These subjects generally are not excluded. However, it is important to ask specifically if a contact allergy has been confirmed by a physician or if there is a history of problems with a certain category of products or certain ingredients like fragrances or preservatives before a final judgment concerning eligibility is made (Stotts, 1980). The clear intent is only to exclude subjects with a known or suspected pre-existing sensitization to the product or ingredient being tested.

Consenting subjects are asked to complete a medical/dermatological history questionnaire to allow evaluation of their eligibility for participation in the study. Direct questioning may also be used to supplement or clarify answers to the questionnaire.

3.7. Number of subjects

Each test consists of 80–120 subjects, with approximately 100 completing the test, being the most typical number. It is important to bear in mind that a number of people may drop out during the course of the 6 weeks test (usually for reasons unrelated to the test) and so the number of enrolled volunteers should take this possibility into account. No more than 20% of the test group typically is over 65 years of age due to speculation that immunological reactivity declines with increasing age (Robinson, 1999). Test volunteers are typically healthy adults who are enrolled without restriction as to gender or ethnicity, although composition of the test panels tends towards being predominantly Caucasian middle-aged females.

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. As early as 1945, Henderson and Riley discussed how the total number of test participants employed can affect the predictive accuracy of the data obtained when statistically extrapolating from a small test population to a large test population (Henderson and Riley, 1945).

The HRIPT is concerned with events (skin sensitization) drawn from a binomial distribution. This distribution has two parameters—the sample size and the % of subjects in the population who would develop sensitization. As such, if a population has a fraction \( p \) who would become sensitized, the probability that one or more of \( n \) independent subjects will react is \( 1 - (1 - p)^n \). The data in Table 2 which illustrates the probability of detecting a response for a range of conditions demonstrates the statistical limitations of the test. It can be seen that a skin sensitization rate of 0.1% is unlikely to be observed even in a study with 200 subjects. With group sizes of 100–200, sensitization rates of <1.0% are not likely to be detected.

To increase the sensitivity of the test whilst using such numbers of subjects, if appropriate a higher concentration of test material may be used 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. Based on industry
experience and incorporating the above factors to increase test sensitivity and reliability, a subject population size of ~100 is reasonable given: (1) the exaggerated exposure conditions of the test; (2) the existence of an extensive database in which cutaneous responses to a wide variety of materials have been detected (Gerberick and Robinson, 1998) and (3) the confirmatory not hazard identification nature of the test.

In cases where pre-clinical exposure cannot provide adequate exposure exaggeration (e.g., transdermal drugs), smaller scale (i.e., fewer panelists) tests are appropriate (Robinson et al., 1991). On occasion, results of pre-clinical testing or other human data suggest that the HRIPT should proceed more cautiously. In such cases, a pilot group of 20–25 subjects begins the induction phase 2 or 3 weeks ahead of the remaining subjects, using the same test conditions that are expected to be used in the definitive study. Any significant irritation or unexpected cutaneous responses suggestive of skin sensitization can be identified and appropriate action taken before exposing the remaining larger group of subjects.

### 3.8. Ethical considerations

Provided that the risk of inducing skin sensitization in the subjects is judged to be minimal (based on an assessment of pre-clinical sensitization test data), human testing can be conducted with appropriate regard to the ethics of such testing and with subjects who have been properly informed of the purpose and any potential risks of the test and who have signed an informed consent statement.

The conduct of human volunteer studies is subject to stringent ethical considerations to ensure that the rights, safety and welfare of the human volunteers involved are protected at all times. The topic of ethics and human testing has been reviewed (Carson and Holt, 2006; Salter 1990; Steadman, 1998; Roggeband et al., 1999).

Human volunteer studies are conducted in accordance with accepted codes of ethics for the conduct of human testing. The Declaration of Helsinki (World Medical Association, 1964), Good Clinical Practice (GCP) guidelines (ICH Harmonized Tripartite Guideline for GCP, 1995) and others reflect this approach to human volunteer testing. All test procedures must, in addition, conform to any applicable national regulatory requirements and legislation.

The conduct of human volunteer studies consistent with the principles of GCP ensures that they are designed, conducted and reported in such a way as to fulfill ethical responsibilities to the human volunteers and provide credible scientific data that are useful. Pre-established systematic written procedures [Standard Operating Procedures (SOPs)] for the organization, conduct, data collection and documentation of studies are necessary. By this means, all data, information and documents may be confirmed as being properly generated, recorded and reported.

Three requirements of GCP that merit separate mention are: the pre-study safety assessment, informed consent and institutional board (IRB) or Ethics Committee review.

### 3.9. Pre-study safety assessment

Any study involving human subjects can only be undertaken after a thorough safety review and risk assessment have been conducted and conclude that the study will involve no significant risk for the volunteers and that it satisfies all other ethical requirements. In the assessment of risk, both risks inherent in the study design (method and duration of exposure and experience with such exposure) and those which are a result of knowledge about the test materials involved (nature of the chemical and the pre-clinical and clinical experience with such materials) are to be considered to preclude the occurrence of significant risk to the volunteers.

### 3.10. Informed consent

It is important that fully informed written consent be obtained from the subjects after application of inclusion/exclusion criteria but prior to the conduct of the test. Implicit in such informed consent is that information is provided about: the consent procedure (including the right to withdraw at any time without prejudice); the study, purpose, procedures and the test materials; the risks/benefits; rights and responsibilities under the Declaration of Helsinki and other information such as confidentiality considerations and emergency contact name/number. The informed consent form must be written in a language that is understandable to a potential volunteer, who must be given enough time and help to understand its content to be able to decide to assent or not. The signatories of the informed consent are the person receiving the information (volunteer), the person providing the information (normally the study investigator) and an independent witness.
3.11. IRB/Ethics Committee approval

Before initiation of a human volunteer study, the investigator is required to obtain documented approval from an Institutional Review Board (IRB) (USA) or Ethics Committee (Europe) in compliance with the principles of GCP guidelines. In order to provide approval, the IRB/Ethics Committee reviews the protocol and its objectives, protocol amendments, informed consent procedures and documentation, investigator facilities and personnel involved in the study and all relevant safety information on the test material(s). The study cannot begin until IRB/Ethics Committee approval is given. Approval procedures for protocol modifications and informing the IRB/Ethics Committee of adverse events and a provision of a final report to the IRB/Ethics Committee are conducted according to the IRB’s/Ethics Committee’s standard operating procedures.

4. General interpretation guidelines

The interpretation of the results of an HRIPT is done on a case-by-case basis using immunological principles, general interpretation guidelines and experience. The standard scoring scale used to interpret skin responses is provided in Fig. 3. The following guidelines have been developed for the interpretation of reactions that may occur during an HRIPT.

- Skin sensitization reactions are most frequently erythematous, papular and edematous. Conversely, primary irritation reactions (unless severe), are generally erythematous only. An irritation reaction is usually uniform with a well-defined border, whereas an allergic response (especially if weak) is typically non-uniform and has an irregular border, and a strong response may spread beyond the patch site.
- Responses which are more severe at challenge than in early induction are suggestive of induction of skin sensitization.
- Responses confined to original challenge sites are suggestive of irritation. True allergic reactions will occur at both original and alternate challenge sites and persist through 2 delayed scorings at least 24 h apart. However, unilateral allergic reactions can sometimes be observed. For this reason all reactions considered suggestive of induction of skin sensitization should be followed up, for example, by re-challenge.
- Responses that increase or maintain severity with time from the 48 to 96 h challenge gradings are presumptive of skin sensitization. Those that subside from the 48 to 96 h grading period are generally considered to be irritant in nature.
- Edematous reactions that occur and persist during the latter part of the induction phase and the challenge phase are indicative of induced skin sensitization and should be confirmed by re-challenge.
- Persistent skin responses with papules and/or edema occurring in week 1 of induction suggest pre-existing skin sensitization. Similar reactions that occur later in induction suggest induction of skin sensitization by the test material.
- Reactions and reaction patterns that are suggestive of allergic reactions or questionable/equivocal reactions should be confirmed through appropriate re-challenge procedures.
- The reactions of any subject(s) in question should always be compared with those of all other exposed subjects. Except in rare instances, allergic reactivity occurs in only a very small number of subjects, while irritation occurs more widely throughout the exposed population.
- Provocative use tests can be used to determine the clinical relevance of positive patch test reactions (Robinson et al., 1989). A negative provocative use test does not negate a positive challenge/re-challenge result.

5. Summary

The HRIPT is the most reliable test method by which confirmatory human data can be made available. It is an ethical tool for confirming that under exaggerated but relevant exposure conditions, the lack of skin sensitizing potential of a given concentration of test material.

The HRIPT protocol can provide an exaggeration of exposure through an extended duration of exposure, testing higher than in-use concentrations where appropriate, minor skin irritation of the test material, and through use of an occluded patch. The procedure allows the detection of pre-existing skin sensitization to test materials as confirmed by persistent skin reactions early in the induction period. Although the HRIPT, as used in the risk assessment process, is confirmatory in nature and is not used for the determination of skin sensitization hazard, the method does have sufficient sensitivity, if conducted with an appropriate number of subjects, to detect induced skin sensitization when exposure to skin sensitizing materials is sufficiently high (Robinson et al., 1991; Gerberick and Robinson, 1998; Cardin et al., 1986). Procedures for HRIPT testing may need to be modified on occasion to address specific test material characteristics, for example use of semi-occlusive patches for test materials too irritant to be tested under full occlusion.

It is imperative that the HRIPT is conducted properly and that those factors that are critical to the design of the study and interpretation of results are considered. This review has described such critical factors and their importance to the generation of reliable HRIPT data. Such data are, in turn, an important element of the overall risk assessment process (Felter et al., 2003; Gerberick and Robinson, 2000; Gerberick et al., 2001; Robinson et al., 2000).
**Erythema Scale:**

This scale is used only for grading degree of erythema (redness). A score on this scale will be assigned following every application of a test material.

0  No visible erythema.

1  Mild erythema (faint pink to definite pink).

2  Moderate erythema (definite redness).

3  Severe erythema (very intense redness).

**Designations for Elevated Responses:**

Edema, papules, vesicles, and bullae, if present, are graded as independent responses.

E  Edema - definite swelling.

P  Papules - many small, red, solid elevations; surface of reaction has granular feeling.

V  Vesicles - small, circumscribed elevations having translucent surfaces so that fluid is visible (blister-like). Vesicles are no larger than 0.5 cm in diameter.

B  Bullae - vesicles with a diameter > 0.5 cm; vesicles may coalesce to form one or a few large blisters that fill the patch site.

**Other Responses/Recording Characteristics:**

S  Spreading - evidence of reaction beyond the pad area (does not include obvious signs of leakage of test material away from pad).

W  Weeping - evidence of release of fluid from a vesicular or bullous reaction.

A  Marked reaction to adhesive (patch relocated).

X  Succeeding patch not applied and succeeding grade is for residual reaction.

L  Patch lost (came off) during first 12h.

Fig. 3. HRIPT scoring scale.
Conflict of Interest

The authors declare that they have no conflicts of interest.

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References


The Research Institute for Fragrance Materials’ human repeated insult patch test protocol

Valerie T. Politano *, Anne Marie Api

Research Institute for Fragrance Materials, Inc., Woodcliff Lake, NJ 07677, USA

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Abstract
With implementation of the dermal sensitization QRA approach for fragrance ingredients, IFRA/RIFM are recommending use of the RIFM standard human repeated insult patch test (HRIPT) protocol for generation of confirmatory human data for the induction of dermal sensitization in a normal human population. Details of this standard HRIPT protocol are provided in this paper. The study protocol consists of two phases—Induction and Challenge. In the Induction phase, patches treated with fragrance ingredients in 75% diethyl phthalate/25% ethanol are applied to backs of volunteers for 24 h. Following patch removal there is a 24-h rest period and volunteers are patched again at the same site. This procedure is repeated to achieve 9 applications over a 3-week period. There is an approximate 2-week rest period followed by a Challenge phase of a single 24-h patch application of test article applied to a naïve site on the back. Skin reactions at the naïve site observed at Challenge may be suggestive of dermal sensitization, and a Rechallenge is performed to confirm the nature of the reactivity. This study is designed to confirm the No-Observed-Effect-Level for induction of dermal sensitization in a normal human population.

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Keywords: Dermal sensitization; Fragrance; HRIPT; Human; Patch test; Skin; Human repeated insult patch test

1. Introduction
Historical human data either from human repeated insult patch tests (HRIPT) or human maximization tests are available for raw materials found in consumer products and a variety of those products. This is certainly true for fragrance ingredients.

The HRIPT is a version of the modified Draize procedure (Draize, 1959; Draize et al., 1944; Marzulli and Maibach, 1976) and is a patch test that is now used to confirm the No-Observed-Effect-Level (NOEL) for the induction of dermal sensitization in a normal human population. The protocol described here has been in use by the Research Institute for Fragrance Materials, Inc. (RIFM) for the last twenty years for the testing of individual fragrance materials.

RIFM has a historical database that contains more than 1000 HRIPTs and greater than 1200 human maximization tests conducted on individual fragrance ingredients. This includes more than 200 HRIPTs that have been conducted by RIFM using the same (RIFM standard) protocol. In addition, the RIFM database contains a significant and increasing number of murine local lymph node assays (LLNA) that can be used in combination with confirmatory human dermal sensitization data in a weight of evidence approach for establishing the No Expected Sensitization Induction Level (NESIL) that is used in a dermal sensitization Quantitative Risk Assessment (QRA) approach (Api et al., 2008).

The objective of the HRIPT protocol is to confirm, in healthy human volunteers, the dermal sensitization NOEL that has been obtained from dermal sensitization quantitative structure–activity relationships, animal pre-clinical data, and historical human data. This protocol is not used to determine hazard. The test is not used as a predictive
method nor is it used on substances with unknown dermal sensitization potential. It is a test to confirm the lack of dermal sensitization at an exposure level which was identified as a NOEL in an animal model and/or historical human data. This methodology can also be used to assess skin irritation through repeated patching during the Induction phase.

The test articles are evaluated for the induction of dermal sensitization and irritation by repeated applications to the skin of healthy human volunteers. A review of the HRIPT design and the critical factors that can affect the induction of dermal sensitization can be found in McNamee (2008).

These HRIPT data add an important aspect to the overall evaluation of dermal sensitization, based on a weight of evidence approach, for a fragrance ingredient when conducted a QRA (Api et al., 2008). In fact, currently the HRIPT is the primary way of confirming in humans a predicted dermal sensitization NOEL from animal testing. With implementation of the QRA approach, the International Fragrance Association (IFRA) and RIFM are recommending the use of the RIFM standard HRIPT protocol for generation of confirmatory human data for use in QRA.

2. Protocol

2.1. Test subjects

A sufficient number of subjects (approximately 130), male and female between the ages of 18 and 70, are to be empanelled such that approximately 100 complete the study. The subjects are informed of the nature of the test, including possible adverse reactions. Written informed consent is obtained. Additionally, the subjects must be considered dependable and able to read, understand, and follow instructions. Prior to test initiation, each subject is to complete a medical history form. The subjects should be able to read, understand, and follow instructions. Prior to test initiation, each subject is to complete a medical history form. The subjects should not exhibit any physical or dermatological condition which would preclude application of the test articles. The subjects must fit all of the inclusion and exclusion criteria listed in Table 1.

2.2. Test articles

The amount of test article applied should be expressed as the quantity of chemical per unit area of the skin, µg/cm², and as a percentage. A vehicle that contains ethanol (EtOH) must be used to dilute the test article. The preferred vehicle of RIFM is 75% diethyl phthalate (DEP)/25% EtOH. In addition to the test article, the test subject should also be patched with the vehicle control and a saline control. It has been well documented that the vehicle in which an allergen is presented to the skin has an effect on its skin-sensitizing potency (Kligman, 1966; Lalok et al., 2004; Stotts and Ely, 1977). The DEP and EtOH combination in use by RIFM was selected because the majority of fragrance materials are soluble in DEP and EtOH; it is representative of the matrix often used in commercial products, and the 3:1 DEP/EtOH ratio was selected since on rare occasions increasing levels of EtOH can induce sensitization in humans (Stotts and Ely, 1977).

2.3. Experimental design

2.3.1. Induction phase

The quantity of test article applied per test patch is 0.3 ml or 0.3 g. The test articles are dispensed onto 25 mm Hilltop Chamber patches (Hill Top Research, Miamiville, OH) and the patches are applied to normal skin between the left scapula and the spinal mid-line. Hill Top Chamber patches are composed of a flexible molded plastic chamber with a double rim that fits close to the skin and the chamber is lined with a nonwoven Webril pad. The patch is held in place with semiocclusive tape. Fresh patches of test article are prepared daily. Following application of test article to the patch, the patch must be applied to the skin a minimum of 15 min after preparation but not longer than 40 min after preparation. Sample preparation and volatilization times should be documented.

Test article patch applications to the same patch site are generally made on Monday, Wednesday, and Friday for three consecutive weeks. The test subjects are instructed to return to the Testing Facility on Tuesday and Thursday (approximately 24 h after patch application) for supervised removal of patches. Test subjects are instructed to remove patches that were applied on Friday approximately 24 h after patch application. Twenty-four-hour rest periods follow Tuesday and Thursday removals and 48-h rest periods follow each Saturday removal. The patch sites are scored by a trained evaluator just prior to the next patch application according to the Draize scoring system (Draize et al., 1944) modified by Phillips et al. (1972) and further modified by McNamee (2008, Table 2). This procedure is repeated until nine Induction applications of the test article are made.

Test subjects are free to withdraw from the study at any time or they may be disqualified by the Testing Facility if in the opinion of the Principal Investigator clinical observations indicate it would be unwise for them to continue. A minimum of nine Induction patches are required in order to satisfactorily complete the Induction phase of the study. Test subjects are permitted a maximum of one missed scheduled patch application during the Induction phase of the study which must be made up during the Induction phase of the study. Test subjects will be discontinued by the Testing Facility if they miss more than one Induction patch application and the corresponding make-up patch application. Test subjects are permitted to miss one regularly scheduled supervised patch removal, and they are instructed to remove the patches themselves at home approximately 24 h following application. Test subjects will be discontinued by the Testing Facility if they miss more than one supervised patch removal. Discontinued subject data are reported up to the point of discontinuation, but their data are not used in the results, discussion, or conclusion sections of the final report.

The patch sites are scored by a trained evaluator according to the criteria referenced above (Table 2). Accompanying edema (swelling) or other dermal sequelae is recorded and described as mild, moderate, or severe. If a test subject develops a positive reaction of a 2-level (moderate) or greater during the Induction phase, the patch will then be applied to an adjacent naïve (previously unpatched) site for the next application. If a 2-level or greater reaction occurs at this naïve site, no further Induction applications are made. Reactive subjects are to be subsequently patched with the test article at a naïve test site during the Challenge phase of the study with the rest of the panel, unless in the opinions of the Investigators it would be unwise for them to do so (due to very strong reactions).

2.3.2. Challenge phase

Ten to fourteen days after application of the last Induction patch, a single Challenge patch is applied to a naïve site of normal skin on the contralateral side of the back. Test subjects are instructed to return to the Testing Facility 24 h later for removal of the patch by the trained evaluator. The Challenge site is scored 24, 48, and 72 h after application by a trained evaluator, with both a clinical dermatologist and a study monitor present at the 72-h reading. The evaluators and clinical dermatologist must not know the identity of the test articles, vehicle or negative controls. Test subjects are asked to report any delayed reactions that might occur after the final Challenge patch reading. Test subjects who miss the Challenge patch application are discontinued from the study.

2.3.3. Rechallenge

Test subjects who exhibit skin reactivity suggestive but not clearly indicative of induced allergic contact dermatitis during the Challenge phase of the study are requested to participate in a Rechallenge procedure following a four week rest period. The Rechallenge procedure consists of
Table 1
Inclusion and exclusion criteria

**Inclusion criteria**
1. Males or females, age 18 to 70 years of age and in good general health (not more than 20% of the panel should be greater than 65 years of age)
2. Individuals of any skin type or race provided their degree of skin pigmentation does not significantly interfere with evaluations
3. Individuals free of any systemic or dermatological disorder including known allergies to skin care products or topical drugs, or other medical conditions which, in the opinion of the investigator, might interfere with the conduct of the study, interpretations of the results, or increase the risk of adverse reactions
4. Individuals able to read, understand, and provide written informed consent
5. Individuals who are believed to be dependable, who agree to complete the course of the study, and comply with instructions

**Exclusion criteria**
1. Women who are self-reported pregnant, nursing or planning a pregnancy
2. Individuals with a history of any dermatological disease or condition, including but not limited to active atopic dermatitis, psoriasis, eczema, active seasonal allergies or skin cancer within the past 6 months
3. Individuals with abnormal skin pigmentation at the test sites which might interfere with subsequent evaluations of dermal responsiveness
4. Individuals taking medications which might interfere with the test results, including any regimen of steroidal/non-steroidal anti-inflammatory drugs, antihistamines or immunosuppressive drugs
5. Individuals who have applied any type of topical anti-inflammatory medication to the test sites within two weeks prior to enrollment
6. Individuals with any other skin condition that would interfere with the conduct of the study
7. Individuals who have undergone a bilateral mastectomy with lymph node removal, a unilateral mastectomy with lymph node removal within the last year, or a bilateral axillary lymph node removal
8. Individuals with a history of immune deficiency or auto-immune disease
9. Individuals who are currently receiving allergy injections, have received allergy injections within one week prior to enrollment, or expect to begin receiving allergy injections during the study
10. Individuals treated for malignancy within 6 months prior to enrollment
11. Individuals who are currently under treatment for asthma or diabetes
12. Current enrollment in any other research study or participation in a patch test study within 30 days prior to the start of this study
13. Individuals who have ever participated on a patch test study with a cologne or perfume
14. Individuals who are known tape/adhesive reactors

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Table 2
HRIPT scoring scale

<table>
<thead>
<tr>
<th>Erythema scale</th>
<th>Description</th>
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<tbody>
<tr>
<td>0 No visible erythema</td>
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<td>2 Moderate erythema (definite redness)</td>
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<th>Designations for elevated responses</th>
<th>Description</th>
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<tr>
<td>Edema, papules, vesicles, and bullae, if present, are graded as independent responses</td>
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<tr>
<td>E Edema—definite swelling</td>
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<td>P Papules—many small, red, solid elevations; surface of reaction has granular feeling</td>
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<tr>
<td>V Vesicles—small circumscribed elevations having translucent surfaces so that fluid is visible (blisters-like); vesicles are no larger than 0.5 cm in diameter</td>
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<tr>
<td>B Bullae—vesicles with a diameter &gt;0.5 cm; vesicles may coalesce to form one or a few large blisters that fill the patch site</td>
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<tr>
<td>L Patch lost (came off) during first 12 h</td>
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The results of the HRIPT protocol are presented as the number of induced sensitization reactions observed out of the total number of volunteers who completed the study. The results of the individual patch sites are presented in tabular format.

The induction of dermal sensitization is determined by enhancement of the skin reaction observed at Challenge greater than that observed during Induction. Low grade reactions observed during Induction but which are not observed at Challenge are considered to be irritant in nature. If a volunteer has an erythematous and edematous reaction during the early part of the Induction phase that is confirmed at Challenge, the subject is considered to be presensitized and the reaction is not considered having been induced during the Induction phase.

If a test subject reacted during the earliest part of the Induction phase and returns to confirm the reactivity at Challenge, the subject is considered to have exhibited...
Henderson and Riley (1945) investigated statistical calculations of patch tests adapted for the detection and evaluation of chemical agents. If no reactions were observed in a group of 100 test subjects, then the rate of positive reactions in a larger population is not likely to exceed 2.9%, based on a confidence level of 95%, under identical conditions (Henderson and Riley, 1945). The likely maximum rate of 2.9% positive reactions is often misinterpreted to mean that there would be an expected rate of 2.9% in the marketplace. The test conditions in the HRIPT are not identical to real life scenarios. To increase the sensitivity of the test whilst using such numbers of subjects, if appropriate one generally tests a higher concentration of test material and usually more exaggerated exposure conditions 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, in some HRIPT protocols, are exaggerated through possible minor skin irritation of a test material, use of occluded patches, and vehicle effects from the test conditions (Basketter et al., 2006; McNamee et al., 2008).

The induction of human dermal sensitization from the HRIPT is rare. Hall (2006) estimated the rate of dermal sensitization induction to be 0.09% of volunteers in tests on cosmetic products. In addition, Hall (2006) identified there has been no evidence of adverse sequelae from these tests.

**Conflict of Interest**

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Dose metrics in the acquisition of skin sensitization: Thresholds and importance of dose per unit area

Ian Kimber a,1, Rebecca J. Dearman a,1, David A. Basketter b,2, Cindy A. Ryan c, G. Frank Gerberick c, Pauline M. McNamee d, Jon Lalko e, Anne Marie Api e,*

a Syngenta Central Toxicology Laboratory, Alderley Park, Cheshire, UK
b Unilever Safety and Environmental Assurance Centre, Bedfordshire, UK
c Procter & Gamble Company, Cincinnati, OH, USA
d Procter & Gamble Company, Surrey, UK
e Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ, USA

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Abstract

Allergic contact dermatitis is a common occupational and environmental health problem and many hundreds of chemicals have been implicated as skin sensitizers. Sensitization is acquired following topical exposure to a contact allergen and induction of a cutaneous immune response of an appropriate magnitude. For effective assessment and management of human health risks there is a need to appreciate the dose metrics that drive the induction of skin sensitization. The available evidence suggests that under most normal conditions of exposure it is the dose per unit area of chemical that has over-riding impact on the effectiveness of sensitization. The exception to this rule is when the area of the application site drops below a certain critical level. Here we review in detail the evidence which supports dose per unit area as being the critical exposure metric in the induction of skin sensitization, and the mechanistic bases for this relationship.

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Keywords: Dermal exposure; Langerhans cells; Skin sensitization; Quantitative risk assessment

1. Introduction

Skin sensitization is acquired following topical exposure of an inherently susceptible individual to an appropriate amount of a contact allergen. An appropriate amount in this context can be described operationally as being that required to induce a cutaneous immune response of the magnitude necessary for a degree of immunological priming (sensitization) that will result in a dermal inflammatory reaction being provoked following subsequent exposure.

The amount of chemical necessary to result in the acquisition of skin sensitization will be determined primarily by two factors: (a) the inherent potency of the contact allergen, and (b) the conditions of exposure (the site, matrix, extent, frequency and duration of exposure). These aspects of skin sensitization have been considered extensively elsewhere, particularly as they relate to risk assessment, and it is not the purpose of this article to review again these variables (Basketter et al., 2002, 2006; Felter et al., 2002, 2003; Gerberick et al., 2001; Kimber et al., 2001; Robinson et al., 2000). Rather, our purpose here is to explore how best to describe the relevant dosimetry for skin sensitization. This is not a trivial question since, in principle at least, there are a number of dose metrics that could be relevant, including for instance that of dose per unit body weight, a descriptor that frequently finds favour in studies of systemic toxicity.
As a prelude to examination of relevant dosimetry it is helpful to survey briefly the immunobiological bases for the induction of skin sensitization.

2. The immunobiology of skin sensitization

The acquisition of skin sensitization is dependent upon the initiation of a cutaneous immune response of the appropriate quality, and of the necessary magnitude. The central event in sensitization is specific priming of the immune system such that there is a selective clonal expansion of allergen-reactive T lymphocytes. This quantitative increase in the frequency of allergen-specific T lymphocytes equips the immune system with the ability to mount an accelerated and more aggressive secondary immune response following subsequent encounter with the inducing chemical allergen (at the same or a distant skin site) such that a cutaneous inflammatory reaction is provoked. This local inflammatory reaction at the site of exposure is recognized clinically as allergic contact dermatitis. There are available elsewhere detailed reviews of the cellular and molecular mechanisms through which the induction and elicitation of contact allergy are achieved (Dearman and Kimber, 2003; Grabbe and Schwarz, 1998; Kimber et al., 2002; Kimber and Dearman, 2002, 2003).

The need here, however, is for a brief consideration of some of the events induced in the skin and regional lymph nodes that are required for the effective induction of skin sensitization. Of particular importance in this process are believed to be epidermal Langerhans cells (LC), and possibly other cutaneous dendritic cells (DC). The roles of these cells in the initiation of cutaneous immune responses include the recognition and processing of antigen encountered at skin surfaces, and the subsequent transport of that antigen to regional lymph nodes where it is presented to responsive T lymphocytes. In the case of skin sensitization, the inducing chemical allergen must gain access to the viable epidermis where, either directly or indirectly, it associates with native protein and is recognized by LC. These cells process internalized antigen and transport it from the skin, via afferent lymphatics, to draining lymph nodes. Following their mobilization and migration from the skin LC lose the properties of antigen processing cells and acquire instead the characteristics of immunostimulatory DC that are able effectively to present antigen to T lymphocytes in the paracortical regions of lymph nodes. In the absence of effective processing, delivery and presentation of antigen cutaneous immune responses, including skin sensitization, fail to develop. Comprehensive accounts of the roles played by epidermal LC in the acquisition of skin sensitization are available (Cumberbatch et al., 2000, 2003, 2005; Griffiths et al., 2005; Kimber et al., 1998, 2000). It is becoming clear that the biology of LC is extremely complex, as are the biological processes that serve to induce and regulate their mobilization and differentiation. There is also some controversy currently about whether for the effective acquisition of skin sensitization there is a mandatory requirement for LC, and evidence that LC can, in different circumstances, display either immunostimulatory or down-regulatory activity (Bennett et al., 2005; Kaplan et al., 2005; Kissenpfennig and Malissen, 2006; Romani et al., 2006). However, under normal conditions it can be assumed that LC play an important, and usually decisive, role in the development of cutaneous immune responses to chemical allergens, and that the behaviour of these cells, and their handling of chemical allergens, is likely to be directly relevant for considerations of thresholds, dose responses and dose metrics in skin sensitization. This will be explored later in this article.

Before turning our attention to an examination of skin sensitization dose metrics it is appropriate to reflect briefly on what is known of dose thresholds as they relate to the induction of skin sensitization and the elicitation of allergic contact dermatitis.

3. Thresholds

An issue about which there is frequently some uncertainty is that of thresholds of exposure. Specifically, the questions are whether there exist thresholds for the induction of skin sensitization in previously (immunologically) naïve subjects, and whether there are thresholds for the elicitation of contact allergic reactions in subjects that are already sensitized. The answer in both cases is yes (Basketter et al., 2002; Boukhman and Maibach, 2001; Kimber et al., 1999), and there are available methods to determine threshold values (Basketter et al., 1997, 2005). Although it is clear that thresholds exist, it is important to acknowledge that these will vary in absolute terms between subjects. Variation among subjects at the induction stage may be due to inter-individual differences in inherent or acquired susceptibility to sensitization and/or exposure conditions. Specifically, differences may result from variations in the extent or duration of exposure, the matrix in which an allergen is experienced, the condition of the skin at the site of contact, and possibly other factors that are largely of unknown cause, but which may be related to, or independent of, the specific contact allergen (Basketter et al., 2002; Friedmann, 1990; Moss et al., 1985). One important determinant of variations between individuals at the elicitation stage of contact allergy is the extent to which sensitization was acquired previously. Thus, there is recognition that, in general terms at least, as the dose of chemical used for sensitization is reduced (and, as a consequence, the degree of sensitization achieved is reduced also), then the higher the dose of the same chemical that will be required to elicit a dermal reaction in the sensitized subject (Friedmann, 1990; Friedmann et al., 1983; Hostynek and Maibach, 2004; Scott et al., 2002).

One other aspect of thresholds is worthy of brief consideration. The acquisition of skin sensitization, and the extent of sensitization achieved, reflects immunological priming. It is theoretically possible therefore that some level of priming might be achieved, but of insufficient vig-
our to result in frank sensitization (such that subsequent challenge would elicit a dermal inflammatory reaction) (Hostynek and Maibach, 2004; Kimber et al., 1999). This appears to be the case in practice also. In studies reported by Friedmann et al. (1990) it was found that subclinical levels of skin sensitization to the contact allergen 2,4-dinitrochlorobenzene (DNCB) could be induced in human volunteers. The approach taken was to expose a panel of normal subjects to a dose of DNCB that, on the basis of previous investigations, was known to result in the sensitization of approximately 50% of naive individuals. When challenged some time thereafter, and consistent with expectations, half of those in which sensitization had been attempted failed to respond to challenge. When these unresponsive individuals were challenged a second time several weeks later some subjects displayed exaggerated skin reactions compared with control subjects that had previously received the initial challenge regimen alone as a sensitizing stimulus (Friedmann et al., 1990).

4. Dose metrics and the induction of skin sensitization

It is commonly the case that in experimental studies of skin sensitization topical exposure is recorded as a function of the concentration of chemical expressed as percentage weight per volume, or percentage volume per volume. The implicit assumption deriving from this is that a constant amount of chemical allergen would be expected to result in a similar level of sensitization, or frequency of sensitization, irrespective of exposure conditions, and the area of skin over which the chemical was encountered. In fact this is not the case, and the widely reported studies of human skin sensitization conducted by Peter Friedmann and his colleagues in the 1980s have provided strong evidence that the important parameter is the dose of chemical allergen per unit area of skin (Friedmann, 1990, 1996).

The experimental approach used by Friedmann and colleagues was to measure skin sensitization to DNCB among healthy volunteers. In these investigations the effectiveness of sensitization was evaluated as a function of the frequency of subjects displaying skin reactions (determined by measurement of induced increases in skin fold thickness) following challenge. Using this approach it was, for instance, possible to demonstrate that among naive volunteers (subjects with no history of contact dermatitis or cutaneous inflammatory disease) the proportion of individuals sensitized showed a positive sigmoid relationship with the log of the sensitizing dose (Friedmann and Moss, 1985). Similar investigations were performed to examine the effect of concentration and surface area on the development of sensitization.

In one study naïve subjects were exposed topically to DNCB over a circular administration site of 3 cm diameter (equivalent to a surface area of 7.1 cm²). Subjects were exposed to different amounts of DNCB within that standard application site such that the concentrations of DNCB per unit area of skin received by different groups of volunteers ranged from 142 µg/cm² to 8.8 µg/cm². As illustrated in Table 1 in those groups that received either 71 µg/cm² or 142 µg/cm² the sensitization rate following subsequent challenge was found to be 100%. However, exposure to lower concentrations of DNCB per unit area of skin were associated with progressively lower rates of sensitization, with 8.8 µg/cm² DNCB resulting in sensitization of only 8% of subjects (Table 1) (White et al., 1986).

Other experiments provided evidence that the observed differences in sensitization rate were not simply a function of the total amount of allergen delivered to the skin. Thus, for instance, as described above and illustrated in Table 1, it was shown that exposure to a total amount of DNCB of 62.5 µg, delivered over an area of 7.1 cm² and resulting in a dose per unit area of 8.8 µg/cm², was associated with a frequency of sensitization of just 8%. However, when the same total amount of DNCB was administered over a smaller surface area (of 1.8 cm²), resulting in an approximately 4-fold increase in the concentration of chemical per unit area (35.4 µg/cm²), then the rate of sensitization was found to be 83% (Friedmann, 1990; White et al., 1986) (Table 1). The interpretation is that the concentration per unit area of skin is of greater importance than the total amount of chemical experienced at the skin surface in driving sensitization.

Further evidence for this conclusion was provided by results deriving from a slightly different experiment with DNCB. In this case groups of volunteers were exposed to different total amounts of DNCB, but at sites of different diameter such that the concentration of DNCB per unit area of skin was the same in each group. Thus, for instance one group received a total of 58 µg of DNCB over an area of 2.1 cm² such as to provide a dose of 16.4 µg/cm². In another group the total amount of DNCB used for exposure was increased to 232 µg, but in this instance was applied over an area of 14.2 cm² to provide again a dose of 16.4 µg/cm². When these groups were challenged subsequently it was found that the rates of sensitization were comparable (Table 1) (Friedmann, 1990; White et al., 1986).

Collectively these data provide compelling evidence that, with DNCB at least, and over a wide range of conditions of exposure, it is the dose of chemical per unit area of skin, rather that total amount of chemical delivered, that is the key metric in terms of the effectiveness with which sensitization is acquired. However, in drawing this conclusion it is necessary to add one important caveat. It is that although the correlation between dose per unit area and the acquisition of skin sensitization appears to hold true over a wide range of exposure conditions (that is over a wide range of dose per unit areas) the relationship is not without limitations. Thus, as discussed previously (Friedmann, 1990) it would be entirely illogical to anticipate that under conditions of a constant dose per unit area the area of application could be reduced to an infinitesimal size without loss of sensitizing potency. In fact, it was found that the relationship between dose per unit area and the
The effectiveness of sensitization does not hold true when very small application sites are considered. Thus, it was shown that exposure of a group of subjects to a total amount of DNCB of 30 μg, delivered over an area of 0.8 cm² (giving a dose per unit area of 38 μg/cm²) resulted in a sensitization rate of approximately 93%. However, in another group of donors where the same dose per unit area was achieved (by applying a total of 3 μg of DNCB over an area of 0.08 cm²) the sensitization rate was found to be only 26% (Rees et al., 1990).

The possible reasons for the failure of dose per unit area to remain the important metric when exposure sites are very small will be considered later.

Persuasive as the evidence deriving from the studies performed with DNCB (and summarized above and in Table 1) is, there has been speculation that DNCB might represent a special case in this regard, or that for some other reason the dose per unit area metric may not apply in some other situations. This appears not to be the case, however, and evidence to support dose per unit area as the most important exposure determinant in driving the induction of skin sensitization derives from studies of human skin sensitization performed some 40 years ago by Albert Kligman (1966).

The relevant publication by Kligman describes heroic and comprehensive analyses of the various factors that may impact on the development of allergic contact dermatitis. Included in his considerations were the impact of inflammation and various aspects of exposure, including the importance of the area of exposure used for sensitization. For these studies Kligman used male prisoner volunteers and a variety of skin sensitizing chemicals (Kligman, 1966).

In common with the investigations of Friedmann and colleagues with DNCB, one focus of attention in the Kligman studies was the relative importance of dose per unit area and the total delivered dose. In one series of experiments the induction of skin sensitization to 4 contact allergens were examined: ammoniated mercury, monobenzyl ether of hydroquinone, nickel sulfate and neomycin sulfate. Each of the chemicals was applied to groups of volunteers over one of 3 application areas: 0.36 cm², 14 cm², or 56 cm². Although different induction concentrations were used for each chemical, for each individual sensitizer the amount applied per unit area of skin was kept constant by adjustment of the total amount applied. Outcomes were measured subsequently as the frequency of sensitization among volunteers, judged by challenge-induced erythema.

Using this approach the first important observation was that when application sites with areas of 14 cm² and 56 cm² were compared there was no difference in the rate of sensitization, and this was found to hold true for each of the four sensitizers examined. That is, under these conditions of exposure it is the dose per unit area of chemical, rather than the total delivered amount, that is the important metric for the effectiveness of sensitization.

The second important observation was that, in common with the investigations of Rees et al. (1990) with DNCB, the relationship between dose per unit area and the acquisition of skin sensitization was lost when the area of the site used for sensitization became very small. Thus, in the studies of Kligman, when the application site was reduced to an area of 0.36 cm² (in effect a reduction of some 40-fold in area compared with the application site of 14 cm²) the frequency of sensitization to each of the 4 contact allergens was reduced, despite exposure to the same dose per unit area of skin.

There were two other outcomes of these investigations that are worthy of note. The first of these derives from a comparison with the previous data on the effectiveness of sensitization when chemicals were administered simultaneously over 4 adjacent 14 cm² sites on a single arm (giving a total application area of 56 cm²). It was found that the frequency of sensitization using the 4 separate, but adjacent, applications sites did not differ either from sensitization achieved with a single application site of 14 cm², or with a single site of 56 cm². Again, this was true with each of the 4 contact allergens used. The conclusion drawn is that those 4 sites accomplish no more in driving sensitization than does one site of the same area, or one site of the cumulative area. Again these data support the importance under these conditions of dose per unit area.

The second observation was based upon analysis of sensitization achieved by attempting to induce at a constant dose per unit area through two identical 14 cm² area sites on different arms. The results were very clear. With all 4 contact allergens, induction under these conditions was no more or less effective than was exposure via a single 14 cm² site, or via 4 adjacent 14 cm² sites on the same arm. Of course this result is not unexpected because what in effect has been done is to increase the area of exposure (albeit at two different sites) without impacting the dose

### Table 1

Impact of dose per unit area on the acquisition of skin sensitization in human volunteers to 2,4-dinitrochlorobenzene (DNCB)

<table>
<thead>
<tr>
<th>Group</th>
<th>Application site</th>
<th>Sensitizing dose</th>
<th>No. of subjects</th>
<th>% Sensitized</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diameter (cm)</td>
<td>Area (cm²)</td>
<td>Total (μg)</td>
<td>Concentration (μg/cm²)</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>7.1</td>
<td>62.5</td>
<td>8.8</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>7.1</td>
<td>125</td>
<td>17.7</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>7.1</td>
<td>250</td>
<td>35.4</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>7.1</td>
<td>500</td>
<td>71</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>7.1</td>
<td>1000</td>
<td>142</td>
</tr>
</tbody>
</table>

Modified from Friedmann (1990) and White et al. (1986).
per unit area, and from previous comparisons it was clear that above a minimum application area the important metric is dose per unit area, rather than total area (Kligman, 1966).

Finally, there is one other inference to draw from Kligman’s investigations. In separate comparative analyses, and using a different series of contact allergens, it was found that four sequential 48-h exposures to different, but adjacent, sites on one extremity were far more effective than were 4 single 48-h exposures of exactly the same type given sequentially to each of 4 different extremities (arms and legs). The conclusion drawn by Kligman at the time was that “bombardment of the same node is superior” ... to “stimulation of four different nodal systems” (Kligman, 1966). This is an interpretation that will be considered later.

Taken together, the studies of Kligman provide compelling support for the conclusions drawn from similar investigations with DNCB. Of particular moment is the fact that the importance of dose per unit area as the key metric (under most conditions of exposure) for the acquisition of sensitization is not peculiar to DNCB. Of equal significance is the observation that dose per unit area is not relevant only for very strong contact allergens such as DNCB. Of the 4 contact allergens used by Kligman (and based on sensitizing potency, rather than prevalence) one can be considered a strong sensitizer (monobenzyl ether of hydroquinone), one a moderate sensitizer (ammoniated mercury), and two comparatively weak sensitizers (nickel sulphate and neomycin sulphate).

Before leaving consideration of the data that support dose per unit area as being the important exposure metric for the induction of skin sensitization, it is appropriate to draw attention to some information available from guinea pig studies that support the same conclusion. In their comprehensive book on guinea pig testing for skin sensitization, it is likely that even 40 years ago Albert Kligman was correct when he discussed the effects of exposure metrics on the activation of lymph nodes draining the site of encounter with contact allergens (Kligman, 1966). It has been appreciated for many years that cutaneous immune responses, including contact sensitization, are initiated in peripheral lymph nodes draining sites of exposure to antigen (Davies et al., 1969; Oort and Turk, 1965; Wiest and Jung, 1970). More recently, the absolute requirement for draining lymph nodes for the induction of skin sensitization has been shown in lymphotoxin-α-deficient mice that lack peripheral lymph nodes and in which contact hypersensitivity fails to develop (Rennert et al., 2001). Lymph nodes are important because they provide the tissue matrix required to support the cellular and molecular interactions necessary for the effective induction of primary immune responses. Of particular importance in this regard in the context of contact sensitization is the presentation to responsive T lymphocytes of the chemical allergen transported to skin draining lymph nodes by epidermal LC (Cumberbatch et al., 2000, 2003, 2005; Griffiths et al., 2005; Kimber et al., 1998, 2000; Macatonia et al., 1986). As antigen-bearing LC become mobilized and migrate from the epidermis they mature into effective antigen presenting cells—and it is possible that skin sensitization is facilitated also by the transport of antigen to other DC already resident within draining lymph nodes that can similarly serve as immunostimulatory antigen presenting cells. In any event, the likelihood is that there is the need for a certain critical number of antigen-bearing DC reaching and being within draining lymph nodes. This is to provide signals of sufficient magnitude and intensity to overcome the threshold required for the triggering of a primary immune response. Translating this into the dose metric found to be relevant, in most instances, for the acquisition of skin sensitisation, the interpretation is that there is a certain critical level of antigen (in this case chemical allergen), and LC availability for transport, required in the area of skin from which there is lymphatic drainage to a single lymph node, or to a single series of lymph nodes. As this critical mass increases then the threshold for effective stimulation is reached and exceeded, and then as the critical mass increases further then so will the vigour of induced immune responses (and the extent to which skin sensitization is acquired). The corollary is that if the same amount

5. Interpretation and conclusion

An appreciation of dose metrics in skin sensitization is of course of real importance in developing accurate risk assessments and effective risk management strategies.
of chemical is distributed over a larger area of skin, where lymphatic drainage is served by several lymph nodes, then the critical mass of available allergen per lymph node will be reduced, and the level of immune activation diminished accordingly.

If this is a correct interpretation then it is necessary to accommodate the observation that the primacy of dose per unit area as the relevant exposure metric holds for most normal conditions of exposure, but becomes irrelevant, or at least less relevant, when the area of the application site falls below a certain level (in practice, a very small area). The suggestion is that under such conditions, where the chemical allergen is available to only a very small area of skin, there will be insufficient numbers of primed LC transporting antigen to the draining lymph node to reach the threshold of activation necessary for an effective immune response.

One final point should be made. A rather different situation may pertain when induction involves repeated application of the same contact allergen at the same skin site, or at different skin sites that drain to a single lymph node, or a single series of lymph nodes. Here the primacy of dose per unit area may be confounded by this second, and potentially important, variable. If in these circumstances repeated exposures at the same site are closely consecutive (such that the first dose has not cleared before the second exposure) then the true dose per unit area is rather more difficult to ascertain, and calculation of the total dose may represent a more pragmatic solution. Although single sequential exposures to separate lymph nodes are still able to induce a cutaneous immune response, the acquisition of skin sensitization will be more vigorous when repeated applications are focused through a single lymph node.

Irrespective of the detailed mechanistic basis for the relevant dose metrics in skin sensitization there is clear evidence that in most normal circumstances it is dose per unit area that will dictate the effectiveness and extent of sensitization achieved.

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References


Axilla surface area for males and females: Measured distribution

Christina Cowan-Ellsberry, Pauline M. McNamee, Tyra Leazer

a The Procter & Gamble Co., Cincinnati, OH, USA
b The Procter & Gamble Co., Whitehall Lane, Egham, Surrey TW20 9NW, UK

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Abstract

With the recent introduction of exposure-based Quantitative Risk Assessment (QRA) as an approach to the evaluation, of materials in finished consumer products that are potential dermal sensitizers, the need for robust exposure data was clearly identified. The objective of this current study is to provide a value for the axilla surface area (SA) that is statistically derived and can be used in dermal sensitization QRA for ingredients of personal care products meant for use on the axilla. The axilla surface area measured for 60 men and 60 women resulted in a median surface area for a single axilla of 64.5 cm² for females and 135.5 cm² for males. These participants were representative of the United States population in their range of heights and weights. Furthermore, combining these surface area data with measured use data from this and other studies has enabled calculations of consumer exposure to solid APDO products on a dose/unit area/day basis (9.1 mg/cm²/d).

1. Introduction

The safety of consumer products is assured through the use of scientifically sound risk assessments for these products and their ingredients. These assessments use state of the scientific toxicological approaches and the most robust data for hazard identification and exposure. This is true both for the evaluation of systemic toxicity and toxicity endpoints related to local dermal effects such as skin sensitization.

With the recent introduction of exposure-based Quantitative Risk Assessment (QRA) as an approach to the evaluation of materials in finished consumer products that are potential dermal sensitizers, the need for robust exposure data was clearly identified (Robinson et al., 2000; Gerberick et al., 2001; Felter et al., 2002; Felter et al., 2003). This need has become even more apparent through the most recent activity of the International Fragrance Association (IFRA). IFRA reviewed the principles of dermal sensitization QRA to determine its applicability to fragrance ingredients identified as potential dermal sensitizers (Api et al., 2008). As a result of this review IFRA is applying the principles of dermal sensitization QRA to fragrance ingredients identified as dermal sensitizers. This work will result in the establishment of IFRA standards for these fragrance ingredients.

The IFRA review of the steps involved in the dermal sensitization QRA approach identified that key considerations for calculation of consumer exposure are: (1) the dose metric used to calculate exposure; (2) habits and practices data that define amount and frequency of product use and (3) human parameters data. The dose metric recommended for use in dermal sensitization risk assessments is dose per unit area (e.g., μg/cm²). This dose metric is supported by an understanding of the immunological mechanisms involved in the induction of dermal sensitization and extensive scientific literature publications from both experimental pre-clinical studies and confirmatory clinical studies (Kligman, 1966; Magnusson and Kligman, 1970; Friedmann and Moss, 1985; White...
et al., 1986; Upadhye and Maibach, 1992). The availability of robust habits and practices data across the broad range of consumer products has historically been somewhat variable. Few external data sources defining amount and frequency of product use were available e.g. SCCP notes of guidance (SCCNFP, 2003). However, even for these external data sources, a significant amount of these exposure data was derived from surveys of habits and practices data conducted by individual companies. More recently, exposure studies have been undertaken to define amount and frequency of product use and these employ a probabilistic rather than deterministic approach (McNamara et al., 2007; Tozer et al., 2004; Lorezt et al., 2005, 2006). The resulting data more accurately reflect realistic consumer exposures to consumer products. However, human parameters data such as surface areas of different body parts remain limited (Bremmer et al., 2006; ECETOC, 2001; U.S. EPA, 1997; SCCNFP, 2003).

One of the areas of improvement identified by IFRA in the dermal sensitization QRA for fragrance ingredients was exposure data. It was identified that improved exposure data (i.e. habits and practices data, and human parameters data) would be needed to further refine the calculated Consumer Exposure Level (CEL) in this dermal sensitization QRA approach. Although identified in the context of dermal QRA for fragrance ingredients, the need for improved exposure data is, of course, relevant for systemic as well as dermal sensitization risk assessment for any material.

The current exposure study was undertaken to: (1) address the lack of robust human parameters data related to surface area (SA) of the axilla; (2) provide an understanding of the distribution of the axilla SA for males and females; and (3) provide a value for the axilla SA that is statistically derived and can be used in dermal sensitization QRA for ingredients of personal care products meant for use in the axilla.

2. Methods

2.1. Subjects

Sixty males and sixty females, who were participating in a larger company Antiperspirant/Deodorant (APDO) study, volunteered to take part in this study designed to measure the axilla surface area. The subjects were representative of the US population and included Caucasian, African–American, Asian and Hispanic ethnicities.

2.2. Measurements

Measurements of axilla surface area, body weight and height were collected from each subject. The surface area for one axilla per participant was determined using standard sized rectangular disposable paper templates. The axilla surface area was visually defined in width by the borders of the axilla vault and in length by the region of hair growth and in most cases by the area covered by recently applied APDO product. The measurement was determined by matching the appropriately sized template to the dimensions of the axilla area identified (Fig. 1). Equations for estimating the total body surface area are based on a person’s height and weight, therefore, the participants were also asked for their height while their weight with clothing including shoes was measured on a digital scale.

The disposable paper templates ranged from 2.5 inches in width (W) by 3 inches in length (L) to 3 inches W by 5 inches L with 0.5 inch increments for women representing total axilla surface areas from 48.4 to 96.8 cm², respectively. For men the templates ranged from 3 inches W by 4 inches L to 4.5 inches W by 6 inches L with 0.5 inch increments representing total axilla surface areas from 77.4 cm² to 174.2 cm², respectively. These template ranges were selected based on current axilla surface area values and were expanded based on results from a pilot study. Specific template sizes and respective total axilla surface areas are provided in Tables 1 and 2.

2.3. Models for estimation of body surface area

In US EPA U.S. EPA (1997) and European (ECETOC, 2001) exposure factor handbooks, the surface area for various major body parts (e.g., legs, arms, and torso) is represented as a percentage of the total body surface area. Therefore, one objective of this study was to calculate the percentile of the total body surface area represented by a single axilla for each participant. The total body surface area was determined from each partici-

<table>
<thead>
<tr>
<th>Template size (W x L inches)</th>
<th>Axilla area (cm²)</th>
<th>Frequency of measurement</th>
<th>Average height</th>
<th>Average weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5 x 3</td>
<td>48.4</td>
<td>6</td>
<td>161.71</td>
<td>71.10</td>
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<tr>
<td>2.5 x 3.5</td>
<td>56.4</td>
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<td>168.91</td>
<td>79.04</td>
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<tr>
<td>2.5 x 4</td>
<td>64.5</td>
<td>27</td>
<td>165.76</td>
<td>70.60</td>
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<tr>
<td>2.5 x 4.5</td>
<td>72.6</td>
<td>3</td>
<td>168.49</td>
<td>78.47</td>
</tr>
<tr>
<td>3 x 4</td>
<td>77.4</td>
<td>11</td>
<td>164.29</td>
<td>81.11</td>
</tr>
<tr>
<td>2.5 x 5</td>
<td>80.6</td>
<td>3</td>
<td>173.57</td>
<td>64.64</td>
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<tr>
<td>3 x 4.5</td>
<td>87.1</td>
<td>1</td>
<td>166.37</td>
<td>93.67</td>
</tr>
<tr>
<td>3 x 5</td>
<td>96.8</td>
<td>7</td>
<td>167.46</td>
<td>80.97</td>
</tr>
</tbody>
</table>

These data are derived from a subset (60 females and 60 males) of a larger company Antiperspirant/Deodorant study.
part’s height and weight using different models. The models that were used included the Gehan and George model (1970) which is EPA’s formula of choice (U.S. EPA, 1997), the Costeff (1966) model which is the formula recommended for use in Europe for developing distributions of skin surface areas for adults when only body weight data is available (ECETOC, 2001), the Mosteller (1987) model which is widely used in the medical community and seven other models that have been published (DuBois and DuBois, 1916; Boyd, 1935; U.S. EPA, 1985; Haycock et al., 1978; Mattar, 1989; Livingston and Scott, 2001; Yu et al., 2003). The equation for each of these models is given in Table 3. The basis for these models and a comparison of their body surface area predictions for normal-weight, overweight and obese adults is given in Verbraecken et al. (2006).

2.4. Amount of Antiperspirant/Deodorant applied

This axilla measurements study was conducted concurrently with a larger in-house product self-application study with 141 women and 98 men which evaluated currently marketed APDO product. Therefore the amount of product applied by each participant was available to determine amount applied per axilla. In the larger study product was provided to each participant to apply by their own habits and practices to reflect their typical product use. The women were given an invisible solid product while men were provided with a deodorant stick (i.e., non-antiperspirant) product. The amount of product that the individuals applied was measured by weight difference in the product before and after application. Because the participants applied a different product to the left and right axilla, the amount applied per axilla was measured. Each subject participated from 1 to 3 days in the larger study. The amount of product applied per axilla was calculated as the average over all the applications for that participant. The amount of product applied per axilla surface area (in units of mg/cm²) was calculated from the measured axilla surface area and the average amount of product applied for each participant.

In addition, since the data on amount applied per axilla from the larger study was available, the median and 90th and 10th percentiles of the amount applied per axilla across the larger number of participants were also determined.

2.5. Statistical methods

Shapiro–Wilk normality tests were conducted on both the original and log-transformed response variables. The Shapiro–Wilks test compares very favorably with other goodness-of-fit tests (Shapiro and Wilk, 1965). It has a simple, graphical representation and can be thought of as an approximate measure of the correlation in a normal quantile–quantile plot of the data. Spearman rank correlation analysis was conducted in order to compare axilla area to amount applied. Spearman rank correlation analysis is routinely used when comparing variables that are not normally-distributed, since the correlations are based on the ranks of the data rather than the actual data values (Conover, 1980). In our data set, axilla area is not a continuous, normally-distributed variable, and amount applied cannot always be assumed to be normally-distributed. All statistical analyses were conducted using S-Plus 7.0 (Insightful Corporation, Seattle, WA).

3. Results

3.1. Panel characteristics

Both the female and male panelists were representative of the US population and included Caucasian, African–American, Asian and Hispanic ethnicities. In addition, the female participants ranged in height from 4’11” (149.86 cm) to 6’4” (193.4 cm) and in weight from 101 lbs (45.8 kg) to 242 lbs (109.8 kg). This is comparable to the 5th to 95th percentile ranges of 150.8–173.3 cm in height and 49.8–110.2 kg in weight for females in the US population based on the most recent (1999–2002) NHANES study (US Department of Health and Human Services, 2005). The male participants ranged in height from 5’2” (157.48 cm) to 6’5” (195.58 cm) and in weight from 134 lbs (60.8 kg) to 337 lbs (152.9 kg) while the NHANES data showed 5th and 95th percentile values of 162.9–188.4 cm in height and 60.4–121.2 kg in weight for males (US Department of Health and Human Services, 2005).

Table 2

<table>
<thead>
<tr>
<th>Template size</th>
<th>Axilla area (cm²)</th>
<th>Frequency of measurement</th>
<th>Average height</th>
<th>Average weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 × 4</td>
<td>77.4</td>
<td>1</td>
<td>162.56</td>
<td>82.78</td>
</tr>
<tr>
<td>3 × 5</td>
<td>96.8</td>
<td>4</td>
<td>176.53</td>
<td>91.967</td>
</tr>
<tr>
<td>3.5 × 5</td>
<td>112.9</td>
<td>9</td>
<td>177.24</td>
<td>91.12</td>
</tr>
<tr>
<td>3 × 6</td>
<td>116.1</td>
<td>5</td>
<td>177.8</td>
<td>73.44</td>
</tr>
<tr>
<td>4 × 5</td>
<td>129</td>
<td>5</td>
<td>176.78</td>
<td>102.29</td>
</tr>
<tr>
<td>3.5 × 6</td>
<td>135.5</td>
<td>19</td>
<td>180.14</td>
<td>85.59</td>
</tr>
<tr>
<td>4 × 5.5</td>
<td>141.9</td>
<td>4</td>
<td>175.26</td>
<td>97.24</td>
</tr>
<tr>
<td>4 × 6</td>
<td>154.8</td>
<td>12</td>
<td>181.9</td>
<td>99.96</td>
</tr>
<tr>
<td>4.5 × 6</td>
<td>174.2</td>
<td>1</td>
<td>170.18</td>
<td>109.99</td>
</tr>
</tbody>
</table>

These data are derived from a subset (60 females and 60 males) of a larger company Antiperspirant/Deodorant study.

Table 3

<table>
<thead>
<tr>
<th>Surface area model Reference</th>
<th>Equation (H = height in cm; W = weight in kg; SA = surface area in m²)</th>
<th>Male median percentage with 10th and 90th percentiles</th>
<th>Female median percentage with 10th and 90th percentiles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gehan and George (1970)</td>
<td>SA = 0.02355H⁰.⁴₂₂⁴⁶W⁰.⁵¹₄₅⁶</td>
<td>0.63 (0.48; 0.72)</td>
<td>0.38 (0.29; 0.46)</td>
</tr>
<tr>
<td>Costeff (1966)</td>
<td>SA = (4W + 7)/(W + 90)</td>
<td>0.48 (0.38; 0.55)</td>
<td>0.26 (0.21; 0.34)</td>
</tr>
<tr>
<td>Mosteller (1987)</td>
<td>SA = (H × W/5000)(⁰.⁵)</td>
<td>0.64 (0.48; 0.73)</td>
<td>0.37 (0.29; 0.46)</td>
</tr>
<tr>
<td>DuBois and DuBois (1916)</td>
<td>SA = 0.00718H⁰.⁷₂₅W⁰.₄₂₅</td>
<td>0.64 (0.49; 0.74)</td>
<td>0.38 (0.30; 0.48)</td>
</tr>
<tr>
<td>Boyd (1935)</td>
<td>SA = 0.00718H⁰.⁷₂₅W⁰.₄₂₅</td>
<td>0.64 (0.49; 0.73)</td>
<td>0.38 (0.29; 0.47)</td>
</tr>
<tr>
<td>U.S. EPA (1985)</td>
<td>SA = 0.0239H⁰.⁴₁⁷W⁰.₅₁₇</td>
<td>0.63 (0.48; 0.73)</td>
<td>0.37 (0.29; 0.46)</td>
</tr>
<tr>
<td>Haycock et al. (1978)</td>
<td>SA = 0.024265H⁰.₇₉₄W⁰.₅₃₇⁸</td>
<td>0.63 (0.48; 0.73)</td>
<td>0.37 (0.29; 0.47)</td>
</tr>
<tr>
<td>Livingston and Scott (2001)</td>
<td>SA = 0.1173W⁰.₆₄₆₆</td>
<td>0.62 (0.47; 0.71)</td>
<td>0.37 (0.28; 0.47)</td>
</tr>
<tr>
<td>Mattar (1989)</td>
<td>SA = (H + W – 60)/100</td>
<td>0.64 (0.48; 0.75)</td>
<td>0.38 (0.30; 0.48)</td>
</tr>
<tr>
<td>Yu et al. (2003)</td>
<td>SA = 0.015925H × W⁰.⁵</td>
<td>0.67 (0.50; 0.76)</td>
<td>0.39 (0.30; 0.49)</td>
</tr>
<tr>
<td>Average of all the models</td>
<td></td>
<td>0.64 (0.47; 0.71)</td>
<td>0.36 (0.28; 0.45)</td>
</tr>
</tbody>
</table>
3.2. Measured axilla surface area

The measured surface area for a single axilla is provided in Table 1 for females and in Table 2 for males. In women, the median surface area for a single axilla was 64.5 cm² with 90th and 10th percentiles of 96.8 and 54.8 cm², respectively. In men, the median surface area for a single axilla was 135.5 cm² with the 90th and 10th percentiles being 141.9 and 109.7 cm², respectively. For three of the female and eight of the male participants, there were two independent measurements of axilla surface area because they participated in the pilot study and this study. Comparing these two measurements, it was found that the potential uncertainty in the axilla area measurement was 10 cm² or less.

3.3. Percentage of body surface area

The percentage of the total body surface area represented by a single axilla using different models for total body surface area is given in Table 3. The axilla represents a very small percentage of the total body surface area. In women the median percentile was 0.26–0.39 depending on the model used to estimate the total body surface area with the median percentile based on the average of the surface area across all the models of 0.36. In males the median percentile ranged from 0.62 to 0.67 depending on the model used with a median percentile based on the average of the surface areas of 0.64. To illustrate the shape of the distribution of the percentile body surface area represented by the axilla, histograms of the distributions for two of the models—the Gehan and George (1970) and Costeff (1966) models for males and females—are shown in Fig. 2. The distributions of the percentile body surface area represented by the axilla for the other models used are also very similar.

3.4. Amount of APDO applied

The axilla measurements were conducted simultaneously with a larger in-house APDO product application study, subsequently the median amount of self-applied product was calculated in grams (g). In women, the median amount of invisible solid product self-applied per axilla by all 140 women was 0.45 g with 10th and 90th percentiles of 0.31 and 0.66 g. In males, the median amount of deodorant stick product applied per axilla by all 98 men participants was 0.52 g with 10th and 90th percentiles of 0.35 and 0.72 g. These are very similar to the amounts applied per axilla in the larger APDO study, which consisted of 141 females and 98 males, of median (10th and 90th percentiles) values of 0.42 (0.30; 0.64) g for women and median (10th and 90th percentiles) value of 0.53 (0.35, 0.74) for men (P&G unpublished study).

The amount of product applied per axilla surface area was calculated for each participant based on their amount of product applied per axilla and their measured axilla surface area. These values are also presented in Table 4.

Fig. 2. Distribution of the ratio of single axilla surface area to total body surface area for males and females determined using two different models—Gehan and George (1970) (a, males; c, females) and Costeff (1966) (b, males; d, females).
Because of the larger axilla area in men, the median value was 0.0038 for men compared to 0.0062 g/cm² for women.

3.5. Statistical distributions and correlations analysis

The axilla surface area and amount of APDO product applied per axilla surface area were tested for normality and lognormality but the results did not suggest that either of these distributions were normally or lognormally distributed. The ratio of axilla surface area to the total body surface area calculated from both the Gehan and George and Costeff models (Fig. 2) were shown to be normally distributed although barely statistically significant. The Spearman Rank correlation coefficient for the axilla surface area versus amount of APDO product applied per axilla showed a weak to moderate correlation (i.e., 0.39 for females and 0.28 for males) but both were statistically significant (p < 0.05).

4. Discussion and conclusion

The current exposure study was able to provide an improved understanding of the distribution of the axilla surface area in females and males that can be used in dermal sensitization QRA for ingredients of personal care products meant for use in the axilla. The median values measured in this study are very different from currently recommended values of 200 cm² for both axillae (Bremmer et al., 2006). Bremmer et al. (2006) states that their recommended value is estimated and that it is based on a single data source supplemented with personal judgment. The measured axilla surface area data determined in this study results in a median surface area for both axillae of 129 cm² for females and 271 cm² for both axillae for males.

Comparing the data for a single axilla, the Bremmer et al., 2006 reported value of 100 cm² is higher than any of the female measurements and represents approximately 10th percentile amount used on a daily basis is a factor of 1.36 larger than the amount applied per application (1.70 g/d, respectively. These data indicate that the 90th percentile amount used on a daily basis is a factor of 1.36 larger than the amount applied per application (1.70 g/d ÷ 1.25 g). There are no published data with which to compare the male product use data.

CTFA recently published the results of a study of females across the United States that included the measured amount of solid APDO product applied per application and amount applied per use day (Loretz et al., 2006) based on a 14 day use study. That study showed a median amount applied per application of 0.45 g with 10th and 90th percentile values of 0.14 and 1.25 g across a wide range of brands and solid product forms. The median value in the CTFA study is very similar to the median amount applied per axilla for invisible solid product (0.42 g) in this study. However the current study has a narrower distribution probably because it involved only one product brand and form whereas the CTFA study included all solid APDO product forms. The CTFA study reports the median amount of APDO product applied per use day as 0.49 g/d with 10th and 90th percentile values of 0.17 and 1.70 g/d, respectively. These data indicate that the 90th percentile amount used on a daily basis is a factor of 1.36 larger than the amount applied per application (1.70 g/d ÷ 1.25 g). There are no published data with which to compare the male product use data.

To use the data from the current axilla measurement study in QRA for solid APDO products, the amount applied per unit surface area of the axillae per day (i.e., mg/cm²/d) needs to be determined. This value is 9.1 mg/cm²/day. All the data used for this exposure measurement and details of the calculation can be found in Table 5. The APDO consumer product exposure value of 9.1 mg/cm²/d

---

**Table 4**

<table>
<thead>
<tr>
<th></th>
<th>Median for female participants with 10th and 90th percentiles</th>
<th>Median for male participants with 10th and 90th percentiles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Axilla surface area (cm²)</td>
<td>64.5 (55.6; 96.8)</td>
<td>135.5 (112.9; 154.8)</td>
</tr>
<tr>
<td>Amount of product applied per axilla (g)</td>
<td>0.45 (0.31; 0.66)</td>
<td>0.52 (0.35; 0.74)</td>
</tr>
<tr>
<td>Amount of product applied per axilla (g)</td>
<td>0.42 (0.30; 0.65)</td>
<td>0.52 (0.35; 0.74)</td>
</tr>
<tr>
<td>Amount of product applied per axilla surface area (g/cm²)</td>
<td>0.0062 (0.0047; 0.0089)</td>
<td>0.0038 (0.0028; 0.0055)</td>
</tr>
</tbody>
</table>

*a* These data are derived from a subset (60 females and 60 males) of a larger company Antiperspirant/Deodorant study as also shown in Tables 2 and 3.

*b* These data are derived from the larger company Antiperspirant/Deodorant study conducted with 141 females and 98 males.
is currently the most robust number to incorporate into an exposure-based QRA of APDO product ingredients identified as potential dermal sensitizers. When conducting such an exposure-based QRA for a specific ingredient, the exposure on per surface area basis can be calculated by multiplying this consumer product exposure value by the fractional concentration of the ingredient in the product.

Throughout this analysis, the choice was to use the 90th percentile of the data to represent the high end user. The choice of the 90th percentile was driven by the type and quality of the data used in the analysis as well as the inherent conservatism of the analysis. For example, the choice to use female data (i.e., amount applied and surface area) to estimate the QRA exposure for males and females is conservative when applied to males even when the difference in product types is accounted for. Similar principles such as evaluating the quality of the raw data and the conservative nature of assumptions made in the analysis were incorporated into the choice of the percentile of exposure data to recommend for assessments in the COLIPA work (Hall et al., 2007).

The overall conclusions—This study represents the only known publicly available study that actually determined the amount of product applied per axilla surface area. The values for male and female axilla surface area have been derived statistically from a large number of participants with a wide range of heights and weights that are representative of the United States population. This enables a more robust surface area value to be available for use in dermal sensitization QRA for consumer products meant to be used on the axillae.

Furthermore, combining these surface area data with measured use data from this and other studies has enabled calculations of consumer exposure to solid APDO products on a dose/unit area/day basis (9.1 mg/cm²/d). This constitutes a significant refinement of the calculation of consumer product exposure, which is necessary for exposure-based risk assessment for APDO products.

Conflict of Interest

The authors declare that they have no conflicts of interest.

References


SCCNFP, 2003. The SCCNFP’s notes of guidance for the testing of cosmetic ingredients and their safety evaluation, 5th Revision. Adopted by the SCCNFP during the 25th plenary meeting of 20 October 2003. SCCNFP/0690/03 Final.


Implementation of the dermal sensitization Quantitative Risk Assessment (QRA) for fragrance ingredients

Anne Marie Api a,*, Matthias Vey b

a Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA
b International Fragrance Association, Brussels, Belgium

Abstract

Significant developments have recently been incorporated in the way dermal sensitization risk assessments are conducted for fragrance ingredients. Based on the RIFM Expert Panel’s recommendation, RIFM and IFRA have formally adopted the QRA approach, refined for fragrance ingredients identified as contact allergens, as the core strategy for primary prevention of dermal sensitization to these materials in consumer products. This new methodology is a major improvement over the former approach because it specifically addresses the elements of exposure-based risk assessment that are unique to the induction of dermal sensitization, while being consistent with the principles of toxicological risk assessment. This methodology will be used to determine global fragrance industry product management practices (IFRA Standards) for potentially sensitizing fragrance ingredients, the first of which was implemented in May 2006 with the 40th Amendment to the IFRA Code of Practice. It contained the first four IFRA Standards based on the QRA, limiting the use of the materials for 11 individual product categories. One of the first four IFRA Standards based on the QRA was on the fragrance material citral. The basis for the acceptable exposure limits are presented in this paper.

1. Introduction

Significant developments have recently been incorporated in the way dermal sensitization risk assessments are conducted for fragrance ingredients. This, in turn, has had a significant impact on the way that IFRA ingredient use restrictions (IFRA Standards) based on dermal sensitization are implemented in the future. For more than a year an expert group, which included representatives from the fragrance and consumer products industries and RIFM, worked on refining this risk assessment methodology for fragrance ingredients. The details about the method for use with fragrance ingredients are explained in Api et al. (2008).

The previous approach for dermal sensitization used by IFRA was qualitative and not an exposure-based risk assessment. This QRA methodology is a major improvement over the former approach because (1) it is consistent with the principles of toxicological risk assessment; (2) can be applied to dermal sensitization since this is a threshold phenomenon and (3) addresses factors that are specific to dermal sensitization. The risk management strategies used in the past by IFRA for fragrance ingredients identified as an allergen limited the use of the fragrance ingredient to the same concentration across all product types that involved skin contact. In the new QRA approach there are 10 different product categories for skin contact products. Category 11 is designated for non-skin or incidental skin contact products. Since exposure is a key element of category determination, this enables maintenance of relevant exposure and therefore safety, while broadening the set of individual limitations and as a side effect also provides greater flexibility to the perfumer because the limit is no longer the same across all skin contact applications. This means that, in some product applications, a higher fragrance ingredient concentration will be possible, while in others, a lower level may be specified, compared to what has been used in the past.

In brief, the key steps of the dermal sensitization QRA process for fragrance ingredients are:

- determination of No Expected Sensitization Induction Level or NESIL;
- application of Sensitization Assessment Factors (SAF) and Calculation of Consumer Exposure (CEL) through product use.

Using these parameters, an Acceptable Exposure Level (AEL) can be calculated and compared with the Consumer Exposure Level (CEL). The ratio of the AEL to CEL must be favorable to support the safe use of the skin sensitizer. This ratio must be calculated for each fragrance ingredient identified as a potential skin sensitizer in each product type. For more details, see Api, 2006; Api et al., 2006, 2008; McNamee, 2006.
2. Definition of IFRA QRA product categories

In the old approach all product types were categorized into two groups—skin contact and non-skin contact products. It is no longer considered sufficient to apply these two groups to the new QRA approach. In the new approach in excess of 50 product types were considered. Since this is such a large number, it is not practical or even desirable to set IFRA Standards based on dermal sensitization for every individual product type. A realistic application of the recommended QRA approach for fragrance ingredients is to use multiple product categories for the implementation of IFRA Standards. This is achieved by grouping consumer product types according to key parameters identified within the QRA approach. These parameters are SAFs and consumer product exposure, which when combined, lead to similar acceptable use levels of a fragrance ingredient. (For a detailed description of the SAFs and the rationale used to determine SAFs see Api et al., 2008) Table 1 provides an illustration of how similar SAFs and consumer product exposures were combined to create the IFRA QRA product categories, using categories 3, 4 and 5 as examples. Using these parameters, Table 2 outlines 11 different IFRA categories for dermal sensitization. For many categories it may appear that there is a wide diversity of product types. However, this is because the categories are based on scientific rationale (SAF and consumer product exposure), and not on the functional similarity of each product type. In cases, where a product is not currently categorized and where the likely consumer product exposure is clearly different or where the matrix may indicate a higher degree of potential penetration or irritation, then it is incumbent on the fragrance supplier to contact the IFRA secretariat (secretariat@ifraorg.org) for advice on appropriate product categorization. This would lead to a modification of the industry guidance available in form of a booklet on the RIFM as well the IFRA websites. In those cases the IFRA membership and stakeholders would be adequately informed about the change(s).

There are several key considerations regarding the product types and categories that must be noted:

- The QRA addresses the protection of human health and is specifically aimed at ideally eliminating the acquisition of dermal sensitization to fragrance ingredients under their conditions of use. The fragrance industry QRA approach defined for dermal sensitization to fragrance ingredients under their conditions of use.
- The products described are all retail consumer products.
- Product types are placed into IFRA product categories on the basis of grouping consumer product types according to key parameters identified within the QRA approach. These parameters are Sensitization Assessment Factors (SAFs) and consumer product exposure, which when combined, lead to similar acceptable use levels of a fragrance ingredient. It is not possible to list every conceivable type of product in the industry guidance document (booklet). Several product types have been placed in specific IFRA categories even in the absence of exposure data by taking into account how the product is used, what it contains and the extent of likely skin exposure. However, should consumer product exposure data become available; these product types may be re-categorized. Also, if additional relevant exposure data become available on any product type, this may also result in re-categorization of the product type. If you are aware of a product type that is not categorized, please contact the IFRA Secretariat (secretariat@ifraorg.org).
- Aerosols:
  - Pressurized aerosols: When calculating fragrance ingredient concentration in pressurized aerosols, to determine compliance with an IFRA Standard (determining the concentration reaching the skin), the propellant should be discounted because it flashes off very rapidly. The basis for the calculation should be the active solution or the mixture of the fragrance compound (fragrance mixture or fragrance oil) and other excipients (e.g. water, ethanol, active components).
  - Aerosol skin contact: Skin contact from aerosol products (e.g. aerosol air freshener) as defined in Category 9 relates to those aerosol products that are not intended for skin contact, but their use may result in skin contact. This excludes deodorant/antiperspirants, hair styling aids and sprays, which are part of other categories.
- After sun creams and self-tanning Products: After sun and sunless tanning products are not addressed separately, but are included in the major product types (e.g. facial cream, body cream) in line with other sun care products. Products used on mildly sunburned skin are also expected to fit into the major product categories without amendment to their QRA which is already sufficiently conservative. Use of products for severely sunburned skin could constitute a different exposure scenario, but since this borders on needing professional medical advice for treatment, this is considered to be outside the scope of this QRA activity.

### Table 1

An example of constructing IFRA categories

<table>
<thead>
<tr>
<th>Product types in IFRA product category</th>
<th>Inter-individual SAF</th>
<th>Matrix SAF</th>
<th>Use SAF</th>
<th>Overall SAF</th>
<th>Exposure(^a) mg/cm(^2)/day</th>
<th>Citral (NESIL = 1400)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroalcoholic products applied to recently shaved skin</td>
<td>10</td>
<td>3</td>
<td>10</td>
<td>300</td>
<td>2.2</td>
<td>0.2%</td>
</tr>
<tr>
<td>Eye products of all types (eye shadow, mascara, eyeliner, eye make-up, etc.)</td>
<td>10</td>
<td>3</td>
<td>10</td>
<td>300</td>
<td>(CTFA, 2005a) 2.17</td>
<td>0.2%</td>
</tr>
<tr>
<td>Men's facial creams and balms</td>
<td>10</td>
<td>3</td>
<td>10</td>
<td>300</td>
<td>(EC, 1996) 2.06</td>
<td>0.2%</td>
</tr>
<tr>
<td>Tampons</td>
<td>10</td>
<td>1</td>
<td>20</td>
<td>200</td>
<td>(RIFM, 2006) 2.9</td>
<td>0.2%</td>
</tr>
<tr>
<td>Category 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroalcoholic products applied to unshaved skin</td>
<td>10</td>
<td>3</td>
<td>3</td>
<td>100</td>
<td>(CTFA) 2.21</td>
<td>0.6%</td>
</tr>
<tr>
<td>Hair styling aids, hair sprays of all types (pumps, aerosol sprays, etc.)</td>
<td>10</td>
<td>3</td>
<td>3</td>
<td>100</td>
<td>(Loretz et al., 2006) 2.20</td>
<td>0.6%</td>
</tr>
<tr>
<td>Body creams</td>
<td>10</td>
<td>3</td>
<td>10</td>
<td>300</td>
<td>(Colipa, 2005) 0.6</td>
<td>0.8%</td>
</tr>
<tr>
<td>Category 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women's facial creams/facial make-up</td>
<td>10</td>
<td>3</td>
<td>3</td>
<td>100</td>
<td>(CTFA, 2002) 3.17</td>
<td>0.5%</td>
</tr>
<tr>
<td>Hand cream</td>
<td>10</td>
<td>3</td>
<td>3</td>
<td>100</td>
<td>(Colipa, 2005) 4.2</td>
<td>0.3%</td>
</tr>
</tbody>
</table>

\(^a\) Source of exposure data.
Table 2
SAF and product type consumer exposure levels that drive the IFRA QRA Category

<table>
<thead>
<tr>
<th>IFRA QRA Category</th>
<th>SAF</th>
<th>Category consumer exposurea(mg/cm²/day)</th>
<th>Product type that drives the category consumer exposure level</th>
<th>Maximum Pragmatic Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category 1</td>
<td>300</td>
<td>11.7</td>
<td>Lip products</td>
<td>Acceptable Exposure Level derived from QRA</td>
</tr>
<tr>
<td>Category 2</td>
<td>300</td>
<td>9.0</td>
<td>Deodorants/Antiperspirants</td>
<td>Acceptable Exposure Level derived from QRA</td>
</tr>
<tr>
<td>Category 3</td>
<td>300</td>
<td>2.2</td>
<td>Hydroalcoholics for unshaved skin</td>
<td>Acceptable Exposure Level derived from QRA</td>
</tr>
<tr>
<td>Category 4</td>
<td>100</td>
<td>2.2</td>
<td>Hydroalcoholics for shaved skin</td>
<td>Acceptable Exposure Level derived from QRA</td>
</tr>
<tr>
<td>Category 5</td>
<td>100</td>
<td>4.2</td>
<td>Hand cream</td>
<td>Acceptable Exposure Level derived from QRA</td>
</tr>
<tr>
<td>Category 6</td>
<td>100</td>
<td>1.4</td>
<td>Mouthwash</td>
<td>Acceptable Exposure Level derived from QRA</td>
</tr>
<tr>
<td>Category 7</td>
<td>300</td>
<td>4.4</td>
<td>Intimate wipes</td>
<td>Acceptable Exposure Level derived from QRA</td>
</tr>
<tr>
<td>Category 8</td>
<td>100</td>
<td>1.0</td>
<td>Hair styling aids</td>
<td>2% The maximum concentration will not exceed 2% and may be lower if determined by the QRA</td>
</tr>
<tr>
<td>Category 9</td>
<td>100</td>
<td>0.2</td>
<td>Rinse-off hair condition</td>
<td>5% The maximum concentration will not exceed 5% and may be lower if determined by the QRA</td>
</tr>
<tr>
<td>Category 10</td>
<td>100</td>
<td>0.1</td>
<td>Hard surface cleaners</td>
<td>2.5% The maximum concentration will not exceed 2.5% and may be lower if determined by the QRA</td>
</tr>
<tr>
<td>Category 11</td>
<td>10</td>
<td>0.00033</td>
<td>Candles</td>
<td>These products result in negligible skin contact. The approach for a pragmatic concentration of fragrance ingredient in this category is explained in the notes section and below in the Frequently Asked Questions section</td>
</tr>
</tbody>
</table>

a The category consumer exposure Level (mg/cm²/day) is driven by the product type in that category with the combined highest consumer exposure level and highest Sensitization Assessment Factor (SAF). In order to identify the product type consumer exposure that drives the category consumer exposure please refer to the Technical Dossier, Table 9.

- **Baby products**: The categorization of baby shampoos and washes includes the assumption that the dose/unit area is similar to this value for adults (i.e., for babies, less product used over a smaller surface area). Should specific exposure and surface area data for babies become available, these product types may be re-categorized.

- **Children’s toys**: This product type has been placed in Category 1 based on the absence of exposure data. Should exposure data become available, these product types may be re-categorized.

Due to the possibility of ingestion of small amounts of fragrance ingredients (if oral exposure is foreseeable), materials present in the fragrance compound for use in this toy category must be approved for use in food, meaning that all ingredients should be listed as having “no safety concern”, for example by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and/or as Generally Recognized As Safe (GRAS) in accordance with the US Federal Food, Drug and Cosmetic Act.

- **Dental Products**
  - **Toothpaste and mouthwash products**: With the implementation of the QRA approach, the IFRA Standards will include oral care products. Mouthwash and toothpastes are the principal oral care products currently identified in IFRA Category 6. Exposure limits for these products are established to reduce the risk of peri-oral dermal sensitization and as such, are not related to considerations of safe levels for ingestion. The safety of flavor/fragrance ingredients present in products intended to be orally ingested is outside the scope of IFRA’s risk assessment process. In the latter cases, salivary dilution and short/variable contact time in the oral cavity would suggest a different risk assessment approach for ingested flavor/fragrance substances. The aspect of safety through ingestion is managed by the International Organization of Flavor Industries (IOFI, see its Code of Practice). Due to the possibility of ingestion of small amounts of fragrance ingredients, materials present in the fragrance compound for use in this category must be approved for use in food, meaning that all ingredients should be listed as having “no safety concern”, for example by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and/or as Generally Recognized As Safe (GRAS) in accordance with the US Federal Food, Drug and Cosmetic Act.

Existing IFRA Standards (not based on the QRA) will not be applied to these oral care product types in IFRA Category 6. As the QRA approach for fragrance ingredient dermal sensitizers is implemented, then maximum use levels of these ingredients in toothpaste and mouthwash products will be introduced through definition of new or revised IFRA Standards.

- **Denture adhesives and tooth whiteners**: These are regulated globally as medical devices. Since medical device regulations include separate safety assessment guidelines, these product types are not included in the IFRA categorization based on the QRA approach.

- **Diapers, feminine hygiene pads, liners and tampons**: As with all other product types, levels of fragrance ingredients in diapers and feminine hygiene products are being based on the final product. For clarification, the final products here are the diaper, feminine hygiene pad or liner or tampon. It is recognized that products such as these involve special considerations because the fragrance mixture or compound is included in the final product based on weight rather than percent concentration. A re-categorization of these product types may be necessary as additional understanding of these special considerations as they relate to the expression of IFRA Standards is further developed.

- **Non-skin contact or incidental skin contact products**: Most of the non-skin contact or incidental skin contact products (as defined in the Code of Practice) are included in Category 11. Due to the expected insignificant skin exposure from such products the risk of induction of dermal sensitization through the normal formulation and use of such products is considered to be negligible. As such, the concentration of fragrance ingredient should not exceed the concentration of the fragrance compound that is stipulated in the fragrance brief for the finished product. For example, if the concentration of the fragrance compound in the final product is at 20%, then any individual fragrance ingredient within the compound would not exceed 20% of the final product. An example of this is further given in the context of the practical example (citral).

The differentiation as defined in the Code of Practice between non-skin contact products and skin contact products will remain until all existing sensitization Standards are transferred into Standards based on the QRA.
<table>
<thead>
<tr>
<th>Category</th>
<th>Product type</th>
<th>Maximum Pragmatic Level</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category 1</td>
<td>Lip products of all types (solid and liquid lipsticks, balms, clear or colored, etc.)</td>
<td>Acceptable Exposure Level derived from QRA</td>
<td>Products that contain sunscreen or sun-block are not listed separately and are included in the major product type (e.g. lip creams containing sunscreen are included in the lip products category). Due to the possibility of ingestion of small amounts of fragrance ingredients, materials present in the fragrance compound for use in this category must be approved for use in food, meaning that all ingredients should be listed as having “no safety concern”, for example by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and/or as Generally Recognized As Safe (GRAS) in accordance with the US Federal Food, Drug and Cosmetic Act.</td>
</tr>
<tr>
<td></td>
<td>Toys</td>
<td></td>
<td>This product type has been placed in Category 1 based on the absence of exposure data. Should exposure data become available, these product types may be re-categorized. Due to the possibility of ingestion of small amounts of fragrance ingredients (if oral exposure is foreseeable), materials present in the fragrance compound for use in this toy category must be approved for use in food, meaning that all ingredients should be listed as having “no safety concern”, for example by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and/or as Generally Recognized As Safe (GRAS) in accordance with the US Federal Food, Drug and Cosmetic Act.</td>
</tr>
<tr>
<td>Category 2</td>
<td>Deodorant and antiperspirant products of all types (spray, stick, roll-on, under-arm and body, etc.)</td>
<td>Acceptable Exposure Level derived from QRA</td>
<td></td>
</tr>
<tr>
<td>Category 3</td>
<td>Hydroalcoholic products applied to recently shaved skin</td>
<td>Acceptable Exposure Level derived from QRA</td>
<td>Products that contain sunscreen or sun-block are not listed separately and are included in the major product type (e.g. lip creams containing sunscreen are included in the lip products category).</td>
</tr>
<tr>
<td></td>
<td>Eye products of all types (eye shadow, mascara, eyeliner, eye make-up, etc.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Men's facial creams, balms</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tampons</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Category 4</td>
<td>Hydroalcoholic products applied to unshaved skin</td>
<td>Acceptable Exposure Level derived from QRA</td>
<td>Products that contain sunscreen or sun-block are not listed separately and are included in the major product type (e.g. lip creams containing sunscreen are included in the lip products category).</td>
</tr>
<tr>
<td></td>
<td>Hair styling aids, hair sprays of all types (pumps, aerosol sprays, etc.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Body creams, oils, lotions, fragrancing creams of all types (including baby creams, lotions, oils)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ingredients of perfume kits</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fragrance compounds for cosmetic kits</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Scent strips for Hydroalcoholic products, “scratch and sniff” samples, other paper products not mentioned elsewhere for which skin exposure is only incidental (e.g. spectacle cleaning tissues)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Category 5</td>
<td>Women's facial creams/Facial make-up</td>
<td>Acceptable Exposure Level derived from QRA</td>
<td>These product types have been placed in Category 4 based on the absence of exposure data, but it is recognized that these products have similarities to hydroalcoholic products applied to unshaved skin. Should exposure data become available, these product types may be re-categorized.</td>
</tr>
<tr>
<td></td>
<td>Hand cream</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Facial masks</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wipes or refreshing tissues for face, neck, hands, body</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Category 6</td>
<td></td>
<td>Acceptable Exposure Level derived from QRA</td>
<td>These product types have been placed in Category 4 based on the absence of exposure data, but it is recognized that this product is similar to hair styling aids and hair sprays. Should exposure data become available, this product type may be re-categorized.</td>
</tr>
<tr>
<td>Product type</td>
<td>Maximum Pragmatic Level</td>
<td>Comments</td>
<td></td>
</tr>
<tr>
<td>-------------</td>
<td>-------------------------</td>
<td>----------</td>
<td></td>
</tr>
<tr>
<td><strong>Mouthwash</strong></td>
<td></td>
<td>Toothpaste and mouthwash products: With the implementation of the QRA approach, the IFRA Standards will include oral care products. Mouthwash and toothpastes are the principal oral care products currently identified in IFRA Category 6. Exposure limits for these products are established to reduce the risk of peri-oral skin sensitization and as such, are not related to considerations of safe levels for ingestion. The safety of flavor/fragrance ingredients present in products intended to be orally ingested is outside the scope of IFRA’s risk assessment process. In the latter cases, salivary dilution and short/variable contact time in the oral cavity would suggest a different risk assessment approach for ingested flavor/fragrance substances. The aspect of safety through ingestion is managed by the International Organization of Flavor Industries (IOFI, see its Code of Practice).</td>
<td></td>
</tr>
<tr>
<td><strong>Toothpaste</strong></td>
<td></td>
<td>Due to the possibility of ingestion of small amounts of fragrance ingredients, materials present in the fragrance compound for use in this category must be approved for use in food, meaning that all ingredients should be listed as having “no safety concern”, for example by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and/or as Generally Recognized As Safe (GRAS) in accordance with the US Federal Food, Drug and Cosmetic Act. Existing IFRA Standards will not be applied to these oral care product types in IFRA Category 6. As the QRA approach for fragrance ingredient dermal sensitizers is implemented, then maximum use levels of these ingredients in toothpaste and mouthwash products will be introduced through definition of new or revised IFRA Standards.</td>
<td></td>
</tr>
<tr>
<td><strong>Category 7</strong></td>
<td></td>
<td>Acceptable Exposure Level derived from QRA</td>
<td></td>
</tr>
<tr>
<td>Intimate wipes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baby wipes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insect repellent (intended to be applied to the skin)</td>
<td>2%</td>
<td>The maximum concentration will not exceed 2% and may be lower if determined by the QRA.</td>
<td></td>
</tr>
<tr>
<td><strong>Category 8</strong></td>
<td></td>
<td>These product types have been placed in Category 8 based on the absence of exposure data, but it is recognized that the exposure would be similar to body creams, lotions. Although the exposure is expected to be similar to body creams, lotions, the overall SAF for powders and talcs is, however, lower and so these products are placed into a different category compared to body creams, lotions. Should exposure data become available, these product types may be re-categorized.</td>
<td></td>
</tr>
<tr>
<td>Make-up removers of all types (not including face cleansers)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hair styling aids non-spray of all types (mousse, gels, leave-in conditioners, etc.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nail care</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All powders and talcs (including baby powders and talcs)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Category 9</strong></td>
<td></td>
<td>5% The maximum concentration will not exceed 5% and may be lower if determined by the QRA.</td>
<td></td>
</tr>
<tr>
<td>Conditioner (rinse-off)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liquid soap</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shampoos of all types (including baby shampoos)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Face cleansers of all types (washes, gels, scrubs, etc.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shaving creams of all types (stick, gels, foams, etc.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depilatory</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body washes of all types (including baby washes) and Shower Gels of all types</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bar soap (Toilet soap)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feminine hygiene—pads</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feminine hygiene—liners</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bath gels, foams, mousse, salts, oils and other products added to Bathwater</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other aerosols (including air fresheners sprays but not including deodorant/antiperspirants, hair styling aids spray)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Category 10</strong></td>
<td>2.5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(continued on next page)</td>
<td></td>
</tr>
</tbody>
</table>
Sunscreens: Products that contain sunscreen or sun-block are not listed separately but are included in the major product type (e.g., lip creams containing sunscreen are included in the lip products category).

Table 2 provides the SAF and product type consumer exposure levels that drive the IFRA QRA category. These data are used with the NESIL to calculate the acceptable exposure levels to individual fragrance ingredients. Table 3 gives the 11 IFRA QRA categories for dermal sensitization based on the QRA approach. It also gives detailed comments for specific product types. It should be noted that both Tables 2 and 3 contain a column which defines a “Maximum Pragmatic Level”. Practical considerations require setting a default maximum level of the fragrance ingredients identified as dermal sensitizers for some product types. This pragmatic level is defined as that “not exceeding the usual concentration of the fragrance compound in the finished product”. If the Acceptable Exposure Level (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 will take precedence and be applied. IFRA and RIFM will determine whether the AEL or the “Maximum Pragmatic Level” should be applied. The appropriate value will be given in the IFRA Standard.

3. Practical application of the QRA approach for fragrance ingredients: Citral

Citral (Fig. 1) has been chosen as an example to demonstrate the practical application of the principles of QRA. This material is one of the four fragrance ingredients that were part of the 40th Amendment to the IFRA Code of Practice for which Standards have been set based on the QRA approach. The dermal sensitization data on citral include the availability of robust animal sensitization data,

### Table 3 (continued)

<table>
<thead>
<tr>
<th>Product type</th>
<th>Maximum Pragmatic Level</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Handwash laundry detergents of all types</td>
<td>The maximum concentration will not exceed 2.5% and may be lower if determined by the QRA.</td>
<td></td>
</tr>
<tr>
<td>Fabric Softeners of all types including fabric softener sheets</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other household cleaning products (fabric cleaners, soft surface cleaners, carpet cleaners,)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Machine wash laundry detergents (liquids, powders, tablets, etc.) including laundry bleaches</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hand dishwashing detergent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hard surface cleaners of all types (bathroom and kitchen cleansers, furniture polish)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diapers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shampoos for pets</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry cleaning kits</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toilet seat wipes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Category 11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All non-skin contact or incidental skin contact.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Including:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Air fresheners and fragancing of all types (plug-ins, solid substrate, membrane delivery, electrical, pot pourri, powders, fragancing sachets, incense, liquid refills)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animal sprays</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cat litter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deodorizers/Maskers not intended for skin contact (e.g. fabric drying machine deodorizers, carpet powders)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Floor wax</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fuels</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insecticides (e.g. mosquito coil, paper, electrical, for clothing)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Joss sticks or incense sticks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Machine dishwash detergent and deodorizers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Machine only laundry detergent (e.g. liquitabs)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Odored distilled water (that can be added to steam irons)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paints</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plastic articles (excluding toys)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoe polishes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toilet blocks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated textiles (e.g. starch sprays, fabric treated with fragrances after wash, deodorizers for textiles or fabrics, tight with moisturizers)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

It was assumed that the exposure to humans from shampoos for pets could be expected to be similar to hand dishwashing liquids.

This product type has been placed in Category 10 based on the absence of exposure data, but it is recognized that this product is similar to fabric softener sheets. Should exposure data become available, this product type may be re-categorized.

This product type has been placed in Category 10 based on the absence of exposure data, but it is recognized that this product is similar to hard surface cleaner. Should exposure data become available, this product type may be re-categorized.

Due to the expected negligible skin exposure from such products the risk of induction of dermal sensitization through the normal formulation and use of such products is considered to be negligible. As such, the concentration of fragrance ingredient is not restricted in the finished product.
confirmatory human sensitization data as well as diagnostic patch test studies. Table 4 provides a summary of the data used to generate the Weight of Evidence (WoE) No Expected Sensitization Induction Level (NESIL). The summary table is based on the detailed data provided in Lalko and Api (2008) and is presented here for convenience of the reader. The LLNA EC3 value is given as a weighted mean value. The weighted mean EC3 value is the average of the mean EC3 values from studies performed in the same vehicle.

Table 5 provides all the summary data used in the risk characterization of citral in two arbitrarily chosen product types. This table demonstrates how the data are used to determine acceptable levels of use in the two product types, a hydroalcoholic product for unshaved skin and a solid antiperspirant. The first product type is one that defines IFRA Product Category 4 and the second defines IFRA Product Category 2. Using these two product types, this table provides a step-by-step illustration of how the acceptable exposure is calculated.

Table 6 shows the practical application of the dermal sensitization QRA approach for fragrance ingredients in the 11 IFRA QRA categories. It lists the acceptable levels for citral in each IFRA QRA category.

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, RIFM sponsored a survey of the patch test database at the Contact Allergy Unit, University Hospital Leuven, Belgium on citral.

Prior to the IFRA Standard on citral the reported average maximum concentration of citral in hydroalcoholic products was 1.7%. The IFRA Standard based on the QRA approach limits the use in this product type to 0.6%. The data from the patch test database at the Contact Allergy Unit, University Hospital Leuven, Belgium on citral supports that citral was being used at a level that exceeded the acceptable exposure concentration for hydroalcohols since the only reactions observed were to toilet water/perfume products. It is interesting to note that reactions to other product types, such as deodorants/antiperspirants were not observed in this database.

### Table 4

<table>
<thead>
<tr>
<th>Fragrance material</th>
<th>CAS No.</th>
<th>LLNA weighted mean EC3 values (µg/cm²)</th>
<th>Potency classification based on animal data</th>
<th>Human Data NOEL-HRIPT (induction) (µg/cm²)</th>
<th>NOEL–MAX (induction) (µg/cm²)</th>
<th>LOELb (induction) (µg/cm²)</th>
<th>WoE NESILc (µg/cm²)</th>
</tr>
</thead>
</table>

**Notes:**
- NOEL, No observed effect level; HRIPT, Human Repeat Insult Patch Test; MAX, Human Maximization Test; LOEL, lowest observed effect level.
- b Data derived from HRIPT or HMT.
- c WoE NESIL limited to three significant figures.

### Table 5

<table>
<thead>
<tr>
<th>Citral</th>
<th>Hydroalcoholic product for unshaved skin</th>
<th>solid antiperspirant</th>
</tr>
</thead>
<tbody>
<tr>
<td>WoE NESIL (from Table 4)</td>
<td>1400 µg/cm²</td>
<td>1400 µg/cm²</td>
</tr>
<tr>
<td>Sensitization Assessment Factor (SAF)</td>
<td>100 (10 × 3 × 3)</td>
<td>300 (10 × 3 × 10)</td>
</tr>
<tr>
<td>Acceptable Exposure Level (AEL)</td>
<td>14.0 µg/cm²</td>
<td>4.7 µg/cm²</td>
</tr>
<tr>
<td>Consumer Exposure Level (CEL) Product</td>
<td>2.2 mg/cm²/day</td>
<td>9.1 mg/cm²/day</td>
</tr>
<tr>
<td>AEL/CEL</td>
<td>AEL/CEL (14.0 µg/cm² × 0.001 mg/µg) ÷ 2.2 mg/cm²/ day = 0.0064</td>
<td>AEL/CEL (4.7 µg/cm² × 0.001 mg/µg) ÷ 9.1 mg/cm²/ (day) = 0.0005</td>
</tr>
<tr>
<td>Concentration of citral in the product giving AEL ≤ CEL</td>
<td>≤0.64%</td>
<td>≤0.05%</td>
</tr>
</tbody>
</table>

**Notes:**
- b Cowan-Ellsberry et al., 2008.
The IFRA survey showed that the use of citral in these product types was at or below the acceptable exposure level determined by the QRA.

4. Concluding remarks

In the future, the dermal sensitization QRA for fragrance ingredients will be used to establish new IFRA Standards for all fragrance ingredients that are potential dermal sensitizers. The prioritization for assessment will be based on criteria outlined in the RIFM human health criteria document (Ford et al., 2000) such as volume of use, dermal exposure and structural alerts for dermal sensitization. There is still a small group of materials that have existing IFRA Standards based on dermal sensitization, which are still using the previous two category approach. Additional data are being obtained on these materials and the implementation of the QRA in the IFRA Standards for these materials will occur in the near future.

As part of the overall objective of IFRA and RIFM to minimize fragrance allergy in the general population, a key goal is to review by 2011 all chemically defined fragrance ingredients that have structural alerts for dermal sensitization and used at greater than 1 metric ton per year on a worldwide basis. In addition, refinement of the method will continue. Updating the categorization of product types including new product types, improved exposure data and the inclusion of cosmetic products used in an occupational/professional environment are important refinements to be considered.

It is important that dermal sensitization QRA for fragrance ingredients will 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.

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.

Funding Source

This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

References


Cano, M.-F., 2006. Personal communication on exposure data for hydroalcoholic products for shaved skin.


Research Institute for Fragrance Materials Inc. (RIFM), 2005a. Memo to A.M. Api from RIFM Member Company, May 2006 on exposure to feminine hygiene products, diapers, intimate wipes and baby wipes.

Citral: Identifying a threshold for induction of dermal sensitization

Jon Lalko *, Anne Marie Api

Human Health, Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

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Abstract

Citral [CAS# 5392-40-5; EINECS# 226-394-6; RIFM # 116; cis- and trans-3,7-dimethyl-2,6-Octadienal] is an important fragrance ingredient appreciated for its powerful lemon-aroma. It is widely used in fragrance formulations and incorporated into numerous consumer products. A comprehensive review of the dermal sensitization data available for citral was undertaken with the goal of identifying a threshold for the induction of dermal sensitization. In 2007, a complete literature search was conducted. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, the toxicologic database of the Research Institute for Fragrance materials, Inc. (RIFM) was searched, which includes numerous unpublished reports. Based on a weight of evidence approach, the data from this survey demonstrate that the human NOEL (No Observed Effect Level) for induction of dermal sensitization to citral is 1400 µg/cm². The identification of this induction threshold will allow for risk assessments to focus on primary prevention of contact allergy to citral based on a new Quantitative Risk Assessment (QRA) paradigm. This subsequent assessment will form the basis of a risk management approach; specifically a new IFRA (International Fragrance Association) standard on the use of citral in consumer products.

Keywords: Citral; Dermal sensitization; Threshold; Induction; Review

1. Introduction

Citral [CAS# 5392-40-5; EINECS# 226-394-6; RIFM # 116; cis- and trans-3,7-dimethyl-2,6-Octadienal] is an important fragrance ingredient appreciated for its powerful lemon-aroma and is widely used in fragrance formulations in numerous consumer products (Arctander, 1969). Citral is a common component of many essential oils, such as lemongrass and litsea cubeba oils (Lawrence, 2003). It may be produced synthetically or obtained from natural oils (Bedoukian, 1967). Citral is generally recognized as a safe food additive and has been approved by the Food and Drug Administration for use in foods (FDA, GRAS, 21 CFR 182.60). The International Fragrance Association (IFRA) has issued a standard on the use of citral in fragrance formulations based on its allergenic potential (IFRA, 1980). Citral is one of 26 fragrance materials identified as a suspected cause of allergic contact dermatitis by the European Commissions advisory committee—the Scientific Committee for Cosmetic and Non-Food Products (SCCNFP—currently known as the Scientific Committee on Consumer Products [SCCP]). In 2003 Directive, 2003/15/EC, the 7th amendment of the European Cosmetic Directive 76/768/EEC was published based on the recommendations of the SCCNFP. Within the European Union, the directive requires that citral be listed on the ingredient label of consumer products when present at greater than 10 ppm for leave-on cosmetic products and 100 ppm for rinse-off cosmetics. The Member States and the European Parliament adopted the above concentrations as a pragmatic approach to labeling ingredients. Little is known about the dose–response relationship between induction and elicitation of contact allergy. The labeling requirement will provide a degree of secondary prevention by assisting consumers and physicians in avoidance of materials demonstrated or suspected of eliciting an individual contact dermatitis. There still remains a need to conduct accurate
risk assessments to prevent the induction of sensitization to these materials—primary prevention.

A review of all safety data on citral was first published in 1979 by Opdyke. Since that time, a significant number of studies investigating the sensitization potential of citral have been conducted. This paper provides a comprehensive review of the dermal sensitization data available on citral. A review is necessary in order to determine the threshold for induction of sensitization in humans. The identification of an induction threshold will allow for risk assessments to focus on primary prevention of contact allergy to citral based on a new Quantitative Risk Assessment (QRA) paradigm. This subsequent assessment [the details of which are outlined in a paper concurrently submitted with this review (Api et al., 2008)] will form the basis of a new IFRA standard on the use of citral in consumer products.

2. Methods

In 2007, a complete literature search was conducted on citral. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, the toxicologic database of the Research Institute for Fragrance materials, Inc. (RIFM) was searched, which includes numerous unpublished reports. All relevant references are included in this review.

3. Structural analysis

QSAR (Quantitative Structure Activity Relationship) analysis of citral (Fig. 1) reveals structural alerts for potential toxic dermal effects such as sensitization (Ford et al., 2000). Application of the rule based DEREK (Deductive Estimation of Risk from Existing Knowledge) system also identifies citral as a potential contact allergen (Hostyn et al., 1998). In order for a chemical to act as a contact allergen, it must first diffuse from the skin surface to the viable epidermis where it may react with skin proteins to form an immunogen. Citral has a molecular weight of 152.24 Da and a calculated octanol/water partition coefficient, log $K_{O/W}$, of 3.45 suggesting that it would permeate fairly readily through human skin. As a volatile organic, it would be expected that (under non-occlusive conditions) evaporative loss of citral would compete with dermal absorption and result in an exposure less than the applied dose. There is little quantitative information available on the dermal absorption of citral; however, what data is available suggests that citral does penetrate readily through both human and animal skin (Mutalik and Udupa, 2003; Barbier and Beneza, 1983; Meyer and Meyer, 1959). Citral is an $\alpha,\beta$-unsaturated carbonyl and, as such, may react with skin proteins via a 1,4 Michael addition or Schiff base formation to create an immunogen (see Fig. 2) (Smith and Hotchkiss, 2001). Studies investigating peptide reactivity have identified citral as being protein reactive (Gerberick et al., 2004).

4. Available data

4.1. Predictive tests in animals

Citral has been evaluated in a total of 16 guinea pig sensitization studies and 11 murine local lymph node assays (LLNAs). The weight of evidence from these animal predictive assay shows that citral is a weak to moderate sensitizer.

4.1.1. Guinea pig studies (Table 1)

Guinea pig maximization tests according to the Magnusson and Kligman (1969) method were conducted on citral. Induction consisted of a series of 6 intradermal injections with and without FCA of the test material followed 6–8 days later by a 48-h occluded patch. The animals were challenged 12–14 days later by an occluded 24 h patch application. Reactions were read 24 and 48 h after patch removal. The following results were obtained.

Induction was conducted with 0.4% for the intradermal injection and 1% for the occluded patch. Challenge application was conducted with 0.25%. No vehicle was reported. Sensitization reactions were observed in 40% (4/10) of the animals (Goodwin and Johnson, 1985).

Induction was conducted with 0.2% in 4:1 acetone:olive oil for the intradermal injection and 5% in 4:1 acetone:olive oil for the occluded patch. Challenge application was conducted with 0.5% in 4:1 acetone:olive oil. Sensitization reactions were observed in 60% (6/10) of the animals (Basketter et al., 1991).

Induction was conducted with 0.2% in 0.9% saline solution for the intradermal injection and 5% in 70:30 acetone/PEG 400 for the occluded patch. Challenge application was conducted with 0.5% in 70:30 acetone/PEG 400. Sensitization reactions were observed in 50% of the animals (Basketter and Scholes, 1992).

Ishihara et al. (1986a) reported that in a maximization test using 10% citral throughout the induction and challenge period resulted in sensitization effects being observed (no further details were provided).

Induction was conducted with 5% for the intradermal injection and 25% in petrolatum for the occluded patch.

![Fig. 1. Citral [CAS# 5392-40-5; EINECS# 226-394-6] is a mixture of two isomers (1) cis- and (2) trans-3,7-dimethyl-2,6-Octadienal typically present at proportions of 1/3 and 2/3, respectively.](image-url)
Challenge application was conducted with a subirritant concentration in petrolatum. Sensitization reactions were observed (no further details were provided) (Klecak et al., 1977).

Citral was tested in a Modified Buehler Delayed Hypersensitivity Test in guinea pigs (Buehler, 1965; Ritz and Buehler, 1980). Induction consisted of three 6-h closed patch applications to the same clipped site on the dorsal surface with 20% citral in petrolatum. Induction applications were made once a week for 3 weeks. Following a 10–14 day rest, the guinea pigs were challenged with 20% citral in petrolatum. Challenge application was a 6-h occluded patch at a naive skin site. Control animals were challenged at the same time in an identical manner. Reactions were read 24 and 48 h after patch removal. Sensitization was observed in 5/5 animals (RIFM, 1973). Under the same conditions, samples of citral were tested to determine if changes in sample storage conditions would affect the sensitization potential. Sensitization reactions (5/5 animals per test) were observed to samples of citral that had been stored under nitrogen, stored with the addition of (butylated hydroxyanisole) BHA and after oxygen saturation (RIFM, 1973).

Modified Draize sensitization tests were conducted on citral. In these studies conducted by Sharp (1978), induction was via four simultaneous intradermal injections with 0.25% citral. Challenge was conducted 14 days later by an intradermal injection of 0.1% citral into one flank and a topical open application of 20% citral on the contralateral flank. The vehicle utilized throughout the study was not reported. Reactions were scored 24 h after challenge. A second challenge was carried out 7 days later. If no sensitization reactions were observed, the test was repeated. No reactions were observed in 0/10 animals (Sharp, 1978). A second assay was conducted under the same conditions utilizing 1% citral during intradermal induction with 0.4% (intradermal) and 20% (topical) challenge concentrations. Sensitization effects were observed (no further details were reported) (Sharp, 1978).

Klecak et al. (1977) reported on a modified Draize test conducted with 10 intradermal induction applications on alternate days and an intradermal challenge conducted on days 35 and 49. Induction and challenge treatments were made with 0.1% citral in saline. Sensitization effects were observed (no further details were reported).

Citral was tested in a Maguire Delayed Hypersensitivity Test in guinea pigs. Induction consisted of open applications of 8% citral in petrolatum to abraded skin on days 0, 2, 4 and 7 with an intradermal injection of Freund's Complete Adjuvant (FCA) on day 4. Following a 14-day rest period, open challenge applications of 8% citral in petrolatum were made to the naïve, contralateral flank. Reactions to challenge were read at 24, 48 and 72 h. Sensitization was observed in 4/5 animals (RIFM, 1973).
### Table 1

<table>
<thead>
<tr>
<th>Method</th>
<th>Induction concentration</th>
<th>Challenge concentration</th>
<th>Results</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximization</td>
<td>0.4% (intradermal; VNR)</td>
<td>0.25% (VNR)</td>
<td>Sensitization observed</td>
<td>Goodwin and Johnson (1985)</td>
</tr>
<tr>
<td></td>
<td>1% (topical; VNR)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximization</td>
<td>0.2% in 4:1 acetone:olive oil (intradermal)</td>
<td>0.5% in 4:1 acetone:olive oil</td>
<td>Sensitization observed</td>
<td>Baskettet et al. (1991)</td>
</tr>
<tr>
<td></td>
<td>5% in acetone:olive oil (topical)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximization</td>
<td>0.2% in 0.9% saline solution (intradermal)</td>
<td>0.5% in 70:30 acetone/PEG 400</td>
<td>Sensitization observed</td>
<td>Baskettet and Scholes (1992)</td>
</tr>
<tr>
<td></td>
<td>5% in 70:30 acetone/PEG 400</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximization</td>
<td>10% for both intradermal and dermal (VNR)</td>
<td>10% (VNR)</td>
<td>Sensitization observed</td>
<td>Ishihara et al. (1986a)</td>
</tr>
<tr>
<td>Maximization</td>
<td>5% (intradermal; VNR)</td>
<td>Dose reported as a subirritant concentration (VNR)</td>
<td>Sensitization observed</td>
<td>Klecak et al. (1977)</td>
</tr>
<tr>
<td>Buehler</td>
<td>25% in petrolatum</td>
<td>20% in petrolatum</td>
<td>Sensitization observed</td>
<td>RIFM (1973)</td>
</tr>
<tr>
<td>Draiize</td>
<td>0.25% (VNR)</td>
<td>0.1% (intradermal injection, VNR)</td>
<td>Sensitization observed</td>
<td>Sharp (1978)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20% (Topical, VNR)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Draiize</td>
<td>1% (VNR)</td>
<td>0.4% (intradermal injection, VNR)</td>
<td>Sensitization observed</td>
<td>Sharp (1978)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20% (Topical, VNR)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maguire</td>
<td>0.1% in saline</td>
<td>0.1% in saline</td>
<td>Sensitization observed</td>
<td>Klecak et al. (1977)</td>
</tr>
<tr>
<td>Maguire</td>
<td>8% in petrolatum</td>
<td>8% in petrolatum</td>
<td>Sensitization observed</td>
<td>RIFM (1974a)</td>
</tr>
<tr>
<td>Maguire</td>
<td>8% in petrolatum</td>
<td>8% in petrolatum</td>
<td>Sensitization observed</td>
<td>RIFM (1974a)</td>
</tr>
<tr>
<td>FCAT</td>
<td>50% citral in FCA</td>
<td>Dose reported as a subirritant concentration in petrolatum</td>
<td>Sensitization observed</td>
<td>Klecak et al. (1977)</td>
</tr>
<tr>
<td>OET</td>
<td>10% citral (VNR)</td>
<td>1% (VNR)</td>
<td>Sensitization observed</td>
<td>Klecak et al. (1977)</td>
</tr>
<tr>
<td>CET</td>
<td>3% citral (VNR)</td>
<td>1% Citral (VNR)</td>
<td>Sensitization observed</td>
<td>Ishihara et al. (1986a)</td>
</tr>
<tr>
<td>SIAT</td>
<td>0.4% in FCA</td>
<td>0.5% (VNR)</td>
<td>Sensitization observed</td>
<td>Goodwin and Johnson (1985)</td>
</tr>
<tr>
<td>SIAT</td>
<td>0.4% in saline with FCA</td>
<td>0.5% in acetone/PEG</td>
<td>Sensitization observed</td>
<td>RIFM (1982)</td>
</tr>
</tbody>
</table>

**Abbreviations:** Buehler, Buehler delayed hypersensitivity test; CET, Closed Epicutaneous Test; Draiize, modified Draiize test; FCA, Freund’s complete adjuvant; FCAT, Freund’s complete adjuvant test; Maximization, Magnusson and Kligman guinea pig maximization test; Maguire, modified Maguire delayed hypersensitivity test; OET, Open Epicutaneous Test; PEG, polyethylene glycol; SIAT, Single Injection Adjuvant Test; VNR, vehicle not reported.

a Goodwin and Johnson (1985) also reported on a modified SIAT using two, three and four induction applications. Positive responses were reported for all induction regimes.

b The same results were obtained when four separate samples of citral were tested. Each was stored under different conditions.

1972a). In a repeat study, on a separate sample of citral, reactions were observed to 8% citral in petrolatum in 6/8 animals (RIFM, 1974a).

A Freund’s Complete Adjuvant Test (FCAT) in guinea pigs was conducted with citral. Induction was via intradermal injection of 0.1 ml of a 50:50 mix of citral and FCA on days 0, 2, 4, 7, and 9. Challenge was made with a 24-h closed patch with a subirritant concentration of citral in petrolatum on days 21 and 35. Sensitization effects were observed (No further details were provided) (Klecak et al., 1977).

The sensitization potential of citral was evaluated in an Open Epicutaneous Test (OET) in guinea pigs (Klecak et al., 1977). Induction applications were made via 21 daily open applications to an 8 cm² area on the clipped flank with 10% citral. Challenge was conducted on days 21 and 35 via open application to the contralateral flank with 1% citral. Induction and challenge vehicles were not reported. Reactions were read at 24, 48 and 72 h. Sensitization reactions (1/6) were observed.

Citril was evaluated in a Closed Epicutaneous Test (CET) in guinea pigs. Induction was made via 48-h occluded patches three times a week for 2 weeks with 3% citral. Challenge was conducted 14 days later by a 48-h occluded patch with 1% citral. Reactions were read at patch removal and again 24 and 48 h after patch removal. Sensitization reactions (1/6) were observed (Ishihara et al., 1986a).

Goodwin and Johnson (1985) reported on the results of citral when tested in the Single Injection Adjuvant Test (SIAT) along with the results of a modified SIAT. The SIAT was conducted in guinea pigs. Induction was made via a single intradermal injection with 0.4% citral in Freund’s complete adjuvant. Twelve days later a single 6-h occluded challenge application of 0.5% citral (vehicle not reported) was made to a clipped, shaved flank. A second occluded challenge application was made to the opposite flank 1 week later. Reactions were read 22 and 46 h after patch removal. Sensitization reactions (3/10) were observed.

A modified SIAT was conducted as above with the addition of two, three and four intradermal induction applications. Sensitization reactions were reported for each of the modifications—2/10 following two induction applications, 1/10 following three, and 5/10 following four (Goodwin and Johnson, 1985).

In another SIAT, induction consisted of a single intradermal injection of 0.4% in saline with Freund’s complete adjuvant followed by a challenge with 0.5% citral in 0.5% acetone/PEG. Up to three rechallenges were also conducted. Sensitization reactions were observed (RIFM, 1982).
4.1.2. Local lymph node assays (Table 2)

Citrail has been evaluated in the LLNA following the method of Kimber and Basketter (1992, 1994) as formalized in OECD Guideline 429 and OPPTS Guideline 870.26 (OECD, 2002; NIH, 1999).

Briefly, groups of mice are dosed topically on the dorsum of both ears with test material or vehicle control. Each group receives up to one of five test concentrations. Dosing occurs daily for three consecutive days. On the sixth day after the first application, all mice are injected intravenously via the tail vein with radiolabeled methyl thymidine (3HTdR). Five hours later, the mice are euthanized and the nodule is excised and prepared for scintillation vials. The incorporation of 3HTdR is measured by β-scintillation counting and expressed as disintegrations per minute (dpm) per lymph node for each experimental group or individual animal (which are then averaged by dose group). For each concentration of test material, a stimulation index (SI) relative to the concurrent vehicle-treated control is calculated. A material is considered a sensitizer if at least one concentration of the test material is observed to have an SI value of 3 or more. The EC3 value, a measure of relative potency, can then be derived from the dose–response curve. The EC3 value is the estimated concentration that is required to elicit an SI value of 3 or more.

The LLNAs reported on citral utilized the Ethanol:DEP vehicle system or 4:1 acetone:olive oil as a vehicle. EC3 values in the range of 1.2% to 13% have been reported in these studies. The weighted mean, based on vehicle, of the reported EC3 values for this data set is 5.7% (1414 µg/cm2). Using the potency estimations of Gerberick et al. (2003) citral is classified as a weak sensitizer in the LLNA.

4.2. Non-diagnostic patch test data in humans (Table 3)

The dermal sensitization potential of citral has been evaluated in humans in both the Human Repeated Insult Patch Test (HRIPT) and the Human Maximization Test (HMT). The weight of evidence from these studies show that a No Observed Effect Level (NOEL) of 1400 µg/cm2 exists in the HRIPT when citral was tested in an ethanol vehicle. In all but one HMT, citral induced sensitization in subjects exposed to 4–8% (2759–5517 µg/cm2) citral in petrolatum. The lowest observed effect level (LOEL) was 2759 µg/cm2 citral in petrolatum in the HMT.

The HRIPT is generally performed utilizing a total of nine 24-h occluded applications over 3-weeks with test material and appropriate controls followed by a 2-week rest period. A single 24-h challenge application is then made to a naïve site with the same materials. Observations at challenge coupled with the patterns of reactivity observed during induction provide the basis for an interpretation of contact allergy (Marzulli and Maibach, 1977; McNamee et al., 2008). Citral has been tested in the HRIPT over a range of concentrations. The following results were obtained.

An HRIPT was conducted on 8 female volunteers with 5% citral in alcohol SDA39C. The patches consisted of a 1in2 webril pad with 0.5 ml of test material; which resulted in a dose of 3876 µg/cm2. Sensitization reactions were observed in 5/8 subjects. Four subjects, who reacted during the initial study, were rechallenged approximately 7 months later with both a patch and a single open application behind one ear. Two subjects (2/4) reacted to the patch at rechallenge, no reactions (0/4) were observed following open application (RIFM, 1964a).

No reactions were observed to 1400 µg/cm2 citral in an HRIPT conducted on 101 subjects (30 male and 71 female). The patches consisted of a 25 mm Hill Top Chamber®, corresponding to a dosing area of 2.54 cm2, with 0.3 ml of 1.2% citral in 3:1 DEP:EtOH (RIFM, 2004b).

When 1240 µg/cm2 citral was tested in an HRIPT, no reactions were observed in 50 subjects. The patches con-


Table 3
Human non-diagnostic patch tests with citral

<table>
<thead>
<tr>
<th>Induction dose (µg/cm²)</th>
<th>Test method</th>
<th>Dose volume/Patch area</th>
<th>Test material concentration and vehicle</th>
<th>Incidence of positive responses</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>3876</td>
<td>HRIPT</td>
<td>0.5 ml/6.45 cm²</td>
<td>5% in SDA39C</td>
<td>5/8</td>
<td>RIFM (1964a)</td>
</tr>
<tr>
<td>1400</td>
<td>HRIPT</td>
<td>0.3 ml/2.54 cm²</td>
<td>1.2% in 3:1 DEP:EtOH</td>
<td>0/101</td>
<td>RIFM (2004b)</td>
</tr>
<tr>
<td>1240</td>
<td>HRIPT</td>
<td>0.2 ml/6.45 cm²</td>
<td>4% in petrolatum</td>
<td>0/50</td>
<td>RIFM (1971a)</td>
</tr>
<tr>
<td>775</td>
<td>HRIPT</td>
<td>0.5 ml/6.45 cm²</td>
<td>1.0% in SDA39C</td>
<td>0/40</td>
<td>RIFM (1965)</td>
</tr>
<tr>
<td>388</td>
<td>HRIPT</td>
<td>0.5 ml/6.45 cm²</td>
<td>0.5% in SDA39C</td>
<td>0/41</td>
<td>RIFM (1964b)</td>
</tr>
<tr>
<td>5517</td>
<td>HMT</td>
<td>1 ml/14.5 cm²</td>
<td>8% in petrolatum</td>
<td>8/24</td>
<td>RIFM (1971b)</td>
</tr>
<tr>
<td>3448</td>
<td>HMT</td>
<td>1 ml/14.5 cm²</td>
<td>5% in petrolatum</td>
<td>16/25</td>
<td>RIFM (1974a)</td>
</tr>
<tr>
<td>3448</td>
<td>HMT</td>
<td>1 ml/14.5 cm²</td>
<td>5% in petrolatum</td>
<td>14/25</td>
<td>RIFM (1974c)</td>
</tr>
<tr>
<td>3448</td>
<td>HMT</td>
<td>1 ml/14.5 cm²</td>
<td>5% in petrolatum</td>
<td>12/25</td>
<td>RIFM (1974c)</td>
</tr>
<tr>
<td>3448</td>
<td>HMT</td>
<td>1 ml/14.5 cm²</td>
<td>5% in petrolatum</td>
<td>8/25</td>
<td>RIFM (1974c)</td>
</tr>
<tr>
<td>3448</td>
<td>HMT</td>
<td>1 ml/14.5 cm²</td>
<td>5% in petrolatum</td>
<td>11/24</td>
<td>RIFM (1974d)</td>
</tr>
<tr>
<td>3448</td>
<td>HMT</td>
<td>1 ml/14.5 cm²</td>
<td>5% in butylene glycol</td>
<td>0/25</td>
<td>RIFM (1974e)</td>
</tr>
<tr>
<td>2759</td>
<td>HMT</td>
<td>1 ml/14.5 cm²</td>
<td>4% in petrolatum</td>
<td>3/25</td>
<td>RIFM (1972b)</td>
</tr>
<tr>
<td>2759</td>
<td>HMT</td>
<td>1 ml/14.5 cm²</td>
<td>4% in petrolatum</td>
<td>3/25</td>
<td>RIFM (1972c)</td>
</tr>
<tr>
<td>2759</td>
<td>HMT</td>
<td>1 ml/14.5 cm²</td>
<td>4% in petrolatum</td>
<td>9/25</td>
<td>RIFM (1971c)</td>
</tr>
<tr>
<td>2759</td>
<td>HMT</td>
<td>1 ml/14.5 cm²</td>
<td>4% in petrolatum</td>
<td>5/25</td>
<td>RIFM (1972c)</td>
</tr>
<tr>
<td>2759</td>
<td>HMT</td>
<td>1 ml/14.5 cm²</td>
<td>4% in petrolatum</td>
<td>4/25</td>
<td>RIFM (1971c)</td>
</tr>
<tr>
<td>2759</td>
<td>HMT</td>
<td>1 ml/14.5 cm²</td>
<td>4% in petrolatum</td>
<td>5/25</td>
<td>RIFM (1971c)</td>
</tr>
<tr>
<td>1379</td>
<td>HMT</td>
<td>1 ml/14.5 cm²</td>
<td>2% in petrolatum</td>
<td>2/24</td>
<td>RIFM (1972d)</td>
</tr>
</tbody>
</table>

Abbreviations: DEP, diethyl phthalate; EtOH, ethanol; HMT, Human Maximization Test; HRIPT, Human Repeated Insult Patch Test; SDA39C, alcohol SDA39C.

* Excludes those studies in which insufficient detail was provided describing the patch conditions resulting in the inability to convert to the dose applied (µg/cm²).

b HRIPTs were generally conducted according to the methods discussed by Marzulli and Maibach (1977) and McNamee et al. (2008). HMTs were generally conducted according to the method described by Kligman and Epstein (1975).

sisted of a 1 in² webril pad with 0.2 ml of 4% citral in petrolatum (RIFM, 1971a).

No reactions were observed to 755 µg/cm² citral in an HRIPT conducted on 40 subjects (11 males and 29 females). The patches consisted of a 1 in² webril pad with 0.5 ml of 1% citral in alcohol SDA39C (RIFM, 1965).

An HRIPT was conducted on 12 male and 29 female volunteers with 0.5% citral in alcohol SDA39C. The patches consisted of a 1 in² webril pad with 0.5 ml of test material; which resulted in a dose of 388 µg/cm². No sensitization reactions (0/41) were observed (RIFM, 1964b).

Steltenkamp et al. (1980) reported on the positive results of HRIPTs conducted with 0.5%, 1% and 5% citral in EtOH. However, little detail regarding the methods employed was provided and the information did not allow for the conversion of the reported concentration to their dose per unit area equivalents.

The HMT is typically conducted on 25 human subjects by utilizing 5 alternate day 48-h occluded induction applications of test material and appropriate controls. Following a 10 to 14-day rest period 48-hour challenge applications are made to naïve sites. Patches may be made with and without pretreatment of sodium lauryl sulfate depending upon the inherent irritancy of the test material. Observations at challenge coupled with the patterns of reactivity observed during induction provide the basis for an interpretation of contact allergy (Kligman and Epstein, 1975). Citral has been tested in the HMT over a range of concentrations. The patches utilized for each of the reported studies consisted of a 14.5 cm² webril pad with 0.5 ml of test material. The following results were obtained.

An HMT was conducted with 8% (5517 µg/cm²) citral in petrolatum on 24 males volunteers. Sensitization reactions were observed in 8/24 subjects (RIFM, 1971b).

In an HMT conducted with 5% (3448 µg/cm²) citral in petrolatum on 16 male and 9 female volunteers. Sensitization reactions (16/25) were observed (RIFM, 1974b).

Samples of citral, which had been treated and stored under differing conditions, were tested in the HMT at 5% (3448 µg/cm²) citral in petrolatum on male and female volunteers. Citral stored under nitrogen both with and without the addition of BHA added resulted in sensitization reactions in 14/25 and 12/25 subjects, respectively (RIFM, 1974c). Citral stored under oxygen resulted in sensitization reactions in 8/25 volunteers (RIFM, 1974c).

A sample of citral was stored under nitrogen with the addition of BHA and tested in the HMT at 5% (3448 µg/cm²) in petrolatum on 24 male volunteers. Sensitization reactions were observed in 11/24 subjects (RIFM, 1974d).

A sample of citral was stored under ambient air with the addition of BHA and tested in the HMT at 5% (3448 µg/cm²) in petrolatum on 16 male and 9 female volunteers. No sensitization reactions (0/25) were observed (RIFM, 1974e).

In an HMT conducted with 4% (2759 µg/cm²) citral in petrolatum on 25 male volunteers. Sensitization reactions (3/25) were observed (RIFM, 1972c).
A series of five HMTs were conducted on samples of citral derived from different processes. Each HMT was conducted with 4% (2759 μg/cm²) citral in petrolatum on male subjects. A sample of citral derived from lemongrass oil resulted in sensitization reactions in 3/25 subjects (RIFM, 1972b). Two samples of citral, described as citral refined, resulted in sensitization reactions of 9/25 (sample # 71-4-3) and 5/25 (sample # DL-4-10R) (RIFM, 1971c, 1972c). A sample described as citral synthetic (sample # 714-2) resulted in sensitization reactions in 4/25 subjects (RIFM, 1971c). A sample identified as citral natural (sample # 744-1) resulted in sensitization reactions in 5/25 subjects (RIFM, 1971c).

In an HMT conducted with 2% (1379 μg/cm²) citral in petrolatum on 24 male volunteers. Sensitization reactions (2/24) were observed (RIFM, 1972d).

4.3. Clinical diagnostic patch tests on patients (Table 4)

Routine clinical diagnostic patch tests with citral have been reported in the literature. The results of studies reported in greater than 100 consecutive patients with varying dermatitis showed a range of positive patch tests reactions to citral in these populations.

Ishihara et al. (1981) reported on the results of patch tests with 5% citral. Reactions were observed in cosmetic dermatitis patients (4/155) and eczema/dermatitis patients (5/159). No reactions were observed in control subjects (0/48).

Patch tests were conducted between the years 1978–1986 in dermatologic patients (Itoh et al., 1986, 1988; Nishimura et al., 1984). When citral was tested at 5% (vehicle not reported), reactions were observed in cosmetic dermatitis patients (8/310), non-cosmetic dermatitis patients (9/408) and in one control subject (1/122). When citral was tested at 2% (vehicle not reported), reactions were observed in cosmetic dermatitis patients (1/240) and non-cosmetic dermatitis patients (2/584). No reactions were observed in control subjects (0/105).

In a series of patch tests conducted in six dermatological centers in Europe, 1701 contact dermatitis patients were patch tested for 48 h using Finn Chambers on Scanpor tape (Frosch et al., 2004, 2005). The patients were tested concurrently with a fragrance mix (FM) and the six individual materials comprising the mixture, which included citral. When patients were patched with the FM at 28% and citral at 2.0%, reactions were observed to citral in (12/1701) patients with doubtful/irritant reactions being reported (40/1701). When patients were patched with the FM at 14% and citral at 1%, reactions were observed to citral in (6/1701) patients with doubtful/irritant reactions being reported (14/1701).

Heydorn et al. (2002) reported on the results of patch tests with 2% citral in petrolatum on 315 patients with hand eczema. Positive reactions were observed in 2% of the patients. In an update by Heydorn et al. (2003a), 2% positive reactions were reported to 2% citral when patch tests were conducted on 586 patients. Additionally Heydorn et al. (2003b) reported that 28 positive reactions were observed to 2% citral in 658 hand eczema patients.

In a multicenter study conducted from September 1998 to April 1999, 1825 patients were patch tested with nine fragrance materials, including citral. Reactions (19/1825) were observed with 2.0% citral in petrolatum (DeGroot et al., 2000).

In a multi center study with 1825 patients, it was reported that 2% citral resulted in 25 doubtful reactions and 21 positive reactions (Frosch et al., 2002).

Mitchell et al. (1982) reported that patch tests conducted on 228 eczema patients resulted in 1% positive reactions.

Frosch et al. (1995) reported on the results of patch tests conducted with 0.1% and 1.0% citral in petrolatum. The test material was applied to the back for 2 days using Finn chambers® on Scanpore®. Reactions were assessed per

<table>
<thead>
<tr>
<th>Concentration/Vehicle</th>
<th>Results</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>5% in petrolatum</td>
<td>4/155 cosmetic dermatitis patients, 5/159 eczema/dermatitis patients, 0/48 control subjects</td>
<td>Ishihara et al. (1981)</td>
</tr>
<tr>
<td>5% VNR</td>
<td>8/310 cosmetic dermatitis patients, 9/408 non-cosmetic patients, 1/122 control subjects</td>
<td>Itoh et al. (1986, 1988), Nishimura et al. (1984)</td>
</tr>
<tr>
<td>2% VNR</td>
<td>21/1825 patients</td>
<td>Frosch et al. (2002)</td>
</tr>
<tr>
<td>2% in petrolatum</td>
<td>19/1825 patients</td>
<td>DeGroot et al. (2000)</td>
</tr>
<tr>
<td>2% VNR</td>
<td>12/1701 patients</td>
<td>Frosch et al. (2004, 2005)</td>
</tr>
<tr>
<td>2% VNR</td>
<td>28/658 patients</td>
<td>Heydorn et al. (2002, 2003a,b)</td>
</tr>
<tr>
<td>2% VNR</td>
<td>1/240 cosmetic dermatitis patients, 2/584 non-cosmetic patients, 0/105 control subjects</td>
<td>Itoh et al. (1986, 1988), Nishimura et al. (1984)</td>
</tr>
<tr>
<td>1% VNR</td>
<td>6/1701 patients</td>
<td>Frosch et al. (2004, 2005)</td>
</tr>
<tr>
<td>1% VNR</td>
<td>4/228 patients</td>
<td>Mitchell et al. (1982)</td>
</tr>
<tr>
<td>1% in petrolatum</td>
<td>8/192 patients</td>
<td>Frosch et al. (1995)</td>
</tr>
<tr>
<td>0.1% VNR</td>
<td>1/192 (reaction was questionable) patients</td>
<td>Frosch et al. (1995)</td>
</tr>
</tbody>
</table>

Abbreviations: VNR, vehicle not reported.

* The table is restricted to the literature reports of clinical diagnostic patch tests to citral in greater than 100 consecutive patients.
International Contact Dermatitis Research Group (ICDRG) guidelines on days 2 and 3 or on days 2 and 4. Eight reactions (8/192) were observed to 1% citral. One questionable reaction (1/92) was observed to 0.1% citral.

4.4. ‘Quenching’ studies (Tables 5 and 6)

Studies on the inhibition of the induction of sensitization to citral by another material, ‘the quenching phenomenon’ (see below for details), have been reported in both human and animal studies. The ‘quenching’ of citral by various materials has been demonstrated in humans. However, ‘quenching’ of citral sensitization has not been demonstrated in animal models of dermal sensitization.

Opdyke (1976) reported that citral was shown to be a sensitizer in the HMT, however essential oils that contained significant amounts of citral did not induce sensitization. It was theorized that some other component(s) of the essential oil inhibited the induction of sensitization. As a test of this hypothesis, several terpenes and alcohols found along with citral in the oils were combined. Opdyke (1976) showed that in the HMT citral produced sensitization reactions when applied alone but produced no reactions when applied in the simple mixtures. This inhibition of sensitization was termed the ‘quenching’ phenomenon and was incorporated into risk management strategies for contact allergy to citral in addition to two other aldehydes—cinnamic aldehyde and phenylacetaldehyde (Opdyke, 1976). Later work showed that while this ‘quenching’ phenomenon could not be duplicated in an HRIPT for cinnamic aldehyde and phenylacetaldehyde, for citral it was demonstrated in both the HMT and HRIPT (Cocchiara et al., 2004; Api and Isola, 2000; Api, 2000). No reactions were observed in HMTs conducted as described above on two samples of 5% lemongrass, 4% citral + 1% citrus terpene, 4% citral + 1% α-pinene, 4% and citral + 1% d-limonene (Opdyke, 1976; Api and Isola, 2000; Api, 2000). No reactions (0/118) were observed in an HRIPT conducted as described above on 4% citral + 1% d-limonene in DEP (Api, 2000; Api and Isola, 2000). The results of the human non-diagnostic patch test studies conducted on ‘quenched’ citral are summarized in Table 5.

The dermal sensitization potential of ‘quenched’ citral has been evaluated in guinea pig assays and the LLNA (Table 6).

A guinea pig maximization test was conducted as described above on a mixture of 4:1 citral:eugenol. Induction was conducted with 0.2% in 4:1 acetone:olive oil for intradermal injection and 5% in 4:1 acetone:olive oil for the occluded patch. Challenge application was conducted with 0.5% in 4:1 acetone:olive oil. Sensitization reactions were observed in 70% (7/10) of the animals (Basketter and Allenby, 1991). When a mixture of 4:1 citral:d-limonene was tested under the same condition, sensitization reactions (7/10) were observed with 2/10 questionable reactions (Basketter and Allenby, 1991; Basketter, 1998).

Hanau et al. (1983) reported on a series of guinea pig assays conducted to evaluate the sensitization potential of a 1:1 solution of citral:d-limonene. Induction applications followed a modified FCAT method. Challenge applications were made both open and with the use of an occluded patch. No significant difference between the mixture of 1:1 citral:d-limonene and citral alone was observed following visual scoring. Histological examination of the closed challenge application sites showed increased total infiltrate and lymphocyte reactions to citral when compared to 1:1 citral:d-limonene. In a follow up to this study, Barbier and Benezra (1983) reported on experiments with radiolabeled citral + d-limonene. When the mixture was applied an increase in binding of citral to insoluble protein was observed, which the authors concluded may play a role in the ‘quenching’ of citral.

No difference in sensitization potential was observed between citral and 4:1 citral + d-limonene when each was tested in the LLNA; both materials resulted in an EC3 value of 13% (Basketter, 1998; Basketter et al., 2000).

A series of LLNAs conducted on ‘quenched’ citral were reported by Lalko and Api (2004). A sample of citral + d-limonene at a constant ratio of 4:1 was tested along with citral alone as a control. No significant difference was observed between the calculated EC3 value for each material—1.2% for citral and 0.8% for 4:1 citral:d-limonene.

LLNAs were conducted on two essential oils that contained significant amounts of citral—lemongrass and litsea cubeba oil—to determine if any difference in the sensitization

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**Table 5**

Citral 'quenching' studies—human non-diagnostic patch tests

<table>
<thead>
<tr>
<th>Method</th>
<th>Test material</th>
<th>Results</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMT</td>
<td>5% Lemongrass (with a 4% citral content) in petrolatum</td>
<td>0/25</td>
<td>Opdyke (1976), Api and Isola (2000), Api (2000)</td>
</tr>
<tr>
<td>HMT</td>
<td>5% Lemongrass (with a 3.2% citral content) in petrolatum</td>
<td>0/25</td>
<td></td>
</tr>
<tr>
<td>HMT</td>
<td>4% citral + 1% citrus terpenes in petrolatum</td>
<td>0/25</td>
<td></td>
</tr>
<tr>
<td>HMT</td>
<td>4% citral + 1% α-pinene in petrolatum</td>
<td>0/25</td>
<td></td>
</tr>
<tr>
<td>HMT</td>
<td>4% citral + 1% d-limonene in petrolatum</td>
<td>0/24</td>
<td></td>
</tr>
<tr>
<td>HRIPT</td>
<td>4% citral + 1% d-limonene in DEP</td>
<td>0/118</td>
<td>Api and Isola (2000), Api (2000)</td>
</tr>
</tbody>
</table>

**Abbreviations:** DEP, Diethyl phthalate; HMT, Human Maximization Test; HRIPT, Human Repeated Insult Patch Test.

* HMTs were generally conducted according to the method described by Kligman and Epstein (1975). HRIPTs were generally conducted according to the methods discussed by Marzulli and Maibach (1977) and McNamara et al. (2008).
potential of citral could be observed based on its exposure in a mixture (Lalko and Api, 2006). Lemongrass oil, which contained 68.8% citral, resulted in an EC3 value of 6.5%. *Litsea cubeba* oil, which contained 85.7% citral, resulted in an EC3 value of 8.4%. Adjusting the EC3 value to account for the amount of citral in the oil showed that the response observed was strongly driven by citral and no evidence of the ‘quenching’ phenomenon could be observed.

### 4.5. Conclusions

The chemical structure and properties of citral suggest that it would have the potential to act as a contact sensitizer. Indeed, hazard studies conducted in guinea pigs utilizing a variety of methods and in the mouse using the LLNA identify citral as a contact allergen. The available guinea pig studies (Table 1) do not provide sufficient dose response data, making the determination of a NOEL for the induction of dermal sensitization impossible in the guinea pig models (though with an adequate design, it would be possible to extract such information from a future study). The guinea pig sensitization data can be used to classify the potency of citral. Based on those studies that provide sufficient data, citral can be classified as a weak to moderate sensitizer in the guinea pig models (ECETOC, 2003; Kimber et al., 2003). There are several reports on the positive results of citral in the LLNA (Table 2). In addition to its application as a method to identify potential contact allergens, the LLNA provides an objective and quantitative measure of relative skin sensitizing potency—the EC3 value (Basketter et al., 1999, 2000). EC3 values in the range of 1.2% (300 μg/cm²) in 1:3 EtOH:DEP to 13% (3250 μg/cm²) in 4:1 acetone:olive oil have been reported. The weighted mean EC3 value, based on vehicle utilized, for citral is 5.7% (1414 μg/cm²) showing that citral is a weak allergen in the LLNA.

In humans, the available data provide for the determination of a NOEL and LOEL (Table 3). In the HRIPT, the NOEL is 1400 μg/cm² for the induction of dermal sensitization to citral when tested in an ethanol vehicle. There is no identifiable NOEL in the HMT over the range of concentrations tested (Table 3). However, the HMT data exhibit a general dose-dependent trend toward decreasing activity with decreasing dose. In the HMT the LOEL for citral is 2759 μg/cm² when tested in petrolatum. The HMT protocol was designed to take advantage of the ease with which compromised skin maybe sensitized with the goal of maximizing the tests ability to identify allergens (Kligman, 1985). This procedure is a harsh method that employs pretreatment with the anionic surfactant sodium lauryl sulfate (SLS) and extended (often 48 h) occlusive patches (Kligman and Epstein, 1975). Human patch testing methodology has evolved over more than 50 years; the current confirmatory safety test most typically conducted on fragrance ingredients in humans is the HRIPT (Api, 2002). The HRIPT protocol utilizes 24 h patches over a 3-week period without the use of SLS (Marzulli and Maibach, 1977; McNamee et al., 2008). The HRIPT was designed and refined to exaggerate normal, realistic use conditions with the goal of confirming safe use levels. The HRIPT used today is a confirmatory assay for the predicted NOEL for induction of sensitization in a population of healthy volunteers (Api, 2002; McNamee et al., 2008). This focus on exaggerated realistic exposure conditions makes the HRIPT more relevant, when

<table>
<thead>
<tr>
<th>Table 6</th>
<th>Citral ‘quenching’ studies in animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method</td>
<td>Test material</td>
</tr>
<tr>
<td>GPMT</td>
<td>4:1 citral:α-limonene</td>
</tr>
<tr>
<td>FCAT</td>
<td>1:1 citral:α-limonene</td>
</tr>
<tr>
<td>LLNA</td>
<td>4:1 citral:α-limonene</td>
</tr>
<tr>
<td>LLNA</td>
<td>4:1 citral:α-limonene</td>
</tr>
<tr>
<td>LLNA</td>
<td>Lemongrass oil (containing 68.8% citral)</td>
</tr>
<tr>
<td>LLNA</td>
<td><em>Litsea cubeba</em> oil (containing 85.7% citral)</td>
</tr>
</tbody>
</table>

**Abbreviations:** FCAT, Freund’s complete adjuvant test; GPMT, guinea pig maximization test; LLNA, murine local lymph node assay.
compared to the HMT, to the identification of a threshold for induction under foreseeable usage scenarios.

Historically, risk management focusing on primary prevention of contact allergy to citral from fragrance use has been based on the ‘quenching’ phenomenon—the inhibition of the induction of sensitization to one material by the presence of another as described by Oddyke (1976) (see Section 4.4). The use of citral in fragrance formulations is currently restricted by an IFRA Standard requiring that “it be used in conjunction with substances preventing sensitization [quenching agents], as for example 25% d-limonene, mixed citrus terpenes or a-pinene” (IFRA, 1980). The ‘quenching’ of the induction of dermal sensitization to citral by various materials has been demonstrated in humans (Table 5). However, ‘quenching’ of citral sensitization has not been demonstrated in animal models of contact allergy (Table 6). Similar contradictory results can be found in the literature with respect to the theory of ‘quenching’ for other fragrance ingredients; data to support and refute the phenomenon have been reported (Karlberg et al., 2000; Nilsson et al., 2002; Basketter, 2000). Since there is conflicting data and no adequate mechanism to explain the phenomenon, it is recently being replaced as a basis for risk management of contact allergy to certain fragrance ingredients—e.g., the IFRA Standard for cinnamic aldehyde has been replaced to limit its use based on the NOEL for induction to the discreet material (IFRA, 2003).

In conclusion, the data reported here show by a weight of evidence that the human NOEL for induction of sensitization to citral is 1400 μg/cm². The available guinea pig studies identify citral as a weak to moderate dermal sensitizer. Studies in the LLNA resulted in positive responses with a vehicle weighted average EC3 value of 1414 μg/cm². Based on this calculated EC3 value citral is classified as a weak sensitizer in the LLNA. The EC3 value has been demonstrated to closely correlate to experimentally determined human NOELs for induction (Basketter et al., 2005; Gerberick et al., 2001; Lalko and Api, 2005). The EC3 value reported here is remarkably close to the human NOEL of 1400 μg/cm² identified for citral in the HRIPT. In the HMT, the LOEL for citral is 2759 μg/cm² when tested in petrolatum. Positive reactions to citral have been reported in dermatologic patients. The goal of primary prevention is to avoid inducing sensitization altogether, thereby avoiding the manifestation of contact dermatitis. Currently risk management strategies for the use of citral as a fragrance ingredient are based on the ‘quenching phenomenon’. Due to the discordance between the human and animal studies on quenching and no mechanistic data to support the observations, ‘quenching’ is currently being discontinued by the fragrance industry as a basis for risk management. The identified NOEL for the induction of sensitization to citral can be applied to risk assessment paradigms such as the QRA procedure outlined in a paper concurrently submitted with this review (Api et al., 2008) and Gerberick et al. (2001); with the aim of refining the acceptable use level of citral in various consumer product types.

Conflict of Interest

Jon Lalko 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.

Funding Source

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