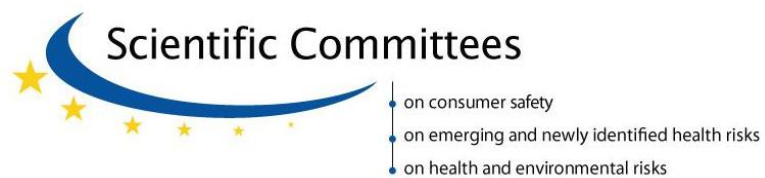




Scientific Committee on Consumer Safety

SCCS

**OPINION**  
**on**  
**Fragrance allergens in cosmetic products**



The SCCS adopted this pre-consultation opinion at its 13<sup>th</sup> plenary meeting

of 13-14 December 2011

### About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

### SCCS

The Committee shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

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## Summary

Contact allergy to fragrance ingredients may develop following skin contact with a sufficient amount of these substances, often through the use of cosmetic products. Contact allergy is an altered specific reactivity in the immune system, which entails recognition of the fragrance allergen(s) in question by immune cells. Contact allergy, which *per se* is a latent condition, i.e. without visible signs or symptoms, persists lifelong. Upon each re-exposure to sufficient amounts of the allergen(s) eczema develops (allergic contact dermatitis), which typically will involve the face, the armpits and/or the hand(s). The disease can be severe and generalised, with a significant impairment of quality of life and potential consequences for fitness for work.

Around 16 % of eczema patients in the European population are sensitised to fragrance ingredients. From studies performed on sectors of the population it can be estimated that the frequency of contact allergy to fragrance ingredients in the general population in Europe is 1-3%. The overall trend of fragrance allergy has been stable during the last 10 years, as some causes of fragrance allergy have decreased and others increased.

Most individuals with contact allergy to fragrance ingredients are aware that they cannot tolerate scented products on their skin and are often able to specifically name product categories that initiated their disease. In this context colognes, eau de toilette, deodorants and lotions are named significantly more often by fragrance allergic eczema patients than by patients without fragrance contact allergy.

Commercially available fragrances and other scented cosmetic products can provoke allergic contact dermatitis under patch test as well as simulated use conditions.

Appropriate diagnostic procedures and patient information are cornerstones in secondary prevention of contact allergy. The SCCNFP identified in 1999 a set of 26 fragrance allergens with a well-recognised potential to cause allergy, for which information should be provided to consumers about their presence in cosmetic products.

This listing has shown to be important in the clinical management of patients who are allergic to one or more of these 26 fragrance chemicals. Listing of the 26 fragrances has also been shown to be beneficial for patients with contact allergy to one or more of the fragrance chemicals, because these are identified on the ingredient listings of cosmetic products, and can thus be avoided.

The present opinion updates the SCCNFP opinion with a systematic and critical review of the scientific literature to identify fragrance allergens, including natural extracts, relevant to consumers. Clinical, epidemiological and experimental studies were evaluated, as well as modelling studies performed, to establish lists of (i) established fragrance allergens, (ii) likely fragrance allergens and (iii) possible fragrance allergens.

The studies since the SCCNFP Opinion on fragrance allergy in consumers confirm that the fragrance allergens identified by SCCNFP in 1999 are still relevant fragrance allergens for consumers from their exposure to cosmetic products. The review of the clinical and experimental data published since then shows that many more fragrance substances have been shown to be sensitisers in humans. Based on the clinical experience alone, 82 substances can be classified as established contact allergens in humans, 54 single chemicals and 28 natural extracts. Of these, 12 chemicals and 8 natural extracts were found to pose a high risk of sensitisation to the consumer, considering the high number of reported cases. In particular one ingredient stood out, hydroxyisohexyl 3-cyclohexene carboxaldehyde, having been the cause of more than 1500 reported cases since the 1999 opinion.

Moreover, animal experiments indicate that additional fragrance substances can be expected to be contact allergens in humans, although human evidence is currently lacking. Additionally, limited *in vivo* evidence together with Structure-Activity Relationship analysis suggests that other fragrance ingredients may be a cause of concern with regard to their potential of causing contact allergy in humans.

The review also lists fragrance substances that can act as prehapten or prohaptens, forming new or more potent allergens by air oxidation and/or metabolic activation. Such

activation processes are of concern as they increase the risk of sensitisation and also the risk for cross reactivity between fragrance substances. In addition to known prehapten fragrance substances, the SCCS performed SAR analyses to identify fragrance substances with structural alerts that indicate that they are possible prehapten. While in the case of prohapten the possibility of becoming activated is inherent to the molecule and cannot be avoided, the activation of prehapten can be prevented by appropriate measures.

The SCCS examined available elicitation dose-response data to decide whether safe thresholds can be established for the fragrance allergens of concern, i.e. those found to pose a high risk of sensitisation to consumers. The SCCS considers that thresholds based on elicitation levels in sensitised individuals will be sufficiently low to protect both the majority of sensitised individuals as well as most of the non-sensitised consumers from developing contact allergy. As data from human dose elicitation experiments are very limited in several respects, no levels that could be considered safe for the majority of contact allergic consumers could be established for individual substances. The studies available, however, indicate that a general level of exposure of up to 0.8 µg/cm<sup>2</sup> (0.01% in cosmetic products) may be tolerated by most consumers, including those with contact allergy to fragrance allergens. The SCCS is of the opinion that this level of exposure (up to 0.01%) would suffice to prevent elicitation for the majority of allergic individuals, unless there is experimental or clinical substance-specific data allowing the derivation of individual thresholds.

It was not possible to provide a safe threshold for natural extracts of concern, as no specific investigations exist and the model providing the general threshold (0.01%) has been based on individual chemicals only. However the SCCS considers that the maximum use concentration applies to the identified chemicals both if added as chemicals or as an identified constituent of a natural ingredient. This will also reduce the risk of sensitisation and elicitation from natural extracts.

The suggested general threshold, although limiting the problem of fragrance allergy in the consumer significantly, would not preclude that the most sensitive segment of the population may react upon exposure to these levels and does not remove the necessity for providing information to the consumer concerning the presence of the listed fragrance substance in cosmetics.

In the case of hydroxyisohexyl 3-cyclohexene carboxaldehyde, the SCCP had recommended limiting the concentration in cosmetics to 200 ppm. Recent voluntary restrictions (recommendations to lower use concentrations, at least for some product types, to the level recommended by the SCCS in 2003) are not reflected in available evidence and are considered insufficient. The SCCS considers that the number of cases of HICC allergy documented over the last decade is exceptionally high and that continued exposure to HICC by the consumer is not considered safe, even at concentrations as low as 200 ppm. Therefore, HICC should not be used in consumer products in order to prevent further cases of contact allergy to HICC and to limit the consequences to those who already have become sensitized.

The SCCP concluded in 2004 that chloroatranol and atranol, the main allergenic constituents of *Evernia prunastri* and *Evernia furfuracea*, should not be present in products for the consumer. The persistently high frequency of contact allergy to *Evernia prunastri* and *Evernia furfuracea* noted in eczema patients does point to a persisting problem with exposure to the allergenic constituents. The SCCS is of the opinion that the presence of the two constituents, chloroatranol and atranol, in cosmetic products are not safe.



## 1. Background

As a result of the public consultation on perfumery materials, which ended on 27 January 2007, there were further requests and information on important and/or frequently used allergens other than those proposed for regulation, such as farnesol, citral, linalool and hydroxyisohexyl-3-cyclohexenecarboxaldehyde. These substances were not part of the consultation, but they all belong to the 26 fragrance substances which should be labelled when present in cosmetic products under certain conditions.

The 26 fragrance substances were introduced into annex III of the Cosmetics Directive by the 7<sup>th</sup> amendment (2003/15/EC) on the basis of the SCCNFP draft opinion (SCCNFP/0017/98) published on 30 September 1999 for public consultation and the final opinion adopted by the SCCNFP during the plenary session of 8 December 1999.

Thirteen of the allergenic fragrance substances listed in this opinion have been frequently reported as well-recognised contact allergens in consumers and are thus of most concern; 11 others are less well documented. See the lists below from the opinion.

*List A: Fragrance chemicals, which according to existing knowledge, are most frequently reported and well-recognised consumer allergens.*

<b>Common name</b>	<b>CAS number</b>
Amyl cinnamal	122-40-7
Amylcinnamyl alcohol	101-85-9
Benzyl alcohol	100-51-6
Benzyl salicylate	118-58-1
Cinnamyl alcohol	104-54-1
Cinnamal	104-55-2
Citral	5392-40-5
Coumarin	91-64-5
Eugenol	97-53-0
Geraniol	106-24-1
Hydroxycitronellal	107-75-5
Hydroxymethylpentyl-cyclohexenecarboxaldehyde	31906-04-4
Isoeugenol	97-54-1

*List B: Fragrance chemicals, which are less frequently reported and thus less documented as consumer allergens.*

<b>Common name</b>	<b>CAS number</b>
Anisyl alcohol	105-13-5
Benzyl benzoate	120-51-4
Benzyl cinnamate	103-41-3
Citronellol	106-22-9
Farnesol	4602-84-0
Hexyl cinnamaldehyde	101-86-0
Lilial	80-54-6
d-Limonene	5989-27-5
Linalool	78-70-6
Methyl heptine carbonate	111-12-6
3-Methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one	127-51-5

Furthermore, two fragrances (natural mixtures) were added

<b>Common name</b>	<b>CAS number</b>
Oak moss	90028-68-5
Tree moss	90028-67-4

At the time there were insufficient scientific data to allow for the determination of dose-response relationships and/or thresholds for these allergens. Nevertheless, in a pragmatic administrative decision the limits of 0.01 and 0.001% were set, for rinse-off and leave-on products respectively.

Scientific information of both a general and a specific nature has been submitted to DG ENTR in order to ask the SCCS for a revision of the 26 fragrances with respect to further restrictions and possible even delisting. A separate request has already been made for hydroxycitronellal, isoeugenol and the content of peroxides in limonene.

## **2. Terms of reference**

- 1. Does the SCCS still consider that the fragrance allergens currently listed in Annex III, entries 67-92, for labelling purposes represent those fragrance ingredients that the consumer needs to be made aware of when present in cosmetic products?*
- 2. Can the SCCS establish any threshold for their safe use based on the available scientific data?*
- 3. Can the SCCS identify substances where processes (e.g. metabolism, oxidation and hydrolysis) may lead to cross-reactivity and new allergens which are relevant for the protection of the consumer?*

### 3. Introduction

#### *Fragrance ingredients*

Fragrance and flavour substances are organic compounds with characteristic, usually pleasant, odours. They are ubiquitously used in perfumes and other perfumed cosmetic products, but also in detergents, fabric softeners, and other household products where fragrance may be used to mask unpleasant odours from raw materials. Flavourings are used in foods, beverages, and dental products. Fragrance substances are also used in aromatherapy and may be present in herbal products, and used as topical medicaments for their antiseptic properties.

Contact allergy to fragrance ingredients occurs when an individual has been exposed, on the skin, to a sufficient degree of fragrance contact allergens. Contact allergy is a life-long, specifically altered reactivity in the immune system. This means that once contact allergy is developed, cells in the immune system will be present which can recognise and react towards the allergen. As a consequence, symptoms, i.e. allergic contact dermatitis, may occur upon re-exposure to the fragrance allergen(s) in question. Allergic contact dermatitis is an inflammatory skin disease characterised by erythema, swelling and vesicles in the acute phase. If exposure continues it may develop into a chronic condition with scaling and painful fissures of the skin. Allergic contact dermatitis to fragrance ingredients is most often caused by cosmetic products and usually involves the face and/or hands. It may affect fitness for work and the quality of life of the individual.

Fragrance contact allergy has long been recognised as a frequent and potentially disabling problem. Prevention is possible as it is an environmental disease and if the environment is modified (e.g. by reduced use concentrations of allergens), the disease frequency and severity will decrease. Ingredient information is a cornerstone in the prevention of allergic contact dermatitis, as knowledge about the allergens which a patient has been exposed to is crucial for including the right substances in the allergy test, and for subsequent information on avoidance of re-exposure. However, the labelling rules in the Cosmetics Directive 76/768/EEC stipulated that perfume and aromatic compositions and their raw materials shall be referred to by the word "perfume" or "aroma", rather than being labelled individually. This is the reason why the SCCNFP in their opinion SCCNFP/0017/98 (1) identified 26 fragrance allergens for which information should be provided to consumers concerning their presence in cosmetic products. This was implemented in the Cosmetics Directive as individual ingredient labelling of the 26 fragrance allergens (Annex III, entries 67-92). However, safe use concentrations of these fragrances in cosmetic products had not yet been determined and much new evidence concerning fragrance allergy has been published since the 1999 opinion. The present request to review the list of recognised fragrance allergens which the consumer needs to be made aware of, to indicate thresholds for their safe use and to consider possible modification of allergens by metabolism and autoxidation, required a thorough review of all relevant scientific data. This includes both published scientific literature as well as unpublished scientific information on fragrances from the industry. The International Fragrance Association (IFRA), as representative of the fragrance industry, was contacted to provide relevant unpublished scientific data on fragrance ingredients. This information, together with the up-to-date published scientific literature, has been critically reviewed for the present SCCS opinion. The relevant data gaps are identified and recommendations for research addressing these gaps are made.

## 4. Clinical aspects of contact allergy to fragrance ingredients

### 4.1. Spectrum of reactions

Adverse reactions to fragrances in perfumes and in fragranced cosmetic products include allergic contact dermatitis, irritant contact dermatitis, photosensitivity, immediate contact reactions (contact urticaria), and pigmented contact dermatitis. Airborne and connubial contact dermatitis occurs.

#### 4.1.1. Allergic contact dermatitis

##### *Mechanism*

Allergic contact dermatitis (ACD) depends primarily on the activation of allergen-specific T-cells. In allergic contact dermatitis, a distinction is made between induction (sensitisation) and elicitation phases. A useful review is available (2).

The induction phase includes the events following initial contact with the allergen and is complete when the individual is sensitised and capable of giving a positive allergic contact dermatitis reaction.

The elicitation phase begins upon re-exposure to the allergen (challenge) and results in clinical manifestation of allergic contact dermatitis.

The entire process of the induction phase requires ca. 10 days to several weeks, whereas an elicitation phase reaction develops within 1–2 days.

Most contact allergens are small, chemically reactive compounds. As these compounds are too small to be directly immunogenic, they act as haptens; i.e. they react with higher molecular weight epidermal and/or dermal biomolecules to form immunogenic adducts. It is usually considered that the biomolecules involved are free or membrane bound proteins, which react via nucleophilic thiol, amino, and hydroxyl groups.

Dendritic cells (DCs) and the local tissue microenvironment are crucial factors in the development of ACD. Langerhans cells (LCs), as epidermal DCs, and dermal DCs are pivotal for the sensitisation and the elicitation phases of ACD. During sensitisation, DCs react with the immunogenic complexes by interaction with neighbouring keratinocytes, migration to the local draining lymph nodes and the priming of naïve T-cells. These reactions are mediated by inflammatory cytokines, chemokines and adhesion molecules. Antigen specific effector T-cells are then recruited into the skin upon contact with the same hapten (elicitation). Following their recruitment these T-cells are activated by antigen-presenting skin cells, including LCs, dermal DCs and keratinocytes, and macrophages.

Although most allergens can form hapten–carrier complexes directly, some need activation, e.g. by enzyme-induced metabolic conversion or abiotic oxidation. Such compounds are termed prohaptens and prehaptens, respectively, and are discussed in more detail in chapter 5. Well known examples of prehaptens and prohaptens are limonene and eugenol. Reduced enzyme activity in certain individuals, related to genetic enzyme polymorphisms, may give an increased or reduced risk of sensitisation to prohaptens (that need enzymatic activation) in certain individuals or populations.

Once sensitised, individuals can develop allergic contact dermatitis upon re-exposure to the contact allergen. Positive patch test reactions mimic this process of allergen-specific skin hyper-sensitivity. Skin contact induces an inflammatory reaction that is maximal within 2–3 days and, without further allergen supply, then declines.

##### *Overview of clinical features*

Perfumes and deodorants are the most frequent sources of sensitisation to fragrance ingredients in women, while aftershave products and deodorants are most often responsible in men (3). Thereafter, eczema may appear or be worsened by contact with other

fragranced products such as cosmetics, toiletries, household products, industrial contacts and flavourings.

Contact allergy to a particular product or chemical is established by means of diagnostic patch testing. When patients with suspected allergic cosmetic dermatitis are investigated, fragrances are identified as the most frequent allergens, not only in perfumes, after-shaves and deodorants, but also in other cosmetic products. Evaluation of perfume allergy may be difficult; a perfume compound may consist of ten to > 300 basic components selected from about 2500 materials.

Between 6 and 14% of patients routinely tested for suspected allergic contact dermatitis react to a standard indicator of fragrance allergy, the Fragrance Mix (4), see also chapter 4.2.2. When tested with ten popular perfumes, 6.9% of female eczema patients proved to be allergic to them (5) and 3.2–4.2% were allergic to fragrances from perfumes present in various cosmetic products (6). The finding of a positive reaction to the Fragrance Mix should be followed by a search for its relevance, i.e. is fragrance allergy the cause of the patient's current or previous complaints, or does it at least contribute to it? Between 50 and 65% of all positive patch test reactions to the mix are relevant. Sometimes, correlation with the clinical picture is lacking and many patients appear to tolerate perfumes and fragranced products without problems (7). This may be explained by: a) irritant (false-positive) patch test reactions to the mix; b) the absence of relevant allergens in those products; and c) the concentration being too low to elicit clinically visible allergic contact reactions. Depending on the degree of sensitivity and exposure, the severity of dermatitis may range from mild to severe with dissemination (8) [pp 158–170].

Clinical studies have shown a highly significant association between reporting a history of visible skin symptoms from using scented products and a positive patch test to the Fragrance Mix (9). Provocation studies with perfumes and deodorants have also shown that fragrance-mix-positive eczema patients often react to use-tests with the products. Subsequent chemical analysis of such products has detected significant amounts of one or more Fragrance Mix ingredients, confirming the relevance of positive patch tests to the Fragrance Mix in these patients (5, 10).

### **Hands**

Contact sensitisation may be the primary cause of hand eczema, or may be a complication of irritant or atopic hand eczema. The number of positive patch tests has been reported to correlate with the duration of hand eczema, indicating that long-standing hand eczema may often be complicated by sensitisation (11). The most common contact allergies in patients with hand eczema are metals, the Fragrance Mix, *Myroxylon pereirae*, and colophonium (12).

Fragrance allergy may be a relevant problem in patients with hand eczema; perfumes are present in consumer products to which their hands are exposed (13). A significant relationship between hand eczema and fragrance contact allergy has been found in some studies based on patients investigated for contact allergy (14). However, hand eczema is a multi-factorial disease and the clinical significance of fragrance contact allergy in (severe) chronic hand eczema may not be clear. A review on the subject has been published (15).

### **Axillae**

Bilateral axillary dermatitis may be caused by perfume in deodorants and, if the reaction is severe, it may spread down the arms and to other areas of the body (8) [pp 158–170]. In individuals who consulted a dermatologist, a history of such first-time symptoms was significantly related to the later diagnosis of perfume allergy (9).

### **Face**

Facial eczema is an important manifestation of fragrance allergy from the use of cosmetic products (16). In men, aftershave products can cause an eczematous eruption of the beard area and the adjacent part of the neck (8) [pp 158–170], and men using wet shaving as opposed to dry have been shown to have an increased risk of 2.9 of being fragrance allergic (17).

#### **4.1.2. Irritant reactions (including contact urticaria)**

Irritant effects of some individual fragrance ingredients, e.g. citral (18, 19), are known. Irritant contact dermatitis from perfumes is believed to be common, but there are no existing investigations to substantiate this (7). Many more people complain about intolerance or rashes to perfumes/perfumed products than are shown to be allergic by testing (9). This may be due to irritant effects or inadequate diagnostic procedures.

Fragrances may cause a dose-related contact urticaria of the non-immunological type (irritant contact urticaria). Cinnamal, cinnamic alcohol, and *Myroxylon pereirae* are well recognised causes of contact urticaria, but others, including menthol, vanillin and benzaldehyde have also been reported (20). The reactions to *Myroxylon pereirae* may be due to cinnamates (21).

A relationship to delayed contact hypersensitivity was suggested (22), but no significant difference was found between a fragrance-allergic group and a control group in the frequency of immediate reactions to fragrance ingredients (20), in keeping with a non-immunological basis for the reactions seen.

#### **4.1.3. Pigmentary anomalies**

The term “pigmented cosmetic dermatitis” was introduced in 1973 for what had previously been known as melanosis faciei feminae when the mechanism (type IV allergy) and causative allergens were clarified (23). It refers to increased pigmentation, usually on the face/neck, often following sub-clinical contact dermatitis. Many cosmetic ingredients were patch tested at non-irritant concentrations and statistical evaluation showed that a number of fragrance ingredients were associated: jasmine absolute, ylang-ylang oil, cananga oil, benzyl salicylate, hydroxycitronellal, sandalwood oil, artificial sandalwood, geraniol, geranium oil (24).

#### **4.1.4. Photo-reactions**

Musk ambrette produced a considerable number of allergic photocontact reactions (in which UV-light is required) in the 1970s (25) and was later banned from use in the EU. Nowadays, photoallergic contact dermatitis is uncommon (26). Furocoumarins (psoralens) in some plant-derived fragrance ingredients caused phototoxic reactions with erythema followed by hyperpigmentation resulting in Berloque dermatitis (8) [pp 417–432]. There are now limits for the amount of furocoumarins in fragrance products. Phototoxic reactions still occur but are rare (27).

#### **4.1.5. General/respiratory**

Fragrances are volatile and therefore, in addition to skin exposure, a perfume also exposes the eyes and naso-respiratory tract. It is estimated that 2–4% of the adult population is affected by respiratory or eye symptoms by such an exposure (28). It is known that exposure to fragrances may exacerbate pre-existing asthma (29). Asthma-like symptoms can be provoked by sensory mechanisms (30). In an epidemiological investigation, a significant association was found between respiratory complaints related to fragrances and contact allergy to fragrance ingredients, in addition to hand eczema, which were independent risk factors in a multivariate analysis (31).

## 4.2. Epidemiology of fragrance allergy

### 4.2.1. Substances used for screening of contact allergy to fragrance ingredients

A fragrance formula may consist of ten to 300 or more different ingredients. The CosIng database lists 2587 ingredients used for perfuming<sup>1</sup>, as well as several other materials classified as odour “masking” agents, which is equivalent with regard to allergy. A mixture of seven fragrance chemicals and one natural extract, which have been identified as major fragrance allergens in the past (32), are used for diagnosing contact allergy to fragrance ingredients (Table 4-1). This mixture is called the Fragrance Mix (FM I) and is included in the standard patch test tray containing the most common allergens in Europe.

**Table 4-1:** Ingredients of Fragrance Mix I (FM I; 8% allergens in petrolatum).

Single constituent: INCI name (common name)	Conc. (%)
Amyl cinnamal (alpha-amyl cinnamal)	1
Cinnamyl alcohol (cinnamic alcohol)	1
Cinnamal (cinnamic aldehyde)	1
Eugenol	1
Geraniol	1
Hydroxycitronellal	1
Isoeugenol	1
Oak moss absolute (a natural extract; INCI: <i>Evernia prunastri</i> )	1
Sorbitan sesquioleate (added as an emulsifier)	5

Note: All single allergens of the above, when used for breakdown testing, are also in petrolatum.

However, due to the introduction of new fragrance ingredients (with allergenic potential), the above Fragrance Mix I was deemed not to be sufficient for the diagnosis of fragrance allergy. Thus, Fragrance Mix II was devised to supplement Fragrance Mix I in a European multicentre study (33, 34). Since then, FM II has been included in the European baseline series. Table 4-2 lists the ingredients of FM II. In addition to being tested in FM II, hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) is also tested separately at 5% test concentration in the baseline series (35).

<sup>1</sup> <http://ec.europa.eu/enterprise/cosmetics/cosing/index.cfm?fuseaction=search.results&function=66&search>, last accessed 2009-10-14.



**Table 4-2:** Ingredients of Fragrance Mix II (FM II; 14% allergens in petrolatum).

<b>Single constituent: INCI name (common name)</b>	<b>Conc. (%)</b>
Citronellol	0.5
Citral	1
Coumarin	2.5
Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)	2.5
Farnesol	2.5
Alpha-hexyl-cinnamal	5

Note: All single allergens of the above, when used for breakdown testing, are also in petrolatum.

Patch test results in patients and in population samples with these two screening mixes, and single allergens, will be presented and discussed in the following two sections.

#### **4.2.2. Clinical epidemiology**

For a number of reasons the bulk of the evidence regarding the frequency of contact allergy to fragrance ingredients relies on clinical data, i.e. the history, clinical presentation and test results of patients patch tested for suspected allergic contact dermatitis – in general, and not specifically due to fragrance ingredients. The frequency of contact allergy to fragrance ingredients (or other contact allergies, for that matter) cannot be related to the population directly, as it is derived from a subgroup (of patients) selected for specific morbidity. Nevertheless, these data can be examined epidemiologically assuming a largely similar selection process: (i) across time in a given department; and (ii) between departments at any point of time. If the notion of similarity, and thus direct comparability, does not appear valid, adjustment or standardisation techniques can be employed to account for differences, e.g. the average age of patients in a time series on a (fragrance) allergen with age-associated risk of sensitisation. In this situation, changes in the age composition of the patients tested may confound a time trend. A distinction must be made between patch testing “consecutive” patients, i.e. all patients who are patch tested for suspected contact sensitisation, and “aimed” patch testing, i.e. application of allergens only in the subset of patients in whom exposure to the particular allergens of the applied “special series” is suspected. For any given allergen, the latter “aimed” approach will usually yield higher sensitisation prevalences than the testing of not-further-selected “consecutive” patients. Thus, information on the inclusion of an allergen either in a baseline series (tested in virtually all patients) or in a special series (applied in an aimed fashion) must be considered and is given in the following tables, where available in the cited references.

Notwithstanding the potential pitfalls of clinical data, they have proven useful in identifying emerging trends or persisting problems, and also in evaluating the effect of preventive action – either regarding the entire population, or subgroups thereof, such as certain occupations. Regarding the fragrance mixes (FM I and FM II) mentioned above, evidence regarding sensitisation frequencies published since 1999 will be outlined below, thus supplementing the data presented in the SCCNFP opinion on Fragrance Allergy in 1999 (1).



**Fragrance Mix I (“Larsen Mix”)****Table 4-3:** Results with screening agents for contact allergy to fragrance ingredients reported since 1999 in patients patch tested for suspected allergic contact dermatitis in Europe: Fragrance Mix “I” (see Table 4-1). If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS <sup>(§)</sup>.

Country (Ref.)	Population	Year(s)	No. tested	Crude % positive (95% CI)
Sweden (36)	Consecutive patients	2000	3790	6.9
Hungary (37)		1998-1999	3604	8.2 (7.3–9.1) <sup>§</sup>
Czech Republic (38)		1997-2001	12058	5.8 (5.4–6.2) <sup>§</sup>
Ljubljana, Slovenia (39)	Consecutive patients	1989-1998	6129	5.9 (5.3–6.5) <sup>§</sup>
Germany (40)	Consecutive IVDK patients	1996-2002	59298	11.3 (11.0–11.5) <sup>§</sup>
Germany (41)	Consecutive IVDK patients	2005-2008	36961	7.3 (7.0–7.6) <sup>§</sup>
Vienna, Austria (16)	Consecutive patients of one clinic	1997-2000	2660	9.1 (8.1–10.3) <sup>§</sup>
Groningen, Netherlands (42)	Patients (fragrance allergy suspected)	04/2005-06/2007	295	5.8 (3.4–9.1) <sup>§</sup>
The Netherlands (43)	Consecutive patients	09/1998-04/1999	1825	10.6 (9.2–12.1)
The Netherlands (44)	Patients (cosmetic allergy suspected)	1994-1998	757	14.8 (12.3–17.5) <sup>§</sup>
Leuven, Belgium (45)	Consecutive patients	1990-2005	10128	9.1 (8.6–9.7) <sup>§</sup>
Coimbra, Portugal (46)	Consecutive patients	07/1989-06/1999	2600	10.9 (9.7–12.2) <sup>§</sup>
Sheffield, UK (47)	Consecutive patients	1994-1995	744	11.4 (9.2–13.9) <sup>§</sup>
St. John’s, London, UK (48)	Consecutive patients	1980-2004	34072	7.7 (7.4–8.0) <sup>§</sup>
Copenhagen, Denmark (49)	Consecutive patients	1985-2007	16173	7.2 (6.8–7.6) <sup>§</sup>
ESSCA (50)	Consecutive patients	2002-2003	9663	7.1 (6.6–7.6) <sup>§</sup>
ESSCA (51)	Consecutive patients	2004	9941	7.6 (7.1–8.2) <sup>§</sup>
ESSCA (52)	Consecutive patients	2005-2006	18542	7.0 (6.6–7.4) <sup>§</sup>

**Table 4-4:** Results with screening agents for contact allergy to fragrance ingredients reported since 1999 in patients patch tested for suspected allergic contact dermatitis in non-European countries: Fragrance Mix "I" (see Table 4-1). If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS <sup>(5)</sup>.

Country (Ref.)	Population	Year(s)	No. tested	Crude % positive (95% CI)
South Korea (53)	Consecutive patients	04/2002–06/2003	422	9.7 (7.1–13.0) <sup>§</sup>
Lahore, Pakistan (54)	Dermatitis patients	2 years prior to 2002	350	7.7 (5.2–11.0) <sup>§</sup>
Manipal, India (55)	Dermatitis patients	1989-1998	1780	3.1 (2.3–4.0) <sup>§</sup>
Tel Aviv, Israel <sup>§</sup> (56)	Consecutive patients	1999-2000	943	8.5 (6.8–10.5) <sup>§</sup>
Tel Aviv, Israel (57)	Consecutive patients	1998-2004	2156	7.1 (6.1–8.3) <sup>§</sup>
Tehran, Iran (58)	Consecutive patients	2002-2004	250	4.0 (1.9–7.2) <sup>§</sup>
Ankara, Turkey (59)	Consecutive patients	1992-2004	1038	2.1 (1.3–3.2) <sup>§</sup>
Beijing, China (60)	Consecutive patients	2000-2003	378	15.9 (12.3–20.0) <sup>§</sup>
USA (Canada) (61)	Probably consecutive patients	2003	1603	5.9
NACDG 2009 (US and Canada) (62)	Consecutive patients	2005-2006	4439	11.5

Note: § Possibly included in (57).

Beyond the studies discussed above, regarding a time trend of sensitisation to FM I, a significant increase of positive results to FM I until 1998, and a significant drop thereafter has been noted in the IVDK study covering 1996 to 2002 (40). A similar drop from 1999 to 2007 has been observed in female, but not male patients from Copenhagen (49). In accordance with these findings, the prevalence of positive reactions to FM I doubled, or thereabouts, from 1989-1993 to 1994-1998 in Ljubljana, Slovenia (39).

Within Europe, a comparison between different countries and clinical departments is possible. An EECDRG study covering 1996-2000 found 9.7% positives to FM I (range: 5.0–12.6% in ten departments from seven European countries (63)). A different European study, covering 10/1997-10/1998, found 11.3% (95% CI: 9.9–12.9%) positive reactions to FM 1 in 1,855 patients; the variation between centres was marked: Gentofte 8.2% vs. Leuven 23.0% as extremes (64). In the first study of the European Surveillance System on Contact Allergies (ESSCA), covering 2002 and 2003, 9663 patients were patch tested with FM I, overall yielding 7.1% positive reactions with marked variation between participating departments. In Dortmund, Germany, the minimum frequency of 3.7% was noted, while in Lahti, Finland, the highest prevalence, namely 10.4%, was found (50). Subsequently, in the year 2004, the overall prevalence was 7.6%, i.e. largely unchanged (51). In the most recent study by ESSCA, based on 2005/2006 PT data across Europe, significant differences were again noted, this time on the aggregated level of European regions, with FM I sensitisation being the least frequent in the Southern countries (4.8% [95% CI: 3.9–5.5%] age- and sex-standardised prevalence) vs. 7.7% (95% CI: 7.0–8.4%) in the central European departments, with the Finnish, Polish and Lithuanian departments (5.7% [95% CI: 4.6 – 6.8%]) and the UK network (6.8% [95% CI: 6.3 – 7.3%]) in an intermediate position (52).

**Fragrance Mix II**

**Table 4-5:** Results with screening agents for contact allergy to fragrance ingredients reported since 1999 in patients patch tested for suspected allergic contact dermatitis: Fragrance Mix "II" (see Table 4-2). The FM II was only conceived in 2005, so results are still sparse). If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS (§).

Country (Ref.)	Population	Year(s)	No. tested	Crude % positive (95% CI)
EU (33)	Six clinical depts.	10/2002-06/2003	1701	2.9 (2.2–3.9) <sup>§</sup>
Germany (65)	IVDK patients	01/2005-12/2008	35633	4.9 (4.7–5.1) <sup>§</sup>
Groningen, Netherlands (42)	Patients (fragrance allergy suspected)	04/2005-06/2007	227	9.3 (5.8–13.8) <sup>§</sup>
Leuven, Belgium (45)	Consecutive patients	2005 only	335	2.1 (0.8–4.3) <sup>§</sup>
Denmark (66) on behalf of the DCDG, 2010	Consecutive patients	2005-2008	12302	4.5 (4.1–4.9) <sup>§</sup>

**Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)**

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) has been the most frequently reported chemical causing fragrance allergy since the 1999 opinion on fragrance allergy. In total, reports of about 1500 cases have been published in the scientific literature (see section 7.1).

HICC was recognised as an allergen in 1995 (67) and later included in the new perfume mixture, Fragrance Mix II (68), which is routinely used for the diagnosis of perfume allergy, see above. Furthermore, it is recommended to test separately with HICC, because it is a very frequent allergen (35) and detects relevant fragrance sensitisation which would otherwise have been missed (69). In the studies performed in European dermatology clinics, 0.5-2.7% of eczema patients have been found to be allergic to HICC with the highest frequency in central Europe (52). For further details see Table 4-6.

**Table 4-6:** Results with fragrance contact allergy screening agents reported since 1999 in patients patch tested for suspected allergic contact dermatitis: **HICC** (5% pet. if not stated otherwise). If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS (§).

Country (Ref.)	Population	Year(s)	No. tested	Crude % positive (95% CI)
Lithuania (70)	Consecutive patients	04/2006-10/2008	816	0.9 (0.3–1.8) <sup>§</sup>
Spain (69)	Consecutive patients	10/2005-06/2008	852	0.8 (0.3–1.7) <sup>§</sup>
Germany (CH, AT) (71)	Consecutive patients	03/2000-02/2001	3245	1.9 (1.5–2.4) <sup>§</sup>
Germany (CH, AT) (72)	Consecutive patients	01/2003-12/2004	21325	2.4 (2.2–2.6) <sup>§</sup>

Country (Ref.)	Population	Year(s)	No. tested	Crude % positive (95% CI)
Germany (CH, AT) (65)	Consecutive patients	01/2005-12/2008	35582	2.3 (2.2–2.5) <sup>§</sup>
Belgium (45)	Consecutive patients	2002-2005	2901	2.1 (1.6–2.7) <sup>§</sup>
Denmark (66)	Consecutive patients	2005-2008	12302	2.4 (2.1–2.7) <sup>§</sup>
South Korea (53)	Consecutive patients	04/2002–06/2003	422	1.7 (0.6–3.4) <sup>§</sup>
USA, Canada (61)	Probably consecutive patients	2003	1603	0.4 (0.2–0.9) <sup>§</sup>

### ***Myroxylon pereirae (Balsam of Peru)***

*Myroxylon pereirae* is a balm obtained from a Central American tree. It is used as a screening substance for fragrance allergy in Europe and other geographical areas. Although the crude balm is not used in Europe in cosmetics, extracts and distillates are used (73). This natural mixture has been employed as screening agent in the baseline series for many decades. Hence, a wealth of data is available; Table 4-7 summarises results of the past 10 years.

**Table 4-7:** Results with fragrance contact allergy screening agents reported since 1999 in patients patch tested for suspected allergic contact dermatitis: *Myroxylon pereirae* resin (Balsam of Peru) (25% pet.). If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS (<sup>§</sup>).

Country (Ref.)	Population	Year(s)	No. tested	Crude % positive (95% CI) <sup>§</sup>
Tel Aviv, Israel (56) #	Consecutive patients	1999-2000	943	6.6 (5.1–8.4) <sup>§</sup>
South Korea (53)	Consecutive patients	04/2002 – 06/2003	422	7.3 (5.1–10.3) <sup>§</sup>
Tel Aviv, Israel (57)	Consecutive patients	1998-2004	2156	3.6 (2.9–4.5) <sup>§</sup>
Manipal, India (55)	Dermatitis patients	1989-1998	1780	1.0 (0.5 – 1.5) <sup>§</sup>
Tehran, Iran (58)	Consecutive patients	2002-2004	250	2.4 (0.9–5.2) <sup>§</sup>
Sevilla, Spain (74)	Consecutive patients	2002-2004	863	5.8 (4.3–7.6) <sup>§</sup>
Ankara, Turkey (59)	Consecutive patients	1992-2004	1038	2.1 (1.3–3.2) <sup>§</sup>
Vienna, Austria (16)	Consecutive patients of one clinic	1997-2000	2660	5.4 (4.6–6.3) <sup>§</sup>
Czech Republic (38)	Consecutive patients	1997-2001	12058	7.3 (6.8–7.8) <sup>§</sup>
Copenhagen, Denmark (49)	Consecutive patients	1985-2007	16173	3.9 (3.6–4.2) <sup>§</sup>

Country (Ref.)	Population	Year(s)	No. tested	Crude % positive (95% CI) <sup>§</sup>
Sweden (36)	Consecutive patients	2000	3790	6.5
Nine European countries (50)	Consecutive patients	2002-2003	9672	6.1
Germany, three Swiss and one Austrian Dept. (41)	Consecutive patients	2005-2008	36919	8.0 (7.7–8.3)
Ten depts. From seven EU countries (63)	Consecutive patients	1996-2000	26210	6.0
USA (Canada) (61)	Probably consecutive patients	2003	1603	6.6
NACDG 2009 (62)	Consecutive patients	2005-2006	4449	11.9

### *Oil of turpentine*

This natural extract is not tested in all baseline series. It is considered as a minor screening allergen for fragrance contact allergy. Moreover, oil of turpentine is used as a raw material in perfumery (see Annex I). Table 4-8 summarises results of the past 10 years with patch testing of consecutive patients.

**Table 4-8:** Results with fragrance contact allergy screening agents reported since 1999 in patients patch tested for suspected allergic contact dermatitis: **Oil of turpentine** (10% pet.) patients patch tested for suspected allergic contact dermatitis. If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS (<sup>§</sup>).

Country	Population	Year(s)	No. tested	Crude % positive (95% CI) <sup>§</sup>
Lisbon, Portugal (75); virtually no .delta.-3-carene	Consecutive patients	1979-1983	4316	2.3 (1.9–2.8) <sup>§</sup>
Birmingham, UK (76)	Potters with occup. hand dermatitis	6 months; prior to 1996	24	14/4 pos. to "Indonesian turpentine"
Austria/Germany (IVDK) (77)	Consecutive patients	1992-1995	27658	0.47 (0.39–0.55) <sup>§</sup>
Austria/Germany (IVDK) (40)	Consecutive patients	1996-2002	59478	Annual prevalence 1.6 to 4.4%
Augsburg, Germany (78)	Population sample	1998	1141	1.2% (on population level!)
Europe (ESSCA) (50)	Consecutive patients	2002/03	3767	1.6%
Austria/Germany/Switzerland (IVDK) (41)	Consecutive patients	2005-2008	37163	1.8%

An "overall burden" of fragrance contact allergy, in terms of the prevalence of contact allergy to at least one of the up-to-five screening allergens present in the baseline series (FM I, FM II, HICC, *Myroxylon pereirae*, oil of turpentine) has not been given in the published studies. A re-analysis of data from the two published studies of the IVDK (41,

65), covering central Europe from 2005 to 2008 (Germany, Austria and Switzerland), yielded an estimate of such overall prevalence of 16.2% (95% CI: 15.8-16.6%) (IVDK technical report, 2011-11-18).

#### 4.2.3. Population-based epidemiology

In principle, the examination of a representative sample of the population is the most valid approach for estimating disease frequency, as there is no systematic selection process. However, in practice, participation of much less than 70% of those approached introduces the possibility of self-selection and thus of biased morbidity (or risk) estimates. Moreover, the resources needed prohibit regular, e.g. yearly, patch test studies in a sample of several thousand persons. For these reasons few studies exist (see Table 4-9).

A Swedish study of hand eczema in an industrial city showed that among 1,087 individuals recruited from the general population with symptoms of present or previous hand eczema, 5.8% were positive to the Fragrance Mix (79). In Denmark, Fragrance Mix sensitivity was found in 1.1% (0.3-2.1%) of 567 persons drawn as a sample from the general Danish population; only nickel sensitivity was more prevalent (80). In Italy, female patients with hand eczema caused by contact with detergents were patch tested. Of 1100 women, 3.1% reacted to Fragrance Mix I (81). A control group of 619 female patients with no eczema disease were also patch tested; 1.3% were positive to the Fragrance Mix (81). On the other hand, in a sample of 593 healthy Italian recruits, only three positive reactions (0.50%) to FM I were observed (82). Among Danish school children, 14-15 years of age, fragrance contact allergy was detected in 1.8% by patch testing with Fragrance Mix I (83). A study of 85 American student nurses showed that 15 (17.6%) had a positive reaction to Fragrance Mix I; 12 of the individuals also had a positive history of contact dermatitis (84). In this study the concentration of Fragrance Mix I was 16% as opposed to the currently recommended concentration of 8% and the study included only young females. Both of these factors may have contributed to the high prevalence of fragrance sensitivity found.

In 1990, 1998 and 2006, samples of the Danish adult population living in the Copenhagen area were patch tested with the European baseline series. In total 4299 individuals aged 18-69 years (18-41 years only in 1998) completed a pre-mailed questionnaire and were patch tested with FM I and *Myroxylon pereirae* (80, 85, 86). In 1990, 1.1% were found positive to FM I and in 2006, 1.6% were positive, which means no general change. However, when the age group of 18-41 years was analysed, the prevalence of FM I sensitisation followed an inverted V-pattern among women, i.e. an increase from 0.7% in 1990 to 3.9% in 1998, followed by a decrease to 2.3% in 2006. The participation rate varied in the three samples from 71.5% in 1990 to 52.4% in 1998, and to 43.7% in 2006 (80, 85, 86).

Contact sensitisation to FM I is strongly age related, with the relative risk more than doubling in the older age groups, compared to younger PT patients. This has been found in both bivariate (87) and adjusted multifactorial analyses (88). Hence, in older samples of the population, the prevalence of contact allergy to fragrance ingredients in general, and to FM I in particular, can be expected to be higher than in younger samples. From this background, the strikingly high prevalence observed in the MONICA/KORA allergy study in Augsburg, Germany (see Table 4-9) (78), may be explained, together with some residual confounding from the rather complex sampling process.

**Table 4-9:** Results from patch testing with Fragrance Mix I in different population based groups.

Country (Ref.)	Population	Year(s)	No. tested	% positive (95% CI)
Italy (81)	Females without eczema	Not given	619	1.3
Italy (82)	Male recruits	Not given	593	0.50

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Country (Ref.)	Population	Year(s)	No. tested	% positive (95% CI)
Denmark (80)	Population sample adults, 15-69 years	1990-91	567	1.1
Denmark (83)	School children 12-16 years old	1995/96	717	1.8
Denmark (80, 85)	Population sample adults, 18-41 years	Jan-Nov 1998	414	2.7
Denmark (86)	Population sample adults, 18-69 years	June 2006–May 2008	3460	1.6
Norway (89)	Population sample adults, 18-69 years. (Results reported in 2007)	1994 (90)	1236	1.8 (1.1–2.7)
Germany (78)	Subgroup of MONICA sample, age 25-74	1994/95	1141	11.4
USA (84)	Student nurses, females	1980	85	17.6*
Sweden (79)	Population sample adults, age 20-65 years reporting hand eczema	1983-84	1087	5.8*

Note: \* Testing performed with Fragrance Mix I, containing 16% allergens; the currently used Fragrance Mix I contains 8% allergens (see above).

**Table 4-10:** Results from patch testing with other fragrance allergens in different population based groups. If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS (5).

Country (Ref.)	Population	Year(s)	Fragrance allergen	No. tested	% positive (95% CI) <sup>§</sup>
Thailand (91)	Convenience sample (via advertisement), age 18-55	Not given	Isoeugenol, <i>Evernia prunastri</i> , <i>Myroxylon pereirae</i> *	2545	Positive to at least one of three allergens: 2.5 (1.9–3.2) <sup>§</sup>
Germany (78)	Subgroup of MONICA sample, age 25-74	1994/95	<i>Myroxylon pereirae</i>	1141	2.4
Denmark (86)	Population sample, age 18-69	1990 2006	<i>Myroxylon pereirae</i>	567 3460	1.1 0.1

Note: \* *Myroxylon pereirae* is a balm obtained from a Central American tree. It is used as a screening substance for fragrance allergy in Europe and other geographical areas. Although the crude balm is not used in Europe in cosmetics, extracts and distillates are used (73).



### **4.3. Consumer products as a cause of fragrance contact sensitisation and allergic contact dermatitis**

#### **4.3.1. Clinical relevance**

Clinical relevance is a concept used to describe the significance of a positive (allergic) patch test reaction for an individual patient: a reaction is deemed relevant if contact allergy to the substance is associated with previous or current episodes of allergic contact dermatitis. Thereby, the evaluation of clinical relevance links past exposure to morbidity. For the evaluation of relevance, past or recent exposure(s) to the allergen need to be identified in the patient's history. The success of this process generally depends on:

- The patient's understanding and awareness;
- The dermatologist's knowledge concerning exposures;
- Ingredient labelling; and
- Information about the actual chemical composition of the implicated product.

As these requirements may be met to a varying extent, the validity of relevance information as reported in clinical studies may also be variable. However, information on clinical relevance is important, in principle, because the proportion of currently relevant sensitisations reflects the amount of current exposure and resulting disease state, which may increase or decrease with time. In this way, current relevance also reflects the direct burden of a fragrance contact allergy to the individual and indirectly to society. Further important aspects of the evaluation of clinical relevance as a final step of patch testing have been discussed (92-95).

Generally, clinical relevance is categorised as "current", "previous" or "unknown". Further differentiation has been introduced by adding information on:

- Occupational versus non-occupational causation; and
- The level of certainty of the relevance statement, e.g. as "certain", "probable", "possible".

In some cases, clinical relevance may not be established due to:

- Immunological cross-reactivity with an individual allergen, diagnosed or not;
- Active sensitisation by the patch testing;
- Contact sensitisation not caused by the substance, but by a contaminating constituent; or
- Failure to test with a true hapten (e.g. haptens formed from prehapten on exposure to air, see chapter 5).

It should be noted that this statement on clinical relevance refers to the past history of a patient. This implies that a lack of, or unknown, clinical relevance does not make future allergen avoidance unnecessary.

In the context of contact allergy to fragrance ingredients, a number of alternative concepts of relevance have been used, for example:

- A history of intolerance to perfume or to perfumed products;
- A history of intolerance to perfume actually containing the allergen diagnosed;
- Detection of the culprit allergen in a perfume previously used.

#### **4.3.2. Elicitation with clinical symptoms/signs, current and past**

In case reports or small series, the clinical relevance of positive patch test reactions is usually well established and presented in detail. Moreover, a number of large-scale clinical



studies on contact allergy to fragrance ingredients have reported results on clinical relevance, which will be presented and discussed in this section. The studies can be subdivided into those which focus on medical history, patch testing with consumer products or detection of specific allergens in consumer products used by patients.

### **Medical history**

A series of studies conducted in the 1990s showed that most individuals with contact allergy to fragrance ingredients were aware that they could not tolerate fragranced products on their skin and were able to specifically name product categories that initiated their disease (9). In this context, colognes, deodorants and lotions were named significantly more often by fragrance allergic dermatitis patients than by patients without fragrance contact allergy (3). These studies are described in the SCCNFP opinion on fragrance allergy of 1999 (1). Newer studies are outlined below.

#### *NACDG 2009 study (62)*

The definition of "present" clinical relevance in this North American network study was strict, requiring:

- A positive use or patch test with the suspected item(s) for "definite" relevance; and
- Verification of the presence of the allergen in known skin contactants, and consistent clinical presentation for "probable".

If these conditions were not met, but skin contact to items generally containing the item was likely, "possible" was used.

Regarding fragrance allergens, the proportions were as described in Table 4-11.

**Table 4-11:** Extract from ((62) Table 3) regarding the proportion of patients with "present clinical relevance" (see text) and "past clinical relevance" (criteria not given).

Fragrance allergen	n (tested)	% (pos.)	Current relevance (%)			Past relevance (%)
			Definite	Probable	Possible	
<i>Myroxylon pereirae</i>	4449	11.9	1.3	33	53	2.7
FM I	4439	11.5	2.0	29.4	54.3	4.3
Cinnamal	4435	3.1	1.5	33.8	50	2.9
Ylang-Ylang oil	4434	1.5	4.6	10.8	73.8	1.5
Jasmine absolute	4447	1.1	0	24.5	67.3	6.1

#### *Frosch 2002 (a) study (64)*

In this study, 1,855 consecutive patients were patch tested with FM I and a series of a further 14 fragrance chemicals. Prior to the test, the history of adverse reactions to fragrances was classified as "certain" (6.6%), "probable" (8.0%), "questionable" (9.2%) or "none" (76.1%) (see (68)).

#### *Frosch 2002 (b) study (96)*

A series of 18 essential oils or components thereof, together with FM I, was assessed in 1,606 consecutive patients. Similar to the above study, the proportions of patients with a "certain" or "probable" history (or otherwise) and positive reactions to either FM I or the special series, or both, were cross-tabulated. Of note, 53.7% of patients with positive reactions to FM I only, had no history. Similarly 54.2% of patients with positive reactions

only to one of the essential oils had no history. However, in cases of reactivity to both FM I and one of the essential oils, the proportion of patients with no history was only 36.5%.

#### *Frosch 2005 study (33)*

The diagnostic properties of FM I and the new FM II were evaluated in 1,701 consecutive patients patch tested in six European centres. Contrasting a "certain" (found in 8.7% of patients) with "no history" (75.3% of patients), the sensitivity of FM I was 25.2%, and the positive predictive value (PPV) 45.1%. In comparison, the sensitivity of FM II at 14% concentration was 13.5% and the PPV was 55.6%. The combination of the two mixes was important, as more patients with a "certain" history, but also independently from history, reacted to just one of the mixes rather than to both.

#### *Danish Contact Dermatitis Group 2005-2008 (66)*

In 12302 consecutive patients patch tested in seven dermatology clinics and three university hospitals, 10.6% were positive to one or more of the fragrance allergy markers (FM I, FM II, *Myroxylon pereirae* or hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)). Clinical relevance covered current and/or past relevance based on: 1) medical history; 2) results of patch and/or use tests; 3) ingredient labelling; or 4) chemical analysis. Clinical relevance was found in 71.0% of cases positive to FM I, 72.2% of those positive to FM II and 76.7% of those positive to HICC. These proportions were higher than the average for other cosmetic allergens such as preservatives and hair dyes, which gave relevant reactions in about 50% of those positive, as did *Myroxylon pereirae*. *Myroxylon pereirae* itself is not used in cosmetics as it is banned, but sensitisation may be caused by exposures to related substances and thus relevance may be difficult to determine.

### **Cosmetic products**

#### *Fragrance formulae from cosmetic products*

Popular fine fragrances (5), as well as toilet soaps, shampoos, lotions, deodorants, and aftershaves have been shown to provoke allergic contact dermatitis in patients when used for patch testing (5, 6, 97, 98). Moreover, commercially available fragrance formulae and dilutions of individual fragrance allergens were potent elicitors of allergic contact dermatitis under simulated use conditions (10, 99, 100).

More recently, deodorants spiked with the fragrance allergens cinnamal, hydroxycitronellal and HICC, respectively, in realistic in-use concentrations were shown to elicit allergic contact dermatitis in 89-100% of the fragrance allergic individuals tested (101-103). In 87.5% of HICC sensitised individuals the use of a cream (and in 82.8% the use of an ethanol solution) spiked with HICC provoked dermatitis (104). These studies are discussed in more detail in chapter 11 on quantitative aspects. Other new studies are mentioned below:

#### *IVDK "own perfumes" study (105)*

A different perspective on clinical relevance is provided by assessing the proportion of positive reactions to the FM I or single fragrance allergens in patients who had not tolerated certain perfumed products, such as deodorants and aftershaves and who were patch test positive to these cosmetics. The following two tables are taken from this publication.

**Table 4-12:** Extract from ((105) Table 2) on the frequency of positive reactions to fragrance allergens in patients with vs. without positive patch test reaction to their own deodorant.

Fragrance allergen	Conc. (%)	Deodorant positive (n=66)		Deodorant negative (n=855)	
		n (test)	% pos. (95% CI)	n (test)	% pos. (95% CI)
Fragrance Mix I	8	61	38.0 (24.1-51.9)	805	15.0 (12.5-17.5)
<i>Myroxylon pereirae</i>	25	60	22.9 (12.7-33.1)	806	9.1 (7.2-11.0)
Hydroxycitronellal	1	33	6.5 (0.7-12.3)	204	4.3 (1.5-7.1)
Isoeugenol	1	33	6.5 (0.7-12.3)	204	7.2 (3.6-10.8)
Cinnamal	1	29	11.3 (0-24.1)	133	1.1 (0-2.7)
Geraniol	1	29	8.3 (0-20.4)	141	0 (0-2.1)

Of the 66 patients with a positive patch test reaction to their own deodorant, most had positive reactions to one or more fragrance allergens. This was much more prevalent than those patients in whom no positive reaction to their deodorant was observed. This observation supports the notion that the respective fragrance allergens are important in contact allergy to fragrance ingredients caused by deodorants, supporting data regarding exposure (chapter 10.1).

**Table 4-13:** Extract from ((105) Table 2) on the frequency of positive reactions to fragrance allergens in patients with vs. without positive patch test reaction to their own aftershave, eau de toilette or perfume.

Fragrance allergen	Conc. (%)	Product positive (n=63)		Product negative (n=819)	
		n (test)	% pos. (95% CI)	n (test)	% pos. (95% CI)
Fragrance Mix I	8	56	57.1 (46.2-68.1)	764	13.9 (11.4-16.4)
<i>Myroxylon pereirae</i>	25	56	13.9 (7.3-20.4)	766	8.8 (6.8-10.7)
HICC	5	20	58.3 (37.5-79.0)	310	1.3 (0-2.7)
<i>Evernia prunastri</i>	1	28	22.1 (7.0-37.2)	153	8.8 (4.2-13.4)
Hydroxycitronellal	1	33	6.5 (0.7-12.3)	204	4.3 (1.5-7.1)
<i>Cananga odorata</i> (ylang-ylang oil)	10	7	16.3 (2.0-30.5)	43	5.0 (0-11.3)

Similar results were obtained from the subgroup of patients with a positive reaction to their eau de toilette, aftershave (hydroalcohol solutions) or perfumes (Table 4-13). However, notable differences were: (i) the greater relative importance of *Evernia prunastri* (Oak moss absolute); and (ii) generally an extremely high proportion of positive reactions to various other fragrance ingredients.

#### 4.3.3. Elicitation in diagnostic patch tests without clinical history

In a variable proportion of patients, a positive patch test reaction does not correlate with recent or past episodes of presumptive allergic contact dermatitis. Apart from particular circumstances, such as cross-reactivity or reactivity to contaminants outlined above, there are several possible explanations for this:

- The patch test reaction was a false-positive (irritant).
- There was erroneous recall/interpretation of the patient's history (false-negative).
- Lack of knowledge concerning exposures.

- If the patient is weakly sensitised (e.g. by a low induction dose), the occlusive exposure during patch testing may have been the only exposure above the individual elicitation threshold capable of eliciting an unequivocal allergic contact reaction. In this situation, clinical relevance would be classified as “unknown”. Nevertheless, there is an alteration of the immune status of the individual.

Sometimes, a repeated open application or provocative use test is employed to mimic “normal” exposure to the allergen. A positive reaction to such a use-related test confirms actual sensitisation. Moreover, the positive result supports the necessity of future allergen avoidance. Apart from the risk of developing allergic contact dermatitis in the future, sensitisation means an alteration of the immune status of the individual.

#### **4.4. Socio-economic impact of contact allergy**

##### **4.4.1. Health related quality of life**

Skin diseases in general are known to affect quality of life significantly (106); this also applies to eczema, where most studies concern atopic dermatitis and hand eczema patients (107, 108). Hand eczema has a poor prognosis and may affect the self-image, limit social activities and lead to occupational restrictions (108, 109). The quality of life in hand eczema patients with fragrance contact allergy is affected in a similar degree as patients with other contact allergies (110).

In a questionnaire study of 117 patients recently diagnosed with contact allergy to fragrance ingredients, most presented with hand or facial eczema. In response to the question if and how fragrance allergy had affected their life situation, 67.5% replied that they often had to take special precautions, 47.0% replied that they were often bothered by eczema and itch, 17.1% said that they had had to take sick leave due to their fragrance contact allergy and 45.3% felt that fragrance contact allergy had significantly influenced their daily living (111).

##### **4.4.2. Occupational restrictions**

Contact allergy is known to influence severity and prognosis of hand eczema (112, 113) including risk of sick leave (110). Fragrance contact allergy is mostly of a non-occupational origin (88) related to the personal use of scented cosmetics, but may have secondary occupational consequences. This may be due to exposure to fragrance ingredients also in the work place or because hand eczema has developed. Hand eczema itself may make it impossible to remain in the trade even if protective equipment is used. In young people, fragrance allergy may limit the choice of occupations, as it will be difficult to work as a hairdresser, cosmetologist or in other occupations with a significant skin exposure to fragranced products.

##### **4.4.3. Costs to health care/health economics**

In a population based study of 3,460 individuals, contact allergy to FM I was found in 1.6%; logistic regression analyses showed that medical consultation due to cosmetic dermatitis (OR 3.37, 95% CI 1.83-6.20) and cosmetic dermatitis within the past 12 months (OR 3.53, CI 2.02-6.17) were significantly associated with sensitisation to FM I (86). Further, as mentioned above, fragrance allergy may lead to sick leave (111). No specific cost estimates for fragrance allergy exist, but the yearly total costs of contact dermatitis in Western Europe was estimated to be 5.2 billion Euro in 1997. Prices were based on the Allergy White Paper (1997) and on results of investigations and extrapolations of known data for Western Europe (114). Fragrance allergy is the second most frequent cause of contact allergy after nickel allergy and is seen in every 10<sup>th</sup> patient investigated for contact allergy. Even a modest reduction in nickel allergy has been estimated to have the value of 12 million Euro/year/million people in Denmark (Environmental Project Nr. 929, 2004; <http://www2.mst.dk/Udgiv/publications/2004/87-7614-295-7/pdf/87-7614-296-5.pdf>, last accessed 2011-11-13). The costs are likely to differ in other countries, some with higher

expenses and some with lower costs. These estimates show that the cost of contact allergy in the population may be considerable.

#### **4.5. Allergen avoidance**

Generally, “allergen avoidance” can be regarded as having two aspects: (i) primary prevention of the acquisition of contact allergy achieved by avoiding or limiting exposure of the general population, or certain parts of it, to allergens; and (ii) secondary prevention in terms of avoiding (re-)elicitation of allergic contact dermatitis in sensitised individuals.

##### **4.5.1. Primary prevention: limiting or eliminating exposure to allergens in the population**

The main aim of public health is the primary prevention of disease in populations. Allergic contact dermatitis (to fragrances) has the potential to have a significant impact on quality of life, including effects on fitness for work (chapter 4.4). Moreover, it is a common phenomenon and therefore a reduction of exposure to potential (fragrance) allergens must be an objective of effective Public Health measures.

Means of limiting or eliminating exposure to fragrance allergens include the following:

- *Prohibition* by regulatory measures or other means.
- *Restriction* by regulatory measures or other means of the maximum permissible concentration of a substance, or a critical component of natural mixtures, possibly according to different uses and product types, respectively.
- *Substitution* with suitable, but less or non-allergenic compounds. Substitution by a component which is chemically different, but effectively not different in terms of allergenicity or cross-reactivity, is not adequate (e.g. an ester) (chapter 5).
- *Formulating the fragrance* with the aim of limiting or eliminating those substances for which a sensitising potential has been shown. One difficulty with this approach is that sometimes no sensitisation data exist for those components of a fragrance formula which are used to replace a “known sensitiser”.
- *Deliberate avoidance* of the use of fragrances where they are not essential to the function of a finished product, but used merely to add to its appeal. Examples could include most cosmetics, topical medicaments, detergents etc., but obviously not perfumes, eau de toilette and other products used for their scent.
- *Information, e.g. labelling* so that the consumer may make an informed choice to avoid exposure to a particular ingredient.

##### **4.5.2. Secondary prevention: avoiding re-exposure to (a) specific sensitiser(s) in clinically diagnosed individuals**

In clinical dermatology, avoidance of re-exposure to an allergen is central to the care of sensitised patients. Contact sensitisation, as a latent condition, persists life-long, and therefore allergen avoidance is the only means of avoiding potentially severe and/or handicapping disease, which affects quality of life and may affect fitness for work, i.e. allergic contact dermatitis.

In this context, the valid diagnosis of sensitisation, by patch testing (95) with standardised materials, is a prerequisite of successful allergen avoidance.

In the case of fragrances, a history clearly indicative of “fragrance dermatitis” but in which patch testing with commercially available test preparations is negative, most probably reflects a shortcoming of the patch test procedure, namely, a false-negative investigation. An important cause is inadequate information on the presence of fragrance substances present in cosmetic products (and consumer products in general). This means that patients cannot be tested for relevant substances.

A false-negative investigation can also be due to a number of other reasons: (i) non-adherence to scientific recommendations (95) or guidelines (e.g. (115)); (ii) sub-optimal patch test concentration; or (iii) use of non-oxidised material if oxidised material is the true allergen.

In an "ideal" case, from the point of view of successful patient management, the test procedure identifies all the allergen(s) to which the patient has developed contact allergy, according to the information on the culprit product(s) brought in by the patient. Such contact sensitisation is termed "clinically relevant" (62), and the need for allergen avoidance in the future is unequivocally evident in these cases. However, not infrequently, clinical relevance of an allergic patch test reaction cannot be ascertained for various reasons, which may be beyond control by the clinician (see chapter 4.3). Nevertheless, future elicitation of allergic contact dermatitis by sufficient contact with the identified "non-relevant" allergen may be expected. Hence, the patient will need to avoid the respective substance(s).

In a less "ideal" case, only part of the fragrance allergens having caused allergic contact dermatitis are identified (and can subsequently be avoided), while another part remains unidentified, for instance because it is: (i) not labelled on the product; and/or (ii) not available for routine diagnostic patch testing (special investigations such as chemical analysis of the culprit product, and break-down patch testing of its individual components, are performed rarely). Such "residual" undetermined sensitisation will hamper the success of secondary prevention of allergic contact dermatitis due to fragrances.

The above consideration raises the question for the patient of how to identify fragrance chemicals in cosmetics and other products coming into contact with the skin, such as detergents and household products, topical medicaments, products used professionally (e.g. by hairdressers, beauticians, masseurs, aromatherapists), and in other industrially used categories of products (7) (see also chapter 9). In this regard, the labelling with "perfume" or "contains fragrances" does not provide sufficient information. Moreover, such general labelling has two main disadvantages:

- It does not aid the identification of past exposure to specific agents when planning a patch test and later, when interpreting possible positive patch test results regarding clinical relevance.
- The diagnosis of allergic contact sensitisation to unidentified fragrance allergens will lead to unnecessary avoidance of other fragrance substances to which the patient is not sensitised, which are, however, included under the label "perfume".

Furthermore, the attribute "fragrance-free" may be misleading, as it merely states that no substance was added to the product to give it a scent, assuming it is used correctly at all. Nevertheless, fragrance substances used for other purposes, e.g. as preservatives, may expose the "fragrance allergic" patient to the allergen even in a "fragrance free" product (116). However, in terms of cosmetic ingredient labelling, such other uses are less problematic, as each ingredient not used as a fragrance component must be labelled. Also the use of natural products (essential oils) as preservatives must be considered in this context.

Ingredient labelling of 26 individual fragrance ingredients, identified as allergens in humans, was introduced for cosmetics in 2005. The intention was to provide a tool for clinicians for optimizing the investigation of patients with suspected fragrance allergy, as well as for fragrance allergic patients for avoiding products containing substances they have been shown to be allergic to. Both these aims are objectives of secondary prevention and seem to have been well accepted. In a study of fragrance allergic patients and their utilisation of ingredient labelling (111), most responded that they used the ingredient labelling (86.3%) and of those who used it, the majority (65.3%) found it helpful (111). Most allergic patients used the ingredient labelling (83.2%) to find out if the product was scented, while 35.6% also looked for specific ingredients. Many (84.9%) found that a clearer labelling, e.g. easier names and a larger font size, would increase their benefit.

#### **4.6. Conclusions**

Contact allergy to fragrances is relatively common, affecting 1 to 3% of the general population, based on limited testing with eight common fragrance allergens and about 16 % of patients patch tested for suspected allergic contact dermatitis. Fragrance contact allergy is mostly non-occupational and related to the personal use of cosmetic products.

Allergic contact dermatitis can be severe and widespread, with a significant impairment of quality of life and potential consequences for fitness for work. Thus, prevention of contact sensitisation to fragrances, both in terms of primary prevention (avoiding sensitisation) and secondary prevention (avoiding relapses of allergic contact dermatitis in those already sensitised), is an important objective of public health risk management measures.



## 5. Activation of weak or non-sensitising substances into sensitisers - prehaptens and prohaptens

Fragrance allergens act as haptens, i.e. low molecular weight chemicals that are immunogenic only when attached to a carrier protein. However, not all sensitising fragrance chemicals are directly reactive, but require previous activation.

A prehapten is a chemical that itself is non- or low-sensitising, but that is transformed into a hapten outside the skin by simple chemical transformation (air oxidation, photoactivation) and without the requirement of specific enzymatic systems.

A prohaptent is a chemical that itself is non- or low-sensitising but that is transformed into a hapten in the skin (bioactivation) usually via enzyme catalysis.

It is not always possible to know whether a particular allergen that is not directly reactive acts as a prehapten or as a prohaptent, or both, because air oxidation and bioactivation can often give the same product (geraniol is an example).

Some chemicals might act by all three pathways. One example is geranial (an isomer of citral) which is a hapten itself with a moderate sensitisation potency, but can be activated to more potent sensitisers via air oxidation (autoxidation) thus acting as a prehapten and also via bioactivation (metabolic activation) thus acting as a prohaptent (117).

Increased understanding of the importance of activation through interaction with the environment that turns non-sensitising compounds into sensitisers has made it important to distinguish between prehaptens and prohaptens. This distinction facilitates discussions by emphasizing the differences in activation mechanisms between the two types of compounds requiring activation to become haptens. It is important to note that prehapten activation, in contrast to bioactivation, can be prevented to a certain extent by avoidance of air exposure during the handling and storage of the chemicals. This concerns the most prominent haptens formed by autoxidation i.e. the hydroperoxides. In bioactivation, hydroperoxides have not been identified as metabolites, but other allergenic oxidation products (in particular aldehydes and epoxides) have been identified as being formed by both activation routes depending on the structure of the compound. One thoroughly studied example is geraniol which forms the aldehyde geranial, epoxy-geraniol, and also epoxy-geranial via both pathways of activation (autoxidation and metabolic oxidation) (118, 119). When haptens are formed by both pathways, the impact on the sensitisation potency depends on the degree of autoxidation in relation to the amount of metabolic oxidation.

Human data on established prehaptens are presented in Table 5-1 and Table 5-2. In Table 5-1 the results from patch testing with air exposed samples of the prehaptens are given. Table 5-2 shows the results from testing with the prehaptens themselves without intended air exposure. In addition to the data given in this chapter, animal data (LLNA) on the pure prehaptens or after controlled air exposure are given in Table 8-2. Possible pro- and prehaptens are identified by SAR analyses in chapter 9.

### 5.1. Prehaptens

Autoxidation is a free radical chain reaction in which hydrogen atom abstraction in combination with addition of oxygen forms peroxy radicals. The reaction shows selectivity for positions where stable radicals can be formed. So far, all fragrance substances that have been investigated with regard to the influence of autoxidation on the allergenic potential, including identification of formed oxidation products, have oxidisable allylic positions that are able to form hydroperoxides and/or hydrogen peroxide as primary oxidation products upon air exposure. Once the hydroperoxides have been formed outside the skin they form specific antigens and act as skin sensitisers (120). Secondary oxidation products such as aldehydes and epoxides can also be allergenic, thus further increasing the sensitisation potency of the autoxidation mixture (121). The process of photoactivation may also play a role, but further research is required to establish whether this activation route is currently underestimated in importance due to insufficient knowledge of the true haptens in this context.



Most terpenes with oxidisable allylic positions can be expected to autoxidise on air exposure due to their inherent properties. Depending on the stability of the oxidation products that are formed, a difference in the sensitisation potency of the oxidised terpenes can be seen. Oxidation products of commonly used fragrance terpenes (limonene, linalool, geraniol, linalyl acetate) have been identified as potent sensitisers in predictive animal tests (118, 122-127) (see chapter 8). This is also demonstrated for alpha-terpinene and citronellol (AT Karlberg, personal communication 2011). The oxidised fragrance terpenes limonene, linalool and linalyl acetate have been tested in consecutive dermatitis patients and give frequent allergic contact reactions (128-133). Details are given in Table 5-1

In contrast, the non-oxidised compounds rarely cause allergic reactions (41-43, 64, 67, 72, 96, 134-136), for details see Table 5-2. Not all oxidised fragrance substances are strong sensitisers, e.g. caryophyllene is readily oxidised but has a low sensitisation potency after autoxidation (137). This is supported by clinical studies showing oxidised caryophyllene to be a less frequent allergen compared to oxidised limonene and oxidised linalool (131).

As oxidised and non-oxidised fragrance terpenes were not patch tested simultaneously in the same patients, the results are presented in two separate tables (Table 5-1 and Table 5-2).

**Table 5-1:** Contact allergic reactions to the autoxidised fragrance substances limonene, linalool, caryophyllene, myrcene and linalyl acetate in consecutive dermatitis patients.

INCI name	CAS no	Test conc. (%)	n Positive/n tested (%)	Comments (Ref.)
<b>D-Limonene (ox.)</b>	5989-27-5	5	18/703 (2.6%)	§ (128)
		3	28/1172 (1.6%)	
		2	3/362 (0.83%)	
<b>D-Limonene (ox.)</b>	5989-27-5	3	63/2273 (2.8%) variation between centres: 0.3-6.5%	§ (129)
<b>D-Limonene (ox.)</b>	5989-27-5, 5989-54-8, 138-86-3	3	49/1812 (2.3%)	§ (132)
<b>L-Limonene (ox.)</b>			36/1812 (2.0%)	
<b>D – and/or L- Limonene (ox.)</b>			63/2411 (2.6%)	
<b>Linalool (ox.)</b>	78-70-6	2	20/1511 (1.3%) variation between centres: 0.4-2.7%	§ (131)
<b>Caryophyllene (ox.)</b>	88-44-5	3.9	2/1511 (0.1%)	
<b>Myrcene (ox.)</b>	123-35-3	3	1/1511 (0.1%)	
<b>Linalool (ox.)</b>	78-70-6	2	14/1693 (0.83%)	§ (133)
		4	67/2075 (3.2%)	
		6	91/1725 (5.3%)	
		11	72/1004 (7.2%)	
<b>Linalool (ox.)</b>	78-70-6	3	11/483 (2.3%)	(138)
<b>Linalyl acetate (ox.)</b>	115-95-7	6	13/1217 (1.1%)	(139)

Notes: § Bicentric or multicentre studies.  
(ox.) Oxidised.

**Table 5-2:** Contact allergic reactions to limonene, linalool, linalyl acetate and caryophyllene in consecutive dermatitis patient. Please observe that several studies have been performed using the test substances without reporting the autoxidation status but it has been intended to be low. For precise information see the original references.

INCI name	CAS number	Test conc. (%)	n Positive/n tested (%)	Comments (Ref.)
Limonene	138-86-3	2	0/1200	(134)
Limonene			3/2396 (0.1%)	§ (72)
DL-Limonene			11/1241 (0.88%)	§ (41)
Limonene			0/320	(42)
DL-Limonene			3/2396 (0.1%)	§ (72)
Linalool	78-70-6	30	0/179	(136)
		20	3/1825 (0.2%)	§ (43)
		10	2/320 (0.6%)	(42)
		10	4/792 (0.5%)	(135)
		5 and 1	0/100	(67)
Linalool, "stabilised" *		10	7/2401 (0.3%)	§ (72)
	10	2/985 (0.2%)	§ (41)	
Linalyl acetate	115-95-7	1, 5	0/100	(67)
		10	4/1855 (0.2%)	§ (64)
beta-Caryophyllene	87-44-5	5	10/1606 (0.6%)	§ (96)

Notes: § Bicentric or multicentre studies.

(ox.) Oxidised.

\* Stabilised: according to the manufacturer contained additional substances aimed at limiting oxidation.

Due to the complexity of scented products, which are mixtures of many different fragrance substances, there are at present no published data identifying the presence of individual hydroperoxides in cosmetic products containing the above fragrance terpenes. However, clinical studies show a clear connection between contact allergy to oxidised limonene and oxidised linalool, and contact allergy to other markers of fragrance contact allergy (128-133); see Table 5-3.

**Table 5-3:** Concomitant reactions to fragrance markers: Fragrance Mix I and II (FM I, FM II), *Myroxylon pereire* (MP) and to colophonium (coloph.) in the baseline series in patients with positive or negative patch test reactions to oxidised fragrance substances.

	Total number of pos. and/or neg. reactions	Pos. to FM I		Pos. to MP		Pos. to coloph.		Ref.		
		n	%	n	%	n	%			
<b>Reactions to ox. D- limonene and/or limonene hydroperoxide fraction</b>	Pos.: 49	20	41	12	24	12	24	(128)*		
	Neg.: 2751	223	8.1	142	5.2	131	4.8			
<b>Reactions to ox. D- limonene and/or limonene hydroperoxide fraction <sup>a</sup></b>	Pos.: 60	22	37	11	18	13	22	(130)*		
	Neg.: 729	141	19	71	9.7	58	8			
<b>Reactions to ox. D- limonene and/or ox. L- limonene <sup>a</sup></b>	Pos. to ox. D- limonene: 41	14	34	11	27	11	27	(132)*		
	Neg. to ox. D- limonene: 1771	113	6.4	91	5.1	62	3.5			
	Pos. to ox. L- limonene: 36	11	31	12	33	9	25			
	Neg. to ox. L- limonene: 1776	116	6.5	80	4.5	64	3.6			
<b>Reactions to any of ox. linalool, myrcene, caryophyllene</b>	Pos. to any of the tested ox. subst.: 31	12	39	6	31	12	39	(131)*		
	Neg. to any of the tested ox. subst: 1480	93	6	63	4	46	3			
		Pos. to FM I		Pos. to FM II		Pos. to MP		Pos. to coloph.		
		n	%	n	%	n	%	n	%	
<b>Reactions to ox. linalool</b>	Pos. at test conc. 4%: 30	8	26.7	5	16.7	10	33.3	5	16.7	(133)*
	Pos. at test conc. 6%: 55	12	21.8	8	14.5	11	20	8	14.5	
	Pos. at test conc. 11%: 72	14	19.4	9	12.5	14	19.4	9	12.5	
	Total pos. at any test conc: 75/1004	n.g.		n.g.		n.g.		n.g.		
	Total neg. at any test conc: 929/1004	56	6.0	29	3.1	45	4.8	24	2.6	

Notes: \* Bicentric or multicentre studies.

n.g. Not given.

(ox.) Oxidised.

Linalool and linalyl acetate are the main components of lavender oil. They autoxidise on air exposure also when present in the essential oil, and form the same oxidation products found in previous studies of the pure synthetic terpenes. Experimental sensitisation studies showed that air exposure of lavender oil increased the sensitisation potency. Patch test results in dermatitis patients showed a connection between positive reactions to oxidised linalool, linalyl acetate and lavender oil (140).

It should be noted that activation of substances via air oxidation results in various haptens that might be the same or cross-reacting with other haptens (allergens). The main allergens after air oxidation of linalool and linalyl acetate are the hydroperoxides. If linalyl acetate is chemically hydrolysed outside the skin it can thereafter be oxidised to the same haptens as seen for linalool. A corresponding example is citronellol and citronellyl acetate. In clinical studies, concomitant reactions to oxidised linalool and oxidised linalyl acetate have been observed (139, 140). Whether these reactions depend on cross-reactivity or are due to exposure to both fragrance substances cannot be elucidated as both have an allergenic effect themselves.

For prohaptens, the activation outside the body can be prevented to a certain extent. This is possible by measures during handling and storage of the ingredients and the final product to avoid air exposure and/or by the addition of suitable antioxidants. Prevention of autoxidation using antioxidants needs thorough investigation, as the autoxidation rate depends not only on the compound itself, but also its purity (141). Furthermore, it should be noted that most antioxidants exert their function by being activated instead of the compound that they protect, thus suggesting that they too could act as prohaptens skin sensitisers. This is a risk to be considered given that antioxidants are now frequently used at increased concentrations in scented products due to a growing awareness of the problem of autoxidation.

## 5.2. Prohaptens

Compounds that are bioactivated in the skin and thereby form haptens are referred to as prohaptens. The human skin expresses enzyme systems that are able to metabolise xenobiotics (142), modifying their chemical structure to increase hydrophilicity and allow elimination from the body. Xenobiotic metabolism can be divided into two phases: phase I and phase II. Phase I transformations are known as activation or functionalisation reactions, which normally introduce or unmask hydrophilic functional groups. If the metabolites are sufficiently polar at this point they will be eliminated. However, many phase I products have to undergo subsequent phase II transformations, i.e. conjugation to make them sufficiently water soluble to be eliminated. Although the purpose of xenobiotic metabolism is detoxification, it can also convert relatively harmless compounds into reactive species. Cutaneous enzymes that catalyse phase I transformations include the cytochrome P450 mixed-function oxidase system, alcohol and aldehyde dehydrogenases, monoamine oxidases, flavin-containing monooxygenases and hydrolytic enzymes. Acyltransferases, glutathione S-transferases, UDP-glucuronosyltransferases and sulfotransferases are examples of phase II enzymes that have been shown to be present in human skin (142). These enzymes are known to catalyse both activating and deactivating biotransformations (143), but the influence of the reactions on the allergenic activity of skin sensitisers has not been studied in detail.

Skin sensitising prohaptens can be recognised and grouped into chemical classes based on knowledge of xenobiotic bioactivation reactions, clinical observations and/or *in vivo* and *in vitro* studies of sensitisation potential and chemical reactivity. Few mechanistic investigations of prohaptens have so far been published. Investigations that are important for the bioactivation of fragrance substances are studies on alkenes, e.g. alpha-terpinene (144-146), the allylic primary alcohols geraniol (119) cinnamyl alcohol (147-151), eugenol and isoeugenol (152).

In order to be able to predict the sensitisation potency of prohaptens, steps of bioactivation have to be included in the predictive tests where intrinsic bioactivating systems are lacking.

So far, no such predictive non-animal methods have been developed that take account of this.

When bioactivation occurs, the risk of cross-reactivity also needs to be considered. Cross-reactivity between certain aldehydes and their corresponding alcohols, e.g. cinnamal - cinnamyl alcohol and geranial - geraniol, due to the metabolic oxidation of the alcohols to the aldehydes in the skin is demonstrated (119, 147-151).

When using derivatives of a fragrance substance, it must be taken into account that the derivative could be metabolically transformed in the skin into the parent or cross-reacting compounds. A prominent example of such bioactivation is the hydrolysis of esters by esterases to the corresponding original alcohols. The metabolic product obtained can act as a hapten or a prohaptent in exactly the same way as the non-esterified parent compound.

Isoeugenol and its derivatives are an important example for this mechanism from which general conclusions may be drawn. As the use of isoeugenol in fragranced products needs to be indicated on the ingredients list, this important fragrance material may be replaced in fragrance formulations by derivatives with a similar scent. In a study it was shown that several EDP/EDT/aftershave lotions contained high levels of isoeugenyl acetate and isoeugenol methyl ether (Table 5-4) (153). Isoeugenyl acetate will be hydrolysed by esterases in the skin to generate isoeugenol. The situation may be similar for eugenyl acetate and geranyl acetate, which might be used in fragrance formulations instead of eugenol and geraniol, respectively.

**Table 5-4:** Mean and median content of isoeugenol and its derivatives in the 29 perfume products.

Fragrance compound INCI Name	Products containing the fragrance		Content (ppm)			
	No.	%	Range	Mean	SD	Median
Isoeugenol	16	55	27-203	71	54	45
Isoeugenyl acetate	10	34	20-4689	985	1570	166
Isoeugenyl methyl ether	13	45	65-1755	360	442.3	222

### 5.3. Conclusions

- Many fragrance substances can act as prehaptens or prohaptens, forming potent allergens by abiotic and/or metabolic activation. Activation can thus increase the risk of sensitisation.
- Fragrance substances of clinical importance known to be prehaptens and to form sensitising compounds by air oxidation are limonene, linalool, and linalyl acetate.
- Fragrance substances of clinical importance known to be prohaptens and to form sensitising compounds by metabolic transformation are cinnamyl alcohol, eugenol, isoeugenol and isoeugenol acetate.
- Fragrance substances of clinical importance with published data known to be both prehaptens and prohaptens and to form sensitising compounds by air oxidation (prehaptens) and by metabolic transformation are geraniol and alpha-terpinene.
- A fragrance substance that sensitises without activation, but forms more potent sensitising compounds by air oxidation and also by metabolic transformation is geranial (one isomer of citral).
- In the case of prehaptens, it is possible to prevent activation outside the body to a certain extent by different measures, e.g. prevention of air exposure during handling and storage of the ingredients and the final product, and by the addition of suitable

antioxidants. When antioxidants are used, care should be taken that they will not be activated themselves and thereby form new sensitisers.

It should be noted that the possibility to reduce the sensitisation potency by preventing air oxidation is also important for a direct acting hapten or prohaptens, if a further activation by air oxidation to more allergenic compounds has been shown.

- In the case of prohaptens, the possibility to become activated is inherent to the molecule and activation cannot be avoided by extrinsic measures. Activation processes increase the risk for cross-reactivity between fragrance substances. Cross-reactivity has been shown for certain alcohols and their corresponding aldehydes, i.e. between geraniol and geranial (citral) and between cinnamyl alcohol and cinnamal.

Cross-reactivity is also expected between ester derivatives and their parent alcohols, as the esters will be hydrolysed by esterases in the skin. Esters of important contact allergens that can be activated by hydrolysis in the skin are isoeugenol acetate, eugenyl acetate and geranyl acetate all of which are known to be used as fragrance ingredients.

- Further experimental and clinical research in the area of abiotic and/or metabolic activation of fragrance substances is clearly needed to increase the safety for the consumer. Compounds suspected to act as prehapten and/or prohaptens should be considered as allergens, unless it could be demonstrated that they do not become activated by one of the described pathways.

## 6. Retrieval of evidence and classification of fragrance substances

For a systematic review, a structured approach of identifying, grading and aggregating available information should be used. Regarding the classification of substances as allergens, a number of approaches have been suggested (154-156). The categorisation of skin sensitisers according to sensitising potency has also been proposed (157). For this opinion, these discussions were extended to reconcile different perspectives and to arrive at a strategy that is both consistent and applicable in practice.

### 6.1. Retrieval of evidence

A systematic search strategy was employed for the retrieval of clinical data, as outlined below. Experimental data are often not published hence the exact definition of the scope considered for the review is necessary and is given below. Additional LLNA data were reviewed, if identified by the search strategy, e.g. in chapter 8.1.2 and, as "additional information", in Annex I of this opinion. This supplemental evidence was, however, not considered for the final categorisation in Table 13-2.

#### 6.1.1. Search strategy for clinical data

Method of literature search:

1. Manual search of the issues of the journal "Contact Dermatitis" up to March 2010 (for the 26 "annex substances" from 1999 to October 2010), identifying all studies with fragrance substances.
2. PubMed search of CAS number identified in the previous opinion, reviews and already identified clinical studies, respectively, and manual screening of identified publications (narrowed for the last 10 years for the 26 "annex substances"), if necessary narrowing the search results by adding "dermatitis" or "allergy". For example, for citral: 5392-40-5 AND (dermatitis or allergy), translated into  
"5392-40-5"[EC/RN Number] AND  
(  
("dermatitis"[MeSH Terms] OR "dermatitis"[All Fields])  
OR  
("hypersensitivity"[MeSH Terms] OR "hypersensitivity"[All Fields] OR "allergy"[All Fields] OR "allergy and immunology"[MeSH Terms] OR ("allergy"[All Fields] AND "immunology"[All Fields]) OR "allergy and immunology"[All Fields])  
)
3. Manual search of all RIFM reviews published in supplement issues of "Food and Chemical Toxicology<sup>2</sup>" in the past 20 years. In case of the least evidence on human sensitisation the substances were preliminarily selected and further research initiated.
4. Consideration of the most important ("top 100") fragrance compounds in terms of volumes used (disregarding functional additives such as solvents) as supplied by the International Fragrance Association IFRA (personal communication 2010).
5. Consideration of fragrance compounds ranking 101 to 200 on the list of use volumes, if they were classified as skin sensitisers (R 43).

#### 6.1.2. Collection of experimental (LLNA) data

The SCCS requested the International Fragrance Association (IFRA) to submit data on animal tests performed with fragrance substances, by the local lymph node assay (LLNA) in mice, guinea pig maximisation test (GPMT) and Buehler test, to be presented in a structured format. In response, industry submitted first a poster (158) and later a report consisting of

<sup>2</sup> Food and Chemical Toxicology, Elsevier Ltd. <http://www.sciencedirect.com/science/journal/02786915>.

LLNA protocol summaries on the 59 fragrance substances in the poster (159). No guinea pig studies were submitted. The SCCS has reviewed and analysed the report and the publications quoted in the report. A summary is given in chapter 8 and full data are given in Annex II. EC3 values on some additional fragrance substances in two published reviews (160, 161) have also been considered. Additional EC3 values may be available in the scientific literature and there may also be other unpublished data.

## 6.2. Grading of evidence

Assembled evidence has to be graded in two steps: (i) the quality of each single study, and (ii) the strength of evidence underlying the eventual classification as an allergen. Generally, studies (published or not) which are eligible for consideration will contribute to the final overall judgement to different degrees.

- Positive human data, if sufficiently demonstrated (point (i) below), will always over rule experimental (animal), *in vitro* or *in silico* data of similar internal validity, as they provide direct evidence on allergenicity in humans.
- Small study groups will contribute less precise information than larger studies of otherwise similar quality. As a minimum requirement, the size of the study groups and the numbers of events must be stated in the reports.

The following subsections will address special aspects of clinical and experimental studies, respectively.

### 6.2.1. Quality of a clinical study

Two major types of clinical studies must be distinguished because they provide a different scope of information:

- (i) Case reports or small case series, focusing on patients with positive (test) reactions to the target substance, sometimes including a set of non-exposed, possibly non-diseased "control patients"; these should present a concise summary of all relevant aspects of the patient's history, diagnostic procedures and possibly further outcomes.
- (ii) Clinical series in which results of a group of patients patch tested with the target substance, often combined with other substances, are presented. In the latter type of report, usually only a minority of patients tested show a positive reaction to the test substance. This implies that the majority of patients can be used to illustrate the proportion of irritant, doubtful and negative reactions. The degree of detail on the patients' histories is usually limited in such studies, compared to case reports.

Some of the basic quality criteria in clinical patch testing which should be considered are:

- Adherence to international patch test guidelines (94, 95).
- Material(s) tested should be characterised.
- Total number of patients tested must be given.
- Patient selection should be described.
- Relevance may be demonstrated either on a case-by-case basis, following pertinent guidelines, or in terms of a significant epidemiological association between sensitisation and exposure or valid markers of exposure.

### 6.2.2. Quality of an experimental study

International guidelines such as the pertinent OECD guidelines for testing sensitisation have been developed and adopted. Experimental studies following these guidelines are considered as valid. However, a vast number of non-guideline studies are available and should be assessed on a case-by-case basis.



### 6.2.3. Quality of “other” evidence

Supporting evidence besides human and animal (experimental) data comprises *in vitro* test systems, *in chemico* experiments and structure activity relationships (SARs).

SAR analysis has at present no formal regulatory validation for skin sensitisation, nevertheless it may provide useful indicative information on sensitising potential when no or limited clinical or animal data are available.

SAR studies must consider a possible formation of haptens (allergens) from compounds able to act as prehapten by, e.g. autoxidation outside the body as well as metabolic activation in the skin of compounds able to act as prohaptens (121, 162).

### 6.3. Aggregating evidence for a final conclusion

The criteria listed below are followed as a flow chart to arrive at a conclusion. This implies that if classification into one category is achieved, subsequent categories need not be considered. Based on the above criteria, fragrance substances were selected to be included in the present opinion if classified in one of the categories defined below.

#### 6.3.1. Established contact allergen in humans

To qualify as an *established contact allergen*, the SCCS considers that *at least one* of the following two criteria must be met:

- At least two clinical series fulfilling the quality criteria from two different centres with cases of sensitisation, or at least three separate clinical series from different centres if a study, or studies, do not meet all quality criteria. (→ *sufficient human evidence present*)  
or
- Case reports from at least two independent centres describing more than two patients altogether in whom clinically relevant contact sensitisation had unequivocally been proven (→ *sufficient human evidence present*)  
or
- At least one clinical series fulfilling the quality criteria, together with at least one case report of clinically relevant contact sensitisation (→ *sufficient human evidence present*);  
or
- Experimentally induced sensitisation (e.g. unequivocally positive human maximisation tests/repeated insult patch test)<sup>3</sup> (→ *sufficient human evidence present*).

#### 6.3.2. Established contact allergen in animals

To qualify as an *established contact allergen*, the following criterion must be met:

- At least one positive result in an animal study carried out according to accepted guidelines, providing unequivocal evidence of a sensitisation potential (→ *sufficient animal evidence present*).

#### 6.3.3. Likely contact allergen, if human, animal and other evidence is considered

To qualify as an *likely contact allergen*, if classification as “established ...” is not applicable, *at least two* of the following criteria must be met:

- Individual cases of allergic patch test reactions not fulfilling the requirements for sufficient evidence (→ *limited human evidence present*)

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<sup>3</sup> It should be noted that the SCCS considers such tests unethical (163).

or

- A positive result in at least one non-guideline animal study, which should be evaluated on a case-by-case basis (→ *limited animal evidence present*)  
or
- Other evidence, e.g. results from *in chemico* experiments or *in vitro* tests or from structure-activity considerations based on sufficiently valid results for closely related compounds (→ *other evidence present*).

#### **6.3.4. Possible contact allergen, if human, animal and other evidence is considered**

To qualify as a *possible contact allergen*, if classification as “established ...” or as “likely ...” contact allergen is not applicable, *at least one* of the following criteria must be met:

- Individual cases of allergic patch test reactions not fulfilling the requirements for sufficient evidence (→ *limited human evidence present*)  
or
- A positive result in at least one non-guideline animal study, which should be evaluated on a case-by-case basis (→ *limited animal evidence present*)  
or
- Other evidence, e.g. results from *in chemico* experiments or *in vitro* tests or from structure-activity considerations based on sufficiently valid results for closely related compounds (→ *other evidence present*).

#### **6.4. Conclusions**

The present opinion includes (i) a well-defined search strategy for retrieving pertinent evidence; (ii) a definition of criteria used to evaluate available evidence; and, finally (iii) a set of rules to categorise the substances with regard to the relevant toxicological endpoint, i.e. sensitisation in man, based on the evidence.

## 7. Reported fragrance allergens from the clinical perspective

In this chapter, clinical evidence regarding sensitisation to individual fragrance chemicals and to natural extracts (essential oils) is tabulated. In this report “single chemicals” refers to chemicals of natural or synthetic origin whose chemical identity is fully known. The term “natural extracts” refers to plant or animal derived mixtures of natural chemicals, for example lavender oil, whose composition may be variable and may or may not have been fully or partly established. Full information, including possible synonyms, structural formulas (in the case of single chemicals only), a short summary of available evidence and further information, e.g. on regulatory status, is presented in Annex I.

### 7.1. Tabular summary of evaluated individual fragrance chemicals

Regarding nomenclature, INCI names are used wherever possible. If an INCI name is not available, the perfuming name as listed by CosIng is used. Detailed information on the publications identified and considered for this report can be found in Annex I. Several substances are currently banned from the use in cosmetic products by Annex II of the Cosmetics Directive, based on concerns regarding one or more toxicological endpoints. While available clinical evidence regarding this set of substances is listed in Annex I, these substances have not been further evaluated and are thus not included in this chapter.

In this section, a tabular overview on the classification of substances considered is presented in four tables listing:

1. Established contact allergens in humans (→ *sufficient human evidence present*).
2. Substances with positive human data, which are, however, not sufficient to categorise as “established contact allergen in humans” (→ *limited human evidence present*).
3. Substances with negative human data, i.e. patch tests of patients with suspected contact allergy to fragrance ingredients which yielded negative results.
4. Substances eligible for inclusion (see beginning of chapter 6) for which no human data are available.

A critical point in understanding this scheme is that there is publication bias in reporting allergens. This is due to the fact that once a substance has been reported and accepted as a contact allergen in humans, further reports are less likely to be published unless they are part of an epidemiological survey or when there is a novel source of exposure. Moreover, the number of patients displaying positive test reactions obviously not only depends on the underlying prevalence of sensitisation, but also on how often a substance is patch tested. This implies that inclusion of an allergen or allergen mixture in the baseline patch test series (as for Fragrance Mix I and II, *Myroxylon pereirae* and HICC, and partly also other substances/mixtures) will yield the maximum possible number of cases. In contrast, patch testing in “special” series, e.g. as a break-down of single constituents of the respective mix in case of a positive reaction to the latter, or with application only in the case of strongly suspected fragrance intolerance, will mostly result in higher relative numbers than testing the same compound consecutively, but also in lower absolute numbers.

In Table 7-1, the single substances are listed with a semi-quantification of their impact which were categorised as established contact allergens in humans according to the criteria given in chapter 6.3.

Established contact allergens in humans, according to the criteria outlined at the beginning of this chapter, were grossly categorised according to the number of patients tested and the number of patients reacting positively, based on the publications considered. The following categories were used:

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+	Up to 10 positive test reactions reported
++	11 to 100
+++	101 to 1000
++++	> 1000

If a test allergen has been tested in less than 1,000 patients, "r.t." (rarely tested) is added in the following tables.

**Table 7-1:** Established contact allergens in humans (summary of evaluation as detailed in chapter 6.3). More detailed information forming the basis of this evaluation can be found in Annex I of this opinion.

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Comment: see text
ACETYLCEDRENE	32388-55-9	+
AMYL CINNAMAL	122-40-7	+
AMYL CINNAMYL ALCOHOL	101-85-9	+
AMYL SALICYLATE	2050-08-0	+
trans-ANETHOLE	4180-23-8	+ (r.t.)
ANISYL ALCOHOL	105-13-5	+
BENZALDEHYDE	100-52-7	+
BENZYL ALCOHOL	100-51-6	+
BENZYL BENZOATE	120-51-4	++
BENZYL CINNAMATE	103-41-3	++
BENZYL SALICYLATE	118-58-1	+
BUTYLPHENYL METHYLPROPIONAL (Lilial®)	80-54-6	++
CAMPHOR	76-22-2 / 464-49-3	+ (r.t.)
beta-CARYOPHYLLENE (ox.)	87-44-5	Non-ox.: +, ox.: +
CARVONE	99-49-0 / 6485-40-1 / 2244-16-8	+ (r.t.)
CINNAMAL	104-55-2	+++
CINNAMYL ALCOHOL	104-54-1	+++
CITRAL	5392-40-5	+++
CITRONELLOL	106-22-9 / 1117-61-9 / 7540-51-4	++
COUMARIN	91-64-5	+++
(DAMASCENONE ) ROSE KETONE-4	23696-85-7	+ (r.t.)
alpha-DAMASCONE (TMCHB) <sup>#</sup>	43052-87-5 / 23726-94-5	++
cis-beta-DAMASCONE <sup>#</sup>	23726-92-3	+

<sup>#</sup> 76/768/EEC Annex III, part 1

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<b>INCI name (or, if none exists, perfuming name according to CosIng)</b>	<b>CAS number</b>	<b>Comment: see text</b>
delta-DAMASCONE #	57378-68-4	+
DIMETHYLBENZYL CARBINYL ACETATE (DMBCA)	151-05-3	+
EUGENOL	97-53-0	+++
FARNESOL	4602-84-0	+++
GERANIOL	106-24-1	+++
HEXADECANOLACTONE	109-29-5	+ (r.t.)
HEXAMETHYLINDANOPYRAN	1222-05-5	++
HEXYL CINNAMAL	101-86-0	++
HYDROXYISOHEXYL 3-CYCLOHEXENE CARBOXALDEHYDE (HICC)	31906-04-4 / 51414-25-6	++++
HYDROXYCITRONELLAL	107-75-5	+++
ISOEUGENOL	97-54-1	+++
alpha-ISOMETHYL IONONE	127-51-5	++
(DL)-LIMONENE	138-86-3	++ (non-ox.); +++ (ox.)
LINALOOL	78-70-6	++ (non-ox.); +++ (ox.)
LINALYL ACETATE	115-95-7	+
MENTHOL	1490-04-6 / 89-78-1 / 2216-51-5	++
6-METHYL COUMARIN#	92-48-8	++ (photo-allergy)
METHYL 2-OCTYNOATE	111-12-6	++
METHYL SALICYLATE	119-36-8	+
3-METHYL-5-(2,2,3-TRIMETHYL-3-CYCLOPENTENYL)PENT-4-EN-2-OL	67801-20-1	++ (r.t.)
alpha-PINENE and beta-PINENE	80-56-8 and 127-91-3, resp.	++
PROPYLIDENE PHTHALIDE	17369-59-4	+ (r.t.)
SALICYLALDEHYDE	90-02-8	++
alpha-SANTALOL and beta-SANTALOL	115-71-9 and 77-42-9, resp.	++
SCLAREOL	515-03-7	+
TERPINEOL (mixture of isomers)	8000-41-7	+
alpha-TERPINEOL	10482-56-1 / 98-55-5	
Terpinolene	586-62-9	+

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INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Comment: see text
TETRAMETHYL ACETYLOCTAHYDRONAPHTHALENES	54464-57-2 / 54464-59-4 / 68155-66-8 / 68155-67-9	+
TRIMETHYL-BENZENEPROPANOL (Majantol)	103694-68-4	++
VANILLIN	121-33-5	++

In Table 7-2, those substances are listed which gave rise to a few reported cases of contact sensitisation only, or where results have been reported from just one clinical department. Thus, the level of evidence, regarding human data, must be regarded as *limited*, according to the definitions given in chapter 6.3.

**Table 7-2:** Fragrance substances with positive human data, which are, however, not sufficient to categorise as “established contact allergen in humans”. More detailed information forming the basis of this evaluation can be found in Annex I of this opinion.

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Comment	Ref.
AMBRETTOLIDE	7779-50-2	3.4% positive reactions in 178 patients	(164)
CARVACROL	499-75-2	2 of 28 patients	(Meynadier, after (165))
CUMINALDEHYDE	122-03-2	3 of 179 patients positive	(136)
CYCLOHEXYL ACETATE	622-45-7	0.5% positive of 218 selected patients	(166)
CYCLOPENTADECANONE	502-72-7	3 of 178 patients positive	(164)
trans-trans-delta-DAMASCONE	71048-82-3	1 positive HRIPT (2/15 with 1%)	(167)
2,3-DIHYDRO-2,2,6-TRIMETHYLBENZALDEHYDE	116-26-7	1 positive HRIPT (5 of 53)	(168).
DIMETHYLTETRAHYDRO BENZALDEHYDE	68737-61-1	2.3% positive reactions isomer mixture in 178 patients	(164)
ETHYLENE DODECANEDIOATE	54982-83-1	2 / 218 positive PT reactions	(166)
ETHYL VANILLIN	121-32-4	1 occupational case	(169)
HELIOTROPINE	120-57-0	6 / 1606 consecutive patients positive	(96)
HYDROXYCITRONELLOL	107-74-4	6.0% positive PT reactions in 218 patients	(166).
ISOAMYL SALICYLATE	87-20-7	1 positive in 179 patients, “excited back syndrome”	(136). (67)

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INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Comment	Ref.
		0 / 95 in another study with $\leq 1/10$ of above test conc.	
ISOLONGIFOLENEKETONE	33407-62-4	1 / 178 patients	(164)
METHOXYCITRONELLAL	3613-30-7	Positive PT data of unknown validity by Nakayama et al. in 22/137 patients.	(170)
METHOXYTRIMETHYLHEPTANOL	41890-92-0	0.9% positive PT	(166)
METHYL p-ANISATE	121-98-2	1 / 182 patients positive	(171)
METHYL CINNAMATE	103-26-4	6 / 142 patients positive	(172)
METHYL DIHYDROJASMONATE	24851-98-7	3 / 1606 patients positive 0 / 100	(96) (67)
METHYLIONANTHEME	55599-63-8	1 case	(173)
5-METHYL-alpha-IONONE	79-69-6	5 / 1606	(96)
METHYL OCTINE CARBONATE	111-80-8	1 case	(174)
MYRCENE	123-35-3	1 / 1511 positive to oxidized myrcene	(131)
MYRTENOL	515-00-4	2 HRIPTs with 1 pos. each	(175)
NEROL	106-25-2	6.0% positive	(166)
Nerolidol (isomer not specified)	7212-44-4	Few, unconfirmed pos. cases according to RIFM review	(176)
NOPYL ACETATE	128-51-8	2 / 179 positive, possibly "excited back syndrome"	(136)
PHENETHYL ALCOHOL	60-12-8	1 / 179; 0 / 100	(136) (67)
PHENYLACETALDEHYDE	122-78-1	1.1% of 182 positive. 1 case	(171) (177).
PHENYLPROPANOL	122-97-4	2 / 218	(166).
PHYTOL	150-86-7	1 case in human max. test	(178)
RHODINOL	6812-78-8	Several pos. HRIPTs, clinical data of uncertain validity	(179)
trans-ROSE KETONE-5	39872-57-6	2 / 22 pos. HRIPT	(180)

For a number of substances negative patch tests results were obtained, usually in rather small patient samples (max. 313 patients). For some of these substances exposure is substantial, according to data submitted from IFRA. It should be noted that a negative result does not rule out a notable sensitisation prevalence, as the study size has to be larger



than, e.g. n=298 to yield a 95% CI which excludes a prevalence of 1% and larger than n=597 to exclude a prevalence of 0.5%.

**Table 7-3:** Fragrance substances with negative human data, i.e. patch tests of patients with suspected contact allergy to fragrance ingredients which yielded negative results.

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Results / Comment	Ref.
6-ACETYL-1,1,2,4,4,7-HEXAMETHYLTETRALINE	21145-77-7	0 / 313 consecutive patients in 2 centres	(67)
AMYL CYCLOPENTANONE	4819-67-4	0 / 178	(164)
BENZYL ACETATE	140-11-4	0 / 100 consecutive patients in 1 centre observed	(67)
2-TERT-BUTYL CYCLOHEXYL ACETATE	88-41-5	0 / 313 consecutive patients in 2 centres	(67)
4-tert.-Butylcyclohexyl acetate	32210-23-4	0 / 107 consecutive patients in 1 centre observed	(67)
6-ETHYLIDENEOCTAHYDRO-5,8-METHANO-2H-BENZO-1-PYRAN	93939-86-7	0 / 178	(164)
3 $\alpha$ ,4,5,6,7,7 $\alpha$ -HEXAHYDRO-4,7-METHANO-1H-INDEN-5(OR 6)-YL ACETATE	54830-99-8	0 / 313 consecutive patients in 2 centres	(67)
HEXYL SALICYLATE	6259-76-3	0 / 218 "top 100" substance and classified as R43	(166)
HIBISCOLIDE	6707-60-4	0 / 178	(164)
alpha-IONONE	127-41-3	0 / 205	(67)
beta-IONONE	79-77-6	0 / 205 "top 100" substance	(67)
ISOBORNYL ACETATE	125-12-2	0 / 107 "top 100" substance	(67)
METHYL ANTHRANILATE	134-20-3	0 / 91 "top 100" substance	(181)
METHYL IONONE (mixture of isomers)	1335-46-2	0 / 100 "top 100" substance	(67)
OXALIDE	1725-01-5	0 / 178	(164)

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<b>INCI name (or, if none exists, perfuming name according to CosIng)</b>	<b>CAS number</b>	<b>Results Comment</b> /	<b>Ref.</b>
TERPINEOL ACETATE (Isomer mixture)	8007-35-0	0 / 106 "top substance 100"	(67)
alpha-TERPINYL ACETATE	80-26-2	0 / 179	(136)
TRIMETHYL-PROPYLCYCLOHEXANEPROPANOL	70788-30-6	0 / 178	(164)

For yet another subset of substances, no human data were publicly available. However, exposure to these substances is important as they are used in high volumes (this being the sole criterion for inclusion in this list) and, therefore their hazard with regard to contact sensitisation should be examined.

**Table 7-4:** Fragrance substances lacking human data and used in high volumes according to industry information.

<b>INCI name (or, if none exists, perfuming name according to CosIng)</b>	<b>CAS number</b>
ANISALDEHYDE	123-11-5
BENZYL ACETONE	2550-26-7
p-tert. -Butyldihydrocinnamaldehyde	18127-01-0
CITRONELLYL NITRILE	51566-62-2
CYCLAMEN ALDEHYDE	103-95-7
alpha-CYCLOHEXYLIDENE BENZENEACETONITRILE	10461-98-0
DECANAL	112-31-2
DIHYDROMYRCENOL	18479-58-8
2,4-DIMETHYL-3-CYCLOHEXEN-1-CARBOXALDEHYDE	68039-49-6
3,7-DIMETHYL-1,6-NONADIEN-3-OL	10339-55-6
DIPHENYL ETHER	101-84-8
ETHYL 2-METHYLBUTYRATE	7452-79-1
2-ETHYL-4-(2,2,3-TRIMETHYL-3-CYCLOPENTEN-1-YL)-2-BUTEN-1-OL	28219-61-6
ETHYLENE BRASSYLATE	105-95-3
EUCALYPTOL	470-82-6
GERANYL ACETATE	105-87-3
HEXAHYDRO-METHANOINDENYL PROPIONATE	68912-13-0
HEXYL ACETATE	142-92-7
IONONE isomeric mixture	8013-90-9
ISOAMYL ACETATE	123-92-2
ISOBERGAMATE <sup>#</sup>	68683-20-5
Longifolene	475-20-7
METHYLENEDIOXYPHENYL METHYLPROPANAL	1205-17-0

<sup>#</sup> Annex III, part 1

<b>INCI name (or, if none exists, perfuming name according to CosIng)</b>	<b>CAS number</b>
METHYLBENZYL ACETATE	93-92-5
METHYL DECENOL	81782-77-6
METHYL beta-NAPHTHYL ETHER	93-04-9
METHYLUNDECANAL	110-41-8
OXACYCLOHEXADECENONE	34902-57-3
PENTADECALACTONE	106-02-5
PHENETHYL ACETATE	103-45-7
PHENOXYETHYL ISOBUTYRATE	103-60-6
PHENYLISOHEXANOL	55066-48-3
Tetrahydrolinalool	78-69-3
TETRAHYDRO-METHYL-METHYLPROPYL)-PYRAN-4-OL	63500-71-0
TRICHLOROMETHYL PHENYL CARBINYL ACETATE	90-17-5
TRICYCLODECENYL PROPIONATE	17511-60-3
TRIMETHYLHEXYL ACETATE	58430-94-7
gamma-UNDECALACTONE	104-67-6
VERDYL ACETATE	2500-83-6/ 5413-60-5

## 7.2. Tabular summary of evaluated natural extracts/essential oils

Natural raw materials in terms of extracts are used in the fragrance and flavour industry for various reasons. Most importantly, several naturally occurring mixtures have a very complex composition and sensory nature which cannot (fully) be achieved by synthetic the demand for perfumes based on natural materials is considerable (182).

The three main methods used to concentrate plant fragrance substances; distillation, mechanical separation ("pressing"), and solvent extraction, yield very different extracts. Essential oils are obtained by water steam, water, ethanol, or water/ethanol distillation. Essence oils are essential oils that separate from the aqueous phase in the distillation receiver during the distillative concentration of fruit, usually citrus, juices. Citrus peel oils, apart from distilled lime oil, are prepared in a special way by pressing the peel to release mostly volatile substances from the pericarp in small oil glands, mostly highly volatile terpene hydrocarbons. However, they also contain small amounts of non-volatile compounds such as dyes, waxes and furocoumarines. The method of solvent extraction is generally applied in the separation of heat-labile materials or if an essential oil can only be obtained in very low yield, e.g. from blossoms. It is also used if the non-volatile components are desired for their fixative properties, e.g. in the preparation of resinoids from exudates. The most important extracts are termed: (i) concretes, an extract of fresh plant material with nonpolar solvents, containing not only volatile, but also a large proportion of non-volatile substances such as waxes; and (ii) absolutes, which are prepared by taking up concretes in ethanol; compounds that precipitate on cooling are removed by filtration, yielding a wax-free residue called absolute. Resinoids, used for their fixative properties, are prepared by extracting plant exudates with alcohols or nonpolar solvents. The products are usually highly viscous and thus sometimes diluted, e.g. with phthalates or benzyl benzoate. Oleoresins are concentrates prepared from spices by solvent extraction (182).

Regarding clinical data in terms of contact allergy to fragrance ingredients, the main focus of case reports or clinical studies on essential oils and natural extracts, respectively, is on general dermatological patients with complaints related to use of cosmetics etc. However,

series of cases with occupational exposure to essential oils with occupational allergic contact dermatitis have also been reported (e.g. masseurs, physiotherapists (183, 184), aromatherapists (185-189), beauticians performing massages (190). For further details, e.g. PT results with various essential oils, see the original case reports.

In this section, a tabular overview on the classification of substances considered is presented in three tables listing:

1. Extracts identified as *established contact allergens* in humans (→ *sufficient human evidence present*).
2. Extracts with positive human data, which are, however, not sufficient to categorise as *established contact allergen* in humans (→ *limited human evidence present*).
3. Extracts with negative human data, i.e. patch tests of patients with suspected contact allergy to fragrance ingredients which yielded negative results.

In Table 7-5, essential oils with sufficient human evidence to categorise these as *established contact allergens* in humans are presented.

**Table 7-5:** Natural extracts classified as established contact allergens in humans (summary of evaluation as detailed in chapter 6.3). More detailed information forming the basis of this evaluation can be found in Annex I of this opinion.

<b>INCI name (or, if none exists, perfuming name according to CosIng)</b>	<b>CAS number</b>	<b>Comment: see text</b>
<i>CANANGA ODORATA</i> and <i>Ylang-ylang oil</i>	83863-30-3; 8006-81-3	+++
<i>CEDRUS ATLANTICA</i> BARK OIL	92201-55-3; 8000-27-9	++
<i>CINNAMOMUM CASSIA</i> LEAF OIL <i>CINNAMOMUM ZEYLANICUM</i> BARK OIL	8007-80-5 84649-98-9	++ (r.t.)
<i>CITRUS AURANTIUM AMARA</i> FLOWER / PEEL OIL	8016-38-4; 72968-50-4	++
<i>CITRUS BERGAMIA</i> PEEL OIL EXPRESSED	89957-91-5	+ (r.t.)
<i>CITRUS LIMONUM</i> PEEL OIL EXPRESSED <sup>#</sup>	84929-31-7	++
<i>CITRUS SINENSIS</i> (syn.: <i>AURANTIUM DULCIS</i> ) PEEL OIL EXPRESSED	97766-30-8; 8028-48-6	++
<i>CYMBOPOGON CITRATUS</i> / <i>SCHOENANTHUS</i> OILS	89998-14-1; 8007-02-1; 89998-16-3	++
<i>EUCALYPTUS</i> SPP. LEAF OIL	92502-70-0; 8000-48-4	++
<i>EUGENIA CARYOPHYLLUS</i> LEAF / FLOWER OIL	8000-34-8	+++
<i>EVERNIA FURFURACEA</i> LICHEN EXTRACT <sup>4</sup> (Tree moss)	90028-67-4	+++
<i>EVERNIA PRUNASTRI</i> (Oak moss) <sup>#</sup>	90028-68-5	+++
<i>JASMINUM GRANDIFLORUM</i> / <i>OFFICINALE</i>	84776-64-7; 90045-94-6; 8022-96-6	+++
<i>JUNIPERUS VIRGINIANA</i>	8000-27-9; 85085-41-2	++
<i>LAURUS NOBILIS</i>	8002-41-3; 8007-48-5; 84603-73-6	++

<sup>#</sup> 76/768/EEC Annex III, part 1

<sup>#</sup> 76/768/EEC Annex III, part 1

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INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Comment: see text
<i>LAVANDULA HYBRIDA</i>	91722-69-9	+ (r.t.)
<i>LAVANDULA OFFICINALIS</i>	84776-65-8	++
<i>MENTHA PIPERITA</i>	8006-90-4; 84082-70-2	++
<i>MENTHA SPICATA</i>	84696-51-5	++
<i>MYROXYLON PEREIRAE</i> (Balsam of Peru) #	8007-00-9;	++++
<i>NARCISSUS SPP.</i>	diverse	++
<i>PELARGONIUM GRAVEOLENS</i>	90082-51-2; 8000-46-2	++
<i>Pinus mugo/ pumila</i> #	90082-72-7; 97676-05-6	++
<i>POGOSTEMON CABLIN</i>	8014-09-3; 84238-39-1	++
<i>ROSE FLOWER OIL (ROSA SPP.)</i>	Diverse	++
<i>SANTALUM ALBUM</i>	84787-70-2; 8006-87-9	+++
<i>TURPENTINE (oil)</i> #	8006-64-2; 9005-90-7; 8052-14-0	++++
<b>Verbena absolute</b> ( <i>Lippia citriodora</i> Kunth.) #	8024-12-2	++

Notes: r.t. Rarely tested.

Table 7-6 lists a number of essential oils, mostly tested in just one clinical department, and thus, or for other reasons, not satisfying the criteria for being categorised as *established contact allergen* in humans (i.e. *limited human evidence present*).

**Table 7-6:** Natural extracts with positive human data, which are, however, not sufficient to categorise as “established contact allergen in humans”. More detailed information forming the basis of this evaluation can be found in Annex I of this opinion.

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Comment	Ref.
<i>ACORUS CALAMUS ROOT OIL</i>	84775-39-3	n=7 pos. reactions to “calamus”	(191)
<i>CEDRUS DEODARA WOOD OIL</i>	91771-47-0	Rudzki 1976/1986 found 3 / 3 positive reactions	(191, 192).
<i>CITRUS AURANTIUM AMARA LEAF OIL</i>	72968-50-4	Several cases in 2 series from 1 centre	(191, 192).
<i>CITRUS TANGERINA ...</i>	223748-44-5	1 case	(193)
<i>CYMBOPOGON NARDUS</i> / <i>WINTERIANUS HERB OIL</i>	89998-15-2; 91771-61-8	Several cases in 2 series from 1 centre	(191, 192).
<i>ILLICIAM VERUM FRUIT OIL</i>	84650-59-9	Cases of active	(194)

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<b>INCI name (or, if none exists, perfuming name according to CosIng)</b>	<b>CAS number</b>	<b>Comment</b>	<b>Ref.</b>
		sensitisation; 34% consecutive patients pos. to 1%	
<b>LAVANDULA SPICA</b>	97722-12-8	Several cases in 2 series from 1 centre	(191, 192).
<b>LITSEA CUBEBA</b>	90063-59-5	Several cases in 2 series from 1 centre	(191, 192).
<b>PELARGONIUM ROSEUM</b>	90082-55-6	2.1% pos. of 1483 patients	(195).
<b>ROSMARINUS OFFICINALIS</b>	84604-14-8	3 cases in 2 series from 1 centre	(191, 192).
<b>SALVIA spp.</b>	<i>Diverse</i>	Several cases in 2 series from 1 centre	(191, 192).
<b>TAGETES PATULA</b>	91722-29-1	1 case (aromatherapist)	(185)
<b>THYMUS spp.</b>	84929-51-1	4 / 84 pos	(191)
<b>VETIVERIA ZIZANOIDES</b>	8016-96-4; 84238-29-9	1 / 200 and 9 / 86 pos.	(191, 192)

The last table is an indicative list of natural extracts which lack published human data, but which are of interest: (i) as high-volume exposure; (ii) due to published positive animal experiments; or (iii) because they contain well-known (established) contact allergens.

**Table 7-7:** Indicative list illustrating natural extracts containing established human allergens or having R43-lable or positive LLNA, lacking published human data.

<b>INCI name (or, if none exists, perfuming name according to CosIng)</b>	<b>CAS number</b>	<b>Comment</b>
<b>CITRUS PARADISI PEEL OIL</b>	8016-20-4	high volume substance, classified as R43
<b>CYMBOPOGON MARTINI HERB EXTRACT</b>	84649-81-0	Pos. LLNA study by RIFM: EC3 value 9.6% (196).
<b>MENTHA ARVENSIS</b>	68917-18-0	high volume, classified as R43
<b>OCIMUM BASILICUM</b>	84775-71-3	Pos. LLNA study by RIFM: EC3 value < 2.5% (196).
<b>PIMENTA RACEMOSA</b>	85085-61-6	Contains, among other substances, the established contact allergen eugenol (42-56%)
<b>SANTALUM SPICATA</b>	8024-35-9	Contains, among other substances, the established contact allergens santalols (75%) and farnesol (10%)

### 7.3. Conclusions

- According to the criteria described in chapter 6.3 a total of 54 individual chemicals and 28 natural extracts (essential oils) can be categorised as *established contact allergens* in humans, including all currently regulated substances.
- Of the 54 individual chemicals which are established contact allergens in humans, 12 are considered to be of special concern due to the high number of reported cases, (> 100, i.e. category +++ or ++++ in Table 7-1). These are further considered in chapter 5 (limonene and linalool) and the remainder in chapter 11. In particular one ingredient stands out, hydroxyisohexyl 3-cyclohexene carboxaldehyde, having been the cause of more than 1,500 reported cases since the 1999 opinion (see also chapter 4.2.1, chapter 11.3 and Annex I).
- For an additional 33 individual chemicals (Table 7-2) and 14 natural extracts (Table 7-6), positive patch test results have been reported. However, they do not qualify for the above category, i.e. only *limited human evidence* is present.
- For a number of fragrance substances (n=18, Table 7-3) patch testing did not yield positive results. However, numbers of patients tested are generally too small to rule out the existence of clinical contact sensitisation with sufficient confidence.
- No clinical evidence has been identified for 39 individual chemicals that have been reported to be frequently used (Table 7-4).
- For the substances (and, if possible, also for the main constituents of the natural mixtures) with limited or no human evidence, additional animal data and/or SAR considerations are taken into account. Aggregated data for these substances are presented in chapter 13.



## 8. Animal data

### 8.1. Predictive tests and sensitising potency categories

The animal test methods used in harmonised classification of substances, according to their potential to cause skin sensitisation, are the guinea pig maximisation test (GPMT), the Buehler test<sup>5</sup> and the local lymph node assay (LLNA)<sup>6</sup>. These methods are used in hazard identification and risk assessment for regulatory purposes under REACH<sup>7</sup>. For registration in REACH, the LLNA is the preferred method for measuring skin sensitisation potential in animals, and justification for the use of other methods needs to be provided. According to the directives on classification and labelling<sup>8</sup>, substances and preparations meeting positive criteria in these tests shall be classified as sensitising and assigned the symbol "Xi" and the risk phrase "R43: May cause sensitisation by skin contact"; or, according to the recent regulation on classification, labelling and packaging (CLP<sup>9</sup>) "H317: May cause an allergic skin reaction".

As yet, there is no validated *in vitro* test method accepted for skin sensitisation. Therefore, for cosmetic ingredients the LLNA, the GPMT and the Buehler test have also been used in risk assessment for regulatory purposes.

Positive results from the OECD guideline animal tests mentioned above which are sufficient to classify a substance as a skin sensitizer (R43) are:

- GPMT; at least 30% of the animals have a positive response.
- Buehler test; at least 15% of the animals have a positive response.
- LLNA; at least a 3-fold increase in lymph node cell proliferative activity is induced, compared to vehicle-treated controls (stimulation index  $SI \geq 3$ ). For positive LLNAs, an EC3 value is calculated which gives the estimated concentration of a chemical necessary to give a 3-fold increase in proliferative activity compared to vehicle-treated controls.

Further categorisation of substances classified with R43 into three groups according to allergen potency (extreme, strong and moderate) has been proposed by a European Commission expert group on skin sensitisation (157, 197). Such categorisation is based on EC3 values in the LLNA, on intradermal induction concentration in the GPMT, and topical induction concentration in the Buehler test. The potency categories and their default concentration values based on EC3 values in the LLNA as defined in (157): extreme sensitizer (EC3 value  $\leq 0.2$ ); strong sensitizer (EC3  $> 0.2 - \leq 2$ ); and moderate sensitizer (EC3 value  $> 2$ ). When LLNA EC3 values are available from more than one study, the lowest value should normally be used. Where multiple animal data sets lead to different categorisation of the same substance, the higher potency category should apply (157, 197).

The potency categorisation of substances based on the LLNA is applied by the SCCP in risk assessment of cosmetic ingredients, particularly hair dye substances (198).

#### 8.1.1. LLNA data

The SCCS requested the International Fragrance Association (IFRA) to submit data on animal tests performed with fragrance substances, by the local lymph node assay (LLNA) in mice, the guinea pig maximisation test (GPMT) and the Buehler test, and presented in a structured format. In response, IFRA submitted first a poster (158) and later a report

<sup>5</sup> OECD Guideline for testing of chemicals. Guideline 406: Skin Sensitisation. OECD, Adopted 12 May 1981, updated 17th July 1992.

<sup>6</sup> OECD Guideline for testing of chemicals. Guideline 429: Skin Sensitisation: Local Lymph Node Assay. OECD, Adopted 22 July 2010.

<sup>7</sup> Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).

<sup>8</sup> Directives 67/548/EEC and 1999/45/EC.

<sup>9</sup> Regulation No. 1272/2008.

consisting of LLNA protocol summaries on the 59 fragrance substances in the poster (159). No guinea pig studies were submitted. The SCCS has reviewed and analysed the report and the publications quoted in the report.

Table 8-1 displays the EC3 values for fragrance substances in the report submitted by industry (159). EC3 values for some additional fragrance substances in two published reviews (160, 161) have also been included in Table 8-1. Table 8-2 presents LLNA results for oxidised substances. Full data are given in Annex II. Table 8-3 summarises the distribution of fragrance substances, by potency category, according to EC3 values.

Additional EC3 values may be available in the scientific literature. Many more animal experiments may have been performed, but have not been published.

**Table 8-1:** Summary of local lymph node assay (LLNA) data on 66 fragrance substances, based on a report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009 (159)) and in published reviews by Gerberick et al. 2005 (160) and Kern et al. 2010 (161), respectively. EC3 values (% and M) are given. The order of substances is by decreasing sensitisation potency as assessed by LLNA EC3 values (lowest EC3 value indicating highest potency).

Substance	CAS no.	EC3 value		Reference
		%	M	
Hexyl salicylate	6259-76-3	0.18	0.008	(159, 161)
Cinnamal	104-55-2	0.2	0.015	(159)
Methyl 2-octynoate	111-12-6	<0.5	<0.032	(159, 161)
Isoeugenol	97-54-1	0.54	0.033	(159)
Citral	5392-40-5	1.2	0.079	(159)
2-Hexylidene cyclopentanone	17373-89-6	2.4	0.14	(159)
Methyl octine carbonate	111-80-8	2.5	0.15	(159)
Peru balsam absolute	8007-00-9	2.5	n/a	(159)
trans-2-Hexenal	6728-26-3	2.6	0.26	(159)
Benzyl Salicylate	118-58-1	2.9	0.23	(159, 161)
Butylphenyl methylpropional (BMHCA)	80-54-6	2.9	0.14	(159)
Phenylacetaldehyde	122-78-1	3	0.25	(159, 160)
Allyl phenoxyacetate	7493-74-5	3.1	0.16	(159)
Benzylideneacetone	122-57-6	3.7	0.25	(160)
3-Propylideneophthalide	17369-59-4	3.7	0.21	(159, 160)
<i>Evernia prunastri</i> extract oak moss	90028-68-5	3.9	n/a	(159)
Balsam oil, Peru ( <i>Myroxylon pereirae</i> Klotzsch)	8007-00-9	4	n/a	(159)
Farnesol	4602-84-0	4.1	0.18	(159)
p-t-Butyl-dihydrocinnamaldehyde	18127-01-0	4.3	0.23	(159)
α-Methyl cinnamic aldehyde	101-39-3	4.5	0.31	(159, 160)
Eugenol	97-53-0	5.3	0.32	(159)
Hexyl cinnamal	101-86-0	5.3	0.25	(159)
Dihydrocoumarin	119-84-6	5.6	0.38	(160)

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Substance	CAS no.	EC3 value		Reference
		%	M	
Geraniol	106-24-1	5.6	0.36	(159)
Carvone	6485-40-1	5.7	0.38	(159)
Diethyl maleate	141-05-9	5.8	0.34	(160)
2-Methoxy-4-methylphenol	93-51-6	5.8	0.42	(159, 160)
Anise alcohol	105-13-5	5.9	0.43	(159, 161)
Jasmine absolute ( <i>Grandiflorum</i> )	8022-96-6	5.9	N/a	(159)
Dibenzyl ether	103-50-4	6.3	0.32	(159)
<i>Cananga odorata</i> leaf/flower oil ylang ylang "extra"	8006-81-3	6.8	N/a	(159)
Isocyclocitral	1335-66-6	7.3	0.48	(159)
2,3-Dihydro-2,2,6-trimethylbenzaldehyde	116-26-7	7.5	0.50	(160)
Amyl cinnamal	122-40-7	7.6	0.38	(159)
Perillaldehyde p-Mentha-1,8-dien-7-al	2111-75-3	8.1	0.54	(159, 160)
p-Isobutyl- $\alpha$ -methyl hydrocinnamaldehyde	6658-48-6	9.5	0.46	(159)
d-Limonene*	5989-27-5	<10	<0.73	(159)
Methylundecanal	110-41-8	10	0.54	(160)
Acetylcedrene	32388-55-9	13.9	0.57	(161)
Methylenedioxyphenyl methylpropanal	1205-17-0	16.4	0.85	(159, 161)
Benzyl benzoate	120-51-4	17	0.80	(160)
Hydroxyisohexyl 3-cyclohexene carboxaldehyde	31906-04-4	17.1	0.81	(159, 160)
Benzyl cinnamate	103-41-3	18.4	0.77	(159, 161)
Hydroxycitronellal	107-75-5	19.3	1.12	(159)
Cinnamyl alcohol	104-54-1	21	1.57	(160)
$\alpha$ -iso-Methylionone	127-51-5	21.8	1.06	(159, 161)
Cyklamen aldehyde	103-95-7	22	1.64	(160)
4-Methoxy- $\alpha$ -methyl benzenpropanal	5462-06-6	23.6	1.32	(159)
Amyl cinnamyl alcohol	101-85-9	~25	~1.22	(159, 161)
Tetramethyl acetyloctahydronaphthalenes (OTNE)	54464-57-2	25.1	1.07	(159)
Ethyl acrylate	140-88-5	28	2.8	(160)
Linalool*	78-70-6	30	1.94	(160)
Trimethylbenzenepropanol Majantol	103694-68-4	30	~1.68	(159)
Jasminum Sambac Flower CERA/Extract/Water	91770-14-8	35.4	N/a	(159)
Citronellol	106-22-9	43.5	2.78	(159, 161)
<b>No EC3 value was established; higher concentrations should also have been tested</b>				
6-Methyl-3,5-heptadien-2-one	1604-28-0	>5	>0.40	(159)

Substance	CAS no.	EC3 value		Reference
		%	M	
<i>Camellia sinensis</i> leaf tea leaf absolute	84650-60-2	>5	N/a	(159)
Cinnamyl nitrile	1885-38-7	>10	>0.77	(159)
Menthadiene-7-methyl formate	68683-20-5	>10	>0.51	(159)
<i>Evernia furfuracea</i> extract tree moss absolute	90028-67-4	>20	N/a	(159)
Isocyclogeraniol	68527-77-5	>25	>1.62	(159)
1-Octen-3-yl acetate	2442-10-6	>30	>1.76	(159)
Benzyl alcohol	100-51-6	>50	>4.62	(159)
Coumarin	91-64-5	>50	>3.42	(159)
Vanillin	121-33-5	>50	>3.3	(159)
<b>No EC3 value calculated</b>				
Benzaldehyde	100-52-7	-		(160)

Notes: \* Material with low levels of oxidation according to (159)

n/a: Not applicable (mixture of compounds).

M: EC3 based on molar concentration

### 8.1.2. LLNA data on oxidised fragrance substances

For fragrance substances that can autoxidise upon air exposure, it is also important to investigate the sensitisation potency after air exposure. The oxidised compounds are clinically relevant as they represent what the consumers could come in contact with from perfumes and fragranced products. In Table 8-2 the LLNA data for some of the most commonly used fragrance substances, pure and after autoxidation, are presented. The EC3 values obtained for the pure substances are 5-10 times higher compared to those obtained for the same substances after air exposure. The experimental air exposure simulated air exposure that can take place during normal handling and storage. In the production process, some perfumes are "matured" aerobically, stirring included. During this process, some fragrance substances may be oxidised. It should be noted that, although only a few substances capable of oxidation have so far been investigated, structural alerts indicating possible autoxidation are common among the fragrance substances listed in this document (see chapter 9). It is important to further investigate this issue for increased understanding of the associated risk.

**Table 8-2:** Local lymph node assay (LLNA) data on four fragrance substances and one essential oil before and after air exposure, comparing the sensitisation potency of the pure (not oxidised) substance with the potency of the oxidised.

Substance	CAS no.	Doses % (w/v) vehicle: A:OO 4:1*	EC3 value (% w/v)	Reference
D-Limonene (ox. 10 w)	5989-27-5	1, 5, 25	3.0	(199)
D-Limonene (pure)	5989-27-5	25, 50, 100	30	
Linalool (ox. 10 w)	78-70-6	5, 10, 25	9.4	(126)
Linalool (ox. 45 w)	78-70-6	2.5, 10, 25	4.8	
Linalool (pure)	78-70-6	25, 50, 100	46.2	

Substance	CAS no.	Doses % (w/v) vehicle: A:OO 4:1*	EC3 value (% w/v)	Reference
Linalyl acetate (ox. 10 w)	115-95-7	0.5, 10, 40	3.6	(127)
Linalyl acetate (pure)	115-95-7	10, 30, 100	25	
Geraniol (ox. 10 w)	106-24-1	1, 3, 6, 10, 20	4.4	(118)
Geraniol (ox. 45 w)	106-24-1	0.5, 1, 3, 6, 10	5.8	
Geraniol (pure)	106-24-1	5, 10, 15, 20, 30	22.4	
Lavender oil (ox. 10 w)		1, 5, 10, 20, 50	11	(140)
Lavender oil (ox. 45 w)		1, 5, 10, 20, 50	4.4	
Lavender oil (not ox.)		5, 25, 100	36	

Notes: Pure: Purified before testing as most commercially available fragrance substances are not pure.

Not ox.: Not purified but used as it was delivered as this is a complex mixture and not a specific substance.

Ox. x w: Oxidised by air exposure during x weeks.

\* Acetone:olive oil.

## 8.2. Methodological considerations

### *EC3 mean values*

In the submitted poster (158) and the report by IFRA (159), the LLNA weighted mean EC3 values ( $\mu\text{g}/\text{cm}^2$ ) are presented. The SCCS considers it is misleading to present EC3 values as mean values from tests performed with different vehicles. It is generally agreed that the lowest EC3 value should be used if there is more than one study fulfilling the OECD guideline requirements (157, 197), and these have been introduced into Table 8-1. The EC3 values in the reviews by Gerberick et al. and Kern et al. (160, 161) were based on single representative experiments with a vehicle described in the OECD guideline 429 (see above), and preferably with acetone:olive oil. EC3 mean values, as in the submission by IFRA, were not presented in these two reviews.

### *Vehicle*

The most frequently used *vehicle* in the submission by IFRA (159) was ethanol:diethyl phthalate (1:3), followed by acetone:olive oil (4:1). In some experiments, antioxidants were mixed with ethanol:diethyl phthalate. The vehicle was not reported in some of the references, and no rationale for using vehicles other than those recommended was given in the report (159). According to the OECD guideline 429 (see above), the recommended vehicles are acetone:olive oil (4:1), N,N-dimethylformamide, methyl ethyl ketone, propylene glycol, and dimethyl sulphoxide, but others may be used if sufficient scientific rationale is provided. It is well known that a difference in the EC3 value can be obtained for the same substance depending on which vehicle is used in the LLNA. Thus as an *additional control*, supplementary to the guideline based LLNA control, a clinically relevant solvent or the commercial formulation in which the test substance is marketed may be used.

### *Number of doses and animals*

According to the OECD guideline 429 (see above), a minimum of three concentrations should be tested. The number of consecutive doses used in the reported data, was generally five, sometimes three and in few experiments two. The SCCS considers that too few concentrations were tested in four studies in which only two concentrations were used. Lower concentrations than those tested should have been used in experiments with five

fragrance substances, in which the EC3 value could not be determined. Higher concentrations than those tested should also have been used in experiments with 12 substances, in which the EC3 value could not be determined.

The *number of animals* per dose group was generally four plus a non-exposed control group, sometimes five, and in few experiments six; the minimum according to the OECD guideline being four.

### **Units for concentrations**

In the submission by IFRA (159) the EC3 values are given in weight per area unit ( $\mu\text{g}/\text{cm}^2$ ). The SCCS considers that the EC3 values (%) are the values of primary interest in communicating risk assessment, as EU legislation, OECD guideline 429 and scientific literature refer to EC3 values (%). However, the SCCS recommends that molar (M) EC3 values should be considered, as they give the concentration based on the molecular weight of substances. They have thus been calculated and introduced into Table 8-1.

EC3 values (%) overestimate the intrinsic molecular sensitisation potency for low molecular weight compounds while compounds with a high molecular weight are underestimated. Regarding the differences in molecular weight between the studied fragrance substances, a variation is seen if the ranking list of the sensitisation potency is based on EC3 (%) or EC3 (M) since some substances have a molecular weight twice as high as others.

From comparisons in Table 8-1, we notice that, e.g. hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) has an EC3 value of 17.1 %, or 0.81 M when the calculation includes its molecular weight, while for trans-2-hexenal the corresponding values are 2.6% and 0.26 M. The example shows that comparing the sensitisation potency between these two substances using the EC3 values in % exaggerates the sensitisation potency of trans-2-hexenal compared to that of HICC. When using the EC3 values in molar concentrations the difference is not so pronounced.

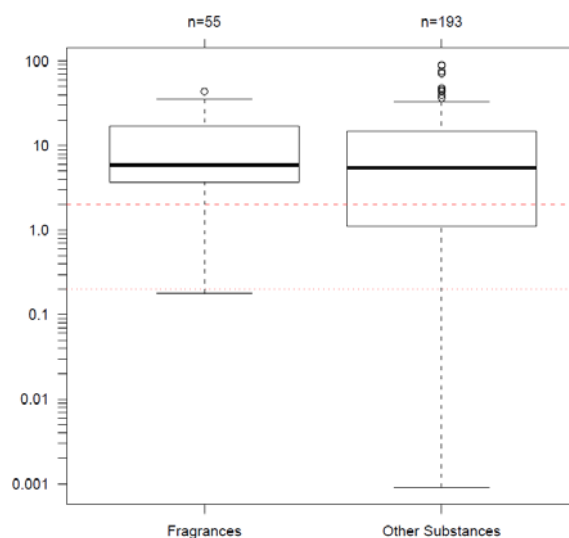
### **8.3. Summary of animal data by LLNA**

The distribution of sensitising potency of fragrance substances compared to other substances, (e.g. biocides, dyes, plastic materials) taken from three references (159-161) as assessed by EC3 values in the LLNA, is shown in Figure 8-1 and Table 8-3.

The median EC3 value of fragrance substances (5.9%) is similar to other substances tested (5.5%). However, very few fragrance substances have low EC3 values ( $\leq 2$ ).

Substances with an EC3 value  $\leq 2$  may be categorised as strong or extreme sensitisers. Such potent sensitisers are comparatively rare among fragrance substances assessed in the LLNA. Nevertheless, fragrances are important allergens in humans, which points to repeated skin exposure to less potent sensitisers as a factor strongly determining sensitisation risk.

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**Figure 8-1:** The distribution of fragrance chemicals and a variety of other chemicals (e.g. biocides, dyes, plastic materials), taken from the three references (159-161), are depicted as boxplots on a logarithmic scale. The bottom of the box denotes the 1<sup>st</sup> quartile (25% percentile), the thick line in the box the median, and the top of the box the 3<sup>rd</sup> quartile (75% percentile). Outliers, i.e. below the 25% and above the 75% percentiles, are shown as whiskers. Beyond the 1.5-fold interquartile range, single values are shown as circles instead of whiskers. The difference in distribution is not significant (Wilcoxon test:  $p=0.061$ ).

Note: EC3 values for the five oxidised fragrances additionally examined (Table 8-2) range from 3.0 to 4.8 (median 4.4) and are lower by a factor of around 7 than EC3 values of the respective non-oxidised material.

**Table 8-3:** Summary of EC3 values for fragrance substances in Table 8-1 and for other substances, all taken from the three references (159-161). The EC3 value intervals for potency categorisation (157, 197) were used for comparison of fragrances substances vs other substances.

EC3 value interval	Fragrance substances		Other substances	
	no.	%	no.	%
≤ 0.2	2	3%	28	11%
> 0.2 - ≤ 2	3	4%	38	15%
> 2	50	71%	127	49%
No EC3 value established *	10	14%	0	0%
No EC3 value calculated (NC)	5	7%	69	26%
<b>All substances</b>	<b>70</b>		<b>262</b>	

Note: \* Substances should have been tested also at higher concentrations.

#### 8.4. Conclusions

- In the event that human data are lacking, the LLNA provides important information on skin sensitising potential and potency.
- Animal data on fragrance substances submitted by IFRA (159) and assessed in this opinion were generated exclusively by LLNA. Other guideline methods are, however, also available.
- The vast majority of the submitted (159) and additional (160, 161) fragrance substances tested by the LLNA are skin sensitisers.
- Several studies in the IFRA report (159) were of insufficient quality, not following the OECD guideline.



- Fragrance substances that can be predicted to autoxidise upon air exposure should also be tested after air exposure, as oxidation may significantly increase their sensitising potency.
- It can be concluded that the skin sensitising potency, as assessed by the LLNA, is only one of several factors that are of importance for sensitisation to fragrance substances. This is illustrated by the fact that only a small fraction of sensitising fragrance substances can be categorised as an extreme allergen based on LLNA test results. Therefore, doses from repeated deposition onto skin must be considered a driving force of sensitisation risk.

## 9. Structure activity relationships (SAR): grouping of substances based on expert judgement

Whether or not a particular chemical will be a sensitiser, and how potent it will be if it is a sensitiser, depends on its ability, either directly or after activation, to react with appropriate proteins in the skin. The ability to predict sensitisation potency, or lack of it, depends on being able to predict reactivity to skin proteins. This is the basis of SAR analysis for skin sensitisation. The prediction can often be made based on the chemical structure, recognising structural features (referred to as **structural alerts**) that are associated with reactivity. Examples of structural alerts are aliphatic aldehydes (alerting to the possibility of sensitisation via a Schiff base reaction with protein amino groups), and  $\alpha,\beta$ -unsaturated carbonyl groups, C=C-CO- (alerting to the possibility of sensitisation via Michael addition of protein thiol groups). Major mechanistic reactivity domains have been discussed in detail by Aptula and Roberts (200). Prediction of the sensitisation potential of compounds that can act via abiotic or metabolic activation (pre- or prohaptens) is more complex compared to that of compounds that act as direct haptens without any activation. The autoxidation patterns can differ due to differences in the stability of the intermediates formed, e.g. it has been shown that autoxidation of the structural isomers linalool and geraniol results in different major haptens/allergens. Moreover, the complexity of the prediction increases further for those compounds that can act both as pre- and prohaptens. In such cases, the impact on the sensitisation potency depends on the degree of abiotic activation (e.g. autoxidation) in relation to the metabolic activation. See also chapter 5.

These structural alerts can be applied by computerized expert systems, i.e. *in silico* or by estimations made by organic chemists (*in cerebro*) using their experience. When an organic chemist looks at a chemical structure, they recognise parts of the structure that they can associate with reactivity, the type of reactivity (i.e. assign the reaction mechanistic domain), and other features of the molecular structure that will affect the reactivity positively or negatively. Human experts should be aware of the complexities, and how structural modification can alter the reactivity associated with structural alerts, etc. Importantly, they can also recognise where there are unfamiliar structural features whose effects they cannot confidently predict. In such cases they can call for experimental chemistry work (*in chemico*) to be done to ascertain the presence or nature of, and degree of reactivity. *In chemico* methods include organic chemistry experimentation to identify chemical reaction products from oxidation and/or reaction with model nucleophiles, identification of mechanisms of reaction. In so called *in chemico* reactivity methods, the ability of a specific chemical to react with selected peptides is determined so as to predict the sensitisation potential of the chemical under investigation (201, 202). To make *in chemico* reactivity methods able to predict the activity of prohaptens, the addition of horseradish peroxidase and hydrogen peroxide oxidation system has been tested to model the enzymatic oxidation in the skin (203, 204).

Although computerized expert systems are derived from input by human experts, they are less well able to capture the subtleties of structure reactivity relationships, and they sometimes fail to detect aspects of chemistry that are obvious to organic chemists. Human experts should be aware of the complexities, as well as how structural modification can alter the reactivity associated with structural alerts, etc. The SAR evaluation made in this section summarised in Table 9-3 and Table 9-4 is based on *in cerebro* alerts applied by organic chemists.

Depending on the type of reactivity (the **reaction mechanistic domain**), it is sometimes possible to make a quantitative prediction of potency in the LLNA, which can be used to predict potency in humans relative to related known human sensitisers. These predictions use quantitative mechanistic models (**QMMs**) based on reactivity expressed quantitatively by model parameters, and sometimes in combination with hydrophobicity. For example, potency of aliphatic aldehydes and ketones (the Schiff base domain) in the LLNA is modelled by a combination of reactivity and hydrophobicity (205), whereas the LLNA potency of DNCB analogues (the  $S_NAr$  domain) is well modelled by reactivity alone (206).

QMMs aiming not only to predict the potential to be a sensitiser but also to predict the potency, promise to be a useful tool in non-animal based risk assessment for skin sensitisation. However, in the field of fragrance substances there are major gaps in our present ability to apply QSAR/QMM. This is largely because many of the fragrance substances of interest have the potential to act via abiotic or metabolic activation (pre- and/or prohaptens, see chapter 5), i.e. they themselves are only weak or non-sensitisers, but have the potential to be activated to form more potent sensitisers. Resulting sensitisation potency will depend on the extent of activation and the nature of the resulting products. We can apply SAR analysis to identify these plausible possibilities, but QSAR modelling for these cases is not yet developed. However, much progress has been made in identifying structural alerts for the various activation mechanisms that have been recognised. This is reviewed by Karlberg et al. (121).

Chemicals with no structural alerts for direct reactivity, or for known activation mechanisms, and no unfamiliar structural features that might be associated with as yet unidentified activation mechanisms, can be predicted to be non-sensitising. Chemicals that do have alerts for reactivity (direct or via activation) are not necessarily sensitisers – they may be insufficiently reactive and/or insufficiently hydrophobic.

Substances meeting the inclusion criteria (see chapter 6), for which, however, no categorisation as established contact allergen in humans or established contact allergen in animals was possible, have been assessed for structural alerts. The results are presented in four tables (Table 9-1 to Table 9-4) based on the prediction made for the actual substance. The following SAR assessments have been used:

- Predicted sensitiser; structural alerts (Table 9-1).
- Possible sensitiser; structural alerts (Table 9-2).
- Predicted non-sensitiser (NS); no obvious structural alerts (Table 9-3).
- Not predictable due to insufficient/conflicting data (Table 9-4).

**Table 9-1:** Predicted sensitisers.

Substance (INCI) name	CAS number	Structural alerts
p-tert.-Butyldihydrocinnamaldehyde <sup>§</sup>	18127-01-0	Schiff base
Citronellal	106-23-0	Schiff base and possible prehapten
Citronellyl nitrile	51566-62-2	Possible prehapten
Decanal	112-31-2	Schiff base
3,7-Dimethyl-1,6-nonadien-3-ol	10339-55-6	Prehapten
Geranyl acetate	105-87-3	Prehapten and prohapten
Isoamyl salicylate	87-20-7	Acyltransfer agent
Methyl cinnamate	103-26-4	Michael acceptor
Methylundecanal	110-41-8	Schiff base
Myrcene	123-35-3	Prehapten
Nerol	106-25-2	Prehapten and prohapten
Nerolidol (isomer not specified)	7212-44-4	Possible prehapten
Oxacyclohexadecenone	34902-57-3	Michael acceptor
Phenethyl salicylate	87-22-9	Acyltransfer agent
trans-Rose ketone-5	39872-57-6	Michael acceptor and possible prehapten

Note: § Classified as R43.

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**Table 9-2:** Possible sensitisers.

<b>Substance (INCI) name</b>	<b>CAS number</b>	<b>Structural alerts</b>
Ambrettolide	7779-50-2	Possible prehapten
Amylcyclopentanone	4819-67-4	Schiff base; the combination of reactivity and hydrophobicity may be enough to confer sensitisation
Benzyl acetate	140-11-4	Prohapten via hydrolysis leading to benzyl alcohol
Carvacrol	499-75-2	Possible prehapten
Cuminaldehyde	122-03-2	Schiff base and possible prehapten
alpha-Cyclohexylidene benzeneacetonitrile	10461-98-0	Possible Michael acceptor
Cyclopentadecanone	502-72-7	Schiff base; the combination of reactivity and hydrophobicity may be enough to confer sensitisation
trans-beta-Damascone	23726-91-2	Possible Michael acceptor
trans-trans-delta-Damascone	71048-82-3	Possible Michael acceptor and possible prehapten
gamma-Damascone	35087-49-1	Possible Michael acceptor and possible prehapten
Dihydromyrcenol	18479-58-8	Possible prehapten
2,3-Dihydro-2,2,6-trimethylbenzaldehyde	116-26-7	Possible Michael acceptor and possible prehapten and possible prohapten
2,4-Dimethyl-3-cyclohexen-1-carboxaldehyde §	68039-49-6	Schiff base and possible prehapten
Dimethyltetrahydro benzaldehyde	68737-61-1	Schiff base and possible prehapten
6-Ethylideneoctahydro-5,8-methano-2H-benzo-1-pyran	93939-86-7	Possible prehapten
2-Ethyl-4-(2,2,3-trimethyl-3-cyclopenten-1-yl)-2-buten-1-ol	19-61-6	Possible prehapten
Ethyl vanillin	121-32-4	Complex
Heliotropine	120-57-0	Possible prohapten
3a,4,5,6,7,7a-Hexahydro-4,7-methano-1H-inden-5(or 6)-yl acetate	54830-99-8	Possible prehapten
Hexahydro-methanoindenyl propionate	68912-13-0	Possible prehapten
Ionone isomeric mixture	8013-90-9	Possible Michael acceptor and possible prehapten
alpha-Ionone	127-41-3	Possible Michael acceptor and possible prehapten
beta-Ionone	79-77-6	Possible Michael acceptor
Isobergamate	68683-20-5	Possible prehapten
Isolongifoleneketone	33407-62-4	Schiff base; the combination of reactivity and hydrophobicity may be enough to confer sensitisation

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Substance (INCI) name	CAS number	Structural alerts
Longifolene <sup>5</sup>	475-20-7	Possible prehapten
Methoxycitronellal	3613-30-7	Schiff base
Methyl decenol	81782-77-6	Possible prehapten
Methyl ionone (mixture of isomers)	1335-46-2	Possible Michael acceptor and possible prehapten
Methylionantheme	55599-63-8	Possible Michael acceptor and possible prehapten
5-Methyl-alpha-ionone	79-69-6	Possible Michael acceptor and possible prehapten
Myrtenol	515-00-4	Possible prehapten
Nopyl acetate	128-51-8	Possible prehapten
Phytol	150-86-7	Possible prehapten and/or prohapten
Rhodinol	6812-78-8	Possible prehapten
Terpineol acetate (isomer mixture)	8007-35-0	Possible prehapten
alpha-Terpinyl acetate	80-26-2	Possible prehapten
Tricyclodecanyl propionate	17511-60-3	Possible prehapten
Verdyl acetate	2500-83-6/ 5413-60-5	Possible prehapten

Note: <sup>5</sup> Classified as R43.

**Table 9-3:** Predicted non-sensitisers with no obvious structural alerts.

Substance (INCI) name	CAS number	Structural alerts
6-Acetyl-1,1,2,4,4,7-hexamethyltetraline	21145-77-7	
Benzyl acetone	2550-26-7	Schiff base; the combination of reactivity and hydrophobicity may <b>not</b> be enough to confer sensitisation
2-tert.-Butylcyclohexyl acetate	88-41-5	
4-tert.-Butylcyclohexyl acetate	32210-23-4	
Cyclohexyl acetate	622-45-7	
Diphenyl ether	101-84-8	
Ethyl 2-methylbutyrate	7452-79-1	
Ethylene dodecanioate	54982-83-1	
Ethylene brassylate	105-95-3	
Eucalyptol	470-82-6	
Hexyl acetate	142-92-7	
Hibiscolide	6707-60-4	
Hydroxycitronellol	107-74-4	However, dehydration followed by autoxidation could give sensitising impurities
Isoamyl acetate	123-92-2	

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Substance (INCI) name	CAS number	Structural alerts
Isobornyl acetate	125-12-2	
Methoxytrimethylheptanol	41890-92-0	
Methyl p-anisate	121-98-2	
Methyl anthranilate	134-20-3	
Methylbenzyl acetate	93-92-5	
Methyl dihydrojasmonate	24851-98-7	Schiff base; the combination of reactivity and hydrophobicity may <b>not</b> be enough to confer sensitisation
Oxalide	1725-01-5	
Pentadecalactone	106-02-5	
Phenethyl acetate	103-45-7	
Phenethyl alcohol	60-12-8	
Phenoxyethyl isobutyrate	103-60-6	
Phenylisohexanol	55066-48-3	
Phenylpropanol	122-97-4	
Tetrahydrolinalool	78-69-3	
Tetrahydro-methyl-methylpropyl)-pyran-4-ol	63500-71-0	
Trimethylhexyl acetate	58430-94-7	
Trimethyl-propylcyclohexanepropanol (tmch)	70788-30-6	
gamma-Undecalactone	104-67-6	

**Table 9-4:** Not predictable.

Substance (INCI) name	CAS number	Structural alerts
Anisaldehyde	123-11-5	Due to insufficient /conflicting data; structural similarities to benzaldehyde suggest certain activity in man
Trichloromethyl phenyl carbonyl acetate	90-17-5	Due to insufficient /conflicting data
Methyl beta-naphthyl ether	93-04-9	Due to insufficient /conflicting data

### 9.1. General results

From this work with the included SAR predictions, the following observations can be made.

- SAR prediction is a useful tool for estimation of the sensitisation potential of those compounds that lack human and animal data as the skin sensitisation potential is closely connected to chemical reactivity.
- For substances for which sufficient experimental/clinical evidence is missing, SAR analyses have been performed to predict a probable or possible risk of allergenic (sensitising) effect. These predictions are based on chemical reactivity and the recognition of structural features in a substance that are in common with the structural features that have been shown to cause sensitisation from other substances. In cases where the SAR analysis indicates a sensitisation potential, the

substance should be investigated further to confirm or reject the conclusion drawn from the SAR analysis.

- Prediction of the sensitisation potential of compounds that can act via abiotic or metabolic activation (pre- or prohaptens) becomes more complex compared to that of compounds that act as direct haptens without any activation.
- The complexity of the prediction increases further for those compounds that can act both as prehaptens and prohaptens.
- Prediction of the sensitisation potential of compounds that can act as prehaptens is further complicated by the fact that the autoxidation patterns can differ due to differences in the stability of the intermediates formed, e.g. it has been shown that autoxidation of the structural isomers of linalool and geraniol results in different major haptens/allergens.

## **9.2. Conclusions**

- Applying only mechanism-based QSAR (QMM) as a tool in non-animal based risk assessment for skin sensitisation is of limited value for fragrance substances. This is due to major information gaps in the present model when addressing substances that act via abiotic or metabolic activation, and the high incidence of such substances in fragrances.
- Quantitative structure activity relationship (QSAR) models should be further developed, combining, as appropriate, information from *in silico*, *in chemico* and *in vitro* methods.



## 10. Exposure

Exposure to fragrance chemicals and other potential allergens is most commonly by direct skin contact. Exposures to fragrance chemicals occur from:

- Personal cosmetic use;
- Detergents and other household products;
- Medicaments;
- Occupation, i.e. personal hygiene, manufacturing ingredient(s), product in work process, plant materials;
- Secondary exposure from another individual (e.g. spouse, child);
- Toys;
- Oral intake;
- Airborne exposure.

Factors that are important for both the induction and elicitation of contact allergy are:

- Dose per unit area;
- Vehicle effects including penetration enhancers;
- Presence of skin irritants;
- Presence of other allergens (combination effects);
- Duration of skin exposure;
- Frequency of applications;
- Anatomical sites of exposure;
- Condition of the skin (barrier function impairment, pre-existing inflammation);
- Occlusion (e.g. in flexures, under clothing and personal protective equipment).

Fragrance mix ingredients are commonly present in cosmetic formulations (68, 207-209). Cosmetics based on natural ingredients may contain fragrance allergens at a higher concentration than other cosmetic products (210). The clinical significance of exposure to natural extracts is difficult to determine as there is often "hidden and variable" exposure to important and potent allergens in natural products.

### 10.1. Concentrations and quantities used

Consumers are exposed in daily life to fragrance chemicals from a large variety of products, such as cosmetics, toys, detergents and other cleaning products, etc. The fragrance exposure may be via dermal and/or inhalation route. With respect to "Terms of Reference" to the SCCS, only dermal exposure from cosmetics is addressed in this opinion. As cosmetics are the perfumed products most commonly used in daily life, potential fragrance allergens identified by the use of cosmetics also represent the exposures of these chemicals from other product categories. In recent years, it has become a trend to add fragrance chemicals to many other types of consumer products, such as children's toys, toilet paper and nappies, which may contribute significantly to the fragrance exposure of the consumer by the dermal route.

Factors for the fragrance exposure assessment by the dermal route require knowledge on:

- Product types (categorisation of scented products) used by the consumer.
- Market survey (impression of the qualitative and quantitative contents of different allergens in consumer products).

- Hydrolysis, metabolism or oxidation of a fragrance material, which may generate a potential skin allergen.
- Chemicals in the product matrix, which may significantly enhance or reduce dermal absorption of a fragrance material.

Fragrance materials, both defined chemical substances and natural mixtures of chemicals (essential oils), are used in all types of cosmetic products: perfumes, eau de cologne, eau de perfume (EDP), and eau de toilette (EDT), aftershave lotion, deodorants, skin care products, skin cleansers, make-up cosmetics, hair care products, and oral care products, etc. However, some unscented cosmetic products have also reached the market in the last decade. Products containing the highest concentration of fragrance chemicals are perfumes, followed by eau de cologne, eau de perfume (EDP) and eau de toilette (EDT). Concentrations of fragrance chemicals in deodorant products are lower than those in EDT/EDP products, but still significant. Aftershave products also contain relatively high amounts of fragrance chemicals. Other cosmetic products contain relatively low amounts, 0.1-1% of perfume oil, compared to up to 30% perfume oils in EDT/EDP (211). The perfume oils are mixtures of 20 to over 200 synthetic fragrance chemicals or natural fragrance materials (essential oils), selected from over 3,000 fragrance materials (211). Perfume oil of the same composition is used in different concentrations in the formulation of various cosmetic products within a brand of cosmetics. For the exposure assessment, levels of fragrance chemicals in cosmetics containing significant amounts of fragrance materials (i.e. EDP/EDT/aftershave/deodorant) should be selected. It may not be possible to detect/measure the amounts of all fragrance chemicals when present in highly diluted form in a cosmetic product such as skin care products, make-up cosmetics etc. On the other hand, if a fragrance is evaluated safe for use when present in significant amounts in a product, it will also be safe for use in other products. Also the analysis of trend of the use of individual fragrance materials should be based on monitoring their contents in fine perfumes and deodorants.

Ninety of the 100 fragrance materials used in annual volumes > 175 tons in perfume formulations are fragrances and the remaining ten are used for other functions such as solvents, antioxidants, and skin penetration enhancers (for example isopropyl myristate), etc. (IFRA, personal communication 2010).

Among the 26 fragrances currently requiring individual labelling, amyl cinnamal, benzyl benzoate, benzyl salicylate, butyl phenyl methyl propional, citral, citronellol, coumarin, eugenol, geraniol, hexyl cinnamal, hydroxyisohexyl 3-cyclohexene carboxyaldehyde (HICC), alpha-isomethyl ionone, and linalool are used in volumes greater than 175 ton.  $\alpha$ -Amylcinnamyl alcohol, anisyl alcohol, benzyl alcohol, benzyl cinnamate, cinnamal, cinnamyl alcohol, farnesol, hydroxycitronellal, isoeugenol, *d*-limonene, methyl-2-octynoate, oak moss (*Evernia prunastri*), tree moss (*Evernia furfuracea*) are used in volumes less than 175 ton.

According to the information from the fragrance industry, 80% of the total fragrance chemical volume is used in cosmetics and 20% in household products.

Since the implementation of the regulation of labelling of 26 fragrance substances in cosmetic products, qualitative information on fragrance exposure from cosmetics is provided in some market surveys performed on cosmetics (Table 10-1, (212)) and (Table 10-2, (213)) and on consumer products including cosmetics (Table 10-3, (214); Table 10-4, (114); and Figure 10-1, (104)). Thus, the implementation of the regulation of fragrance allergens in detergents (Directive 648/2004/EC), similar to that for cosmetics, has also added to the knowledge of fragrance exposure to the consumer. These market surveys revealed that fragrance ingredients which are potent allergens and frequently cause allergies in consumers are used as ingredients in consumer products including cosmetics. The results of these surveys further revealed that limonene and linalool were the most commonly used fragrance chemicals in cosmetics, while anisyl alcohol, cinnamal,  $\alpha$ -amylcinnamyl alcohol, oak moss and tree moss were the least used fragrance ingredients in cosmetics and other consumer products. In general, the most potent allergens were also the most infrequently used ingredients. Prior to the regulation of the 26 allergens, analysis of

21 selected fragrance chemicals in deodorants also revealed additional 66 potential allergens in these products on the basis of structure activity relationship (215).

**Table 10-1:** Presence in children's cosmetics of the 26 fragrance substances that are required to be labelled in cosmetics (212).

Fragrance substance		% Products labelled to contain the fragrance substance
INCI name	CAS number	
Amyl cinnamal	122-40-7	8.2
alpha-Amylcinnamyl alcohol	101-85-9	2.9
Anise alcohol	105-13-5	0
Benzyl alcohol	100-51-6	9.6
Benzyl benzoate	120-51-4	9.1
Benzyl cinnamate	103-41-3	2.9
Benzyl salicylate	118-58-1	9.6
Butyl phenyl methyl propional	80-54-6	7.7
Cinnamal	104-55-2	1
Cinnamyl alcohol	104-54-1	6.7
Citral	5392-40-5	8.2
Citronellol	106-22-9	10.5
Coumarin	91-64-5	4.8
Eugenol	97-53-0	7.2
Farnesol	4602-84-0	2.9
Geraniol	106-24-1	12
Hexyl cinnamal	101-86-0	10.1
Hydroxycitronellal	107-75-5	6.3
Hydroxyisohexyl-3-cyclohexene carboxyaldehyde	31906-04-4	5.8
Isoeugenol	97-54-1	0.5
Alpha-isomethyl ionone	127-51-5	5.8
$\alpha$ -Limonene	5989-27-5	23.1
Linalool	78-70-6	21.6
Methyl-2-octynoate	111-12-6	0
<i>Evernia prunastri</i> /oak moss	90028-68-5	0
<i>Evernia furfuracea</i> /tree moss	90028-67-4	0

## Opinion on fragrance allergens in cosmetic products

**Table 10-2:** Usage trends in deodorants of fragrance chemicals that are required to be labelled in cosmetics.

Fragrance substance		88 products investigated in 2007 (213)			70 products investigated in 1998 (216)	
INCI name	CAS number	% Products labelled to contain the fragrance	Content in 23 selected products		Content in all 70 products	
			% Products found to contain the fragrance	Range(ppm)	% Products found to contain the fragrance	Range (ppm)
Amyl cinnamal <sup>■</sup>	122-40-7	10.2	17	2.3-165	31	1-617
alpha-amyl cinnamyl alcohol	101-85-9	-	-	-	n.a.	n.a.
Anise alcohol	105-13-5	2.3	9	1, 51	n.a.	n.a.
Benzyl alcohol	100-51-6	17.1	26	32-166	76	1-629*
Benzyl benzoate	120-51-4	25.0	48	3-4054	71	1-1075
Benzyl cinnamate	103-41-3	3.4	9	74, 143	n.a.	n.a.
Benzyl salicylate	118-58-1	39.8	48	136-5279	49	1-18758
Butyl phenyl methyl propional	80-54-6	48.9	70	1-5455	51	1-3732
Cinnamal <sup>■</sup>	104-55-2	1.1	4	5	17	1-424
Cinnamyl alcohol <sup>■</sup>	104-54-1	12.5	48	2-503	39	6-1169
Citral <sup>□</sup>	5392-40-5	26.1	44	39-554	n.a.	n.a.
Citronellol <sup>□</sup>	106-22-9	65.9	91	1-5848	81	1-5585
Coumarin <sup>□</sup>	91-64-5	33.0	52	3.8-1255	57	1-1411
Eugenol <sup>■</sup>	97-53-0	27.3	30	1-514	57	1-2355
Farnesol <sup>□</sup>	4602-84-0	14.8	39	9-1791	n.a.	n.a.
Geraniol <sup>■</sup>	106-24-1	48.9	87	1-399	76	1-1178

## Opinion on fragrance allergens in cosmetic products

Fragrance substance		88 products investigated in 2007 (213)			70 products investigated in 1998 (216)	
INCI name	CAS number	% Products labelled to contain the fragrance	Content in 23 selected products		Content in all 70 products	
			% Products found to contain the fragrance	Range(ppm)	% Products found to contain the fragrance	Range (ppm)
Hexyl cinnamal <sup>□</sup>	101-86-0	33.0	48	1-4434	71	2-1684
Hydroxycitronellal <sup>▪</sup>	107-75-5	27.3	70	1-1746	50	1-1023
HICC <sup>□</sup>	31906-04-4	33.0	74	1-4431	53	1-1874
Isoeugenol <sup>▪</sup>	97-54-1	9.1	35	1-138	29	1-458
Alpha-isomethyl ionone	127-51-5	46.6	65	6-2588	61	1-2765
D-Limonene <sup>°</sup>	5989-27-5	53.4	70	1022-11386	n.a.	n.a.
Linalool <sup>°</sup>	78-70-6	53.4	96	8-3447	97	9-1927
Methyl-2-octynoat <sup>°</sup>	111-12-6	1.1	-	-	n.a.	n.a.
<i>Evernia prunastri</i> <sup>▪</sup> /oak moss	90028-68-5	4.6	n.a.	n.a.	n.a.	n.a.
<i>Evernia furfuracea</i> <sup>▪</sup> /tree moss	90028-67-4	2.3	n.a.	n.a.	n.a.	n.a.

Notes: HICC Hydroxyisohexyl-3-cyclohexene carboxyaldehyde.

- Fragrance not detected in any product.

n.a. Not analysed.

\* Benzyl alcohol could not be determined in 49% of the products due to interference.

The most common fragrance allergens are contained in the two mixtures, which are used for diagnosing fragrance allergy, called Fragrance Mix I (▪) and Fragrance Mix II (°), besides the oxidation product of terpens (°), and tree moss extract are common allergens. Methyl-2-octynoate is an extreme, but rare allergen.

## Opinion on fragrance allergens in cosmetic products

**Table 10-3:** Frequency of occurrence in consumer products of the 26 fragrance allergens that are required to be labelled in cosmetics and detergents (214).

INCI name of fragrance	PCP (n = 70)	MP (n = 59)	HP (n = 57)	WP (n = 44)	Cos (n = 39)	Deo (n = 17)	Dent (n = 14)	Total (n = 300)
Linalool	46	47	17	42	26	12	0	190 (63%)
Limonene	34	45	29	43	18	11	9	189 (63%)
Citronellol	23	24	21	37	25	15	0	145 (48%)
Geraniol	19	26	15	36	18	12	0	126 (42%)
BPMP	30	27	21	27	13	8	0	126 (42%)
Hexyl cinnamal	37	20	22	22	14	10	0	125 (42%)
Benzyl salicylate	23	23	10	31	15	12	0	114 (38%)
Alpha-isomethyl ionone	15	20	7	24	28	10	0	104 (35%)
Coumarin	12	27	8	23	12	8	0	90 (30%)
Lyr <sup>TM</sup>	17	24	3	24	15	5	0	88 (29%)
Eugenol	13	26	4	22	6	6	3	80 (27%)
Citral	2	28	6	29	7	2	0	74 (25%)
Benzyl benzoate	8	9	3	31	11	8	0	70 (23%)
Benzyl alcohol	9	8	1	30	9	3	1	61 (20%)
Hydroxycitronellal	5	6	1	30	6	4	0	52 (17%)
Isoeugenol	2	5	0	17	0	3	0	27 (9%)
Cinnamic alcohol	4	2	0	13	4	2	0	25 (8%)
Farnesol	1	3	0	17	2	0	0	23 (8%)
Amyl cinnamal	5	0	3	7	5	2	0	22 (7%)
Cinnamal	3	4	0	7	0	0	3	17 (6%)
Evermia prunastri/oak moss	0	3	0	5	5	0	0	13 (4%)
Benzyl cinnamate	2	0	0	8	0	0	0	10 (3%)
Evermia furfuracea/tree moss	1	5	0	3	0	0	0	9 (3%)
Anisyl alcohol	0	0	0	1	0	0	0	1 (0.3%)
Amyl cinnamic alcohol	0	0	0	0	0	0	0	0
Methyl heptine carbonate	0	0	0	0	0	0	0	0

INCI, International Nomenclature of Cosmetic Ingredients; PCP, personal care products; MP, men's products; HP, household products; WP, women's perfumes; Cos, cosmetics; Deo, deodorants; Dent, dental products; BPMP, butyl phenyl methyl propional; Lyr<sup>TM</sup>, hydroxyisohexyl-3-cyclohexene carboxaldehyde.

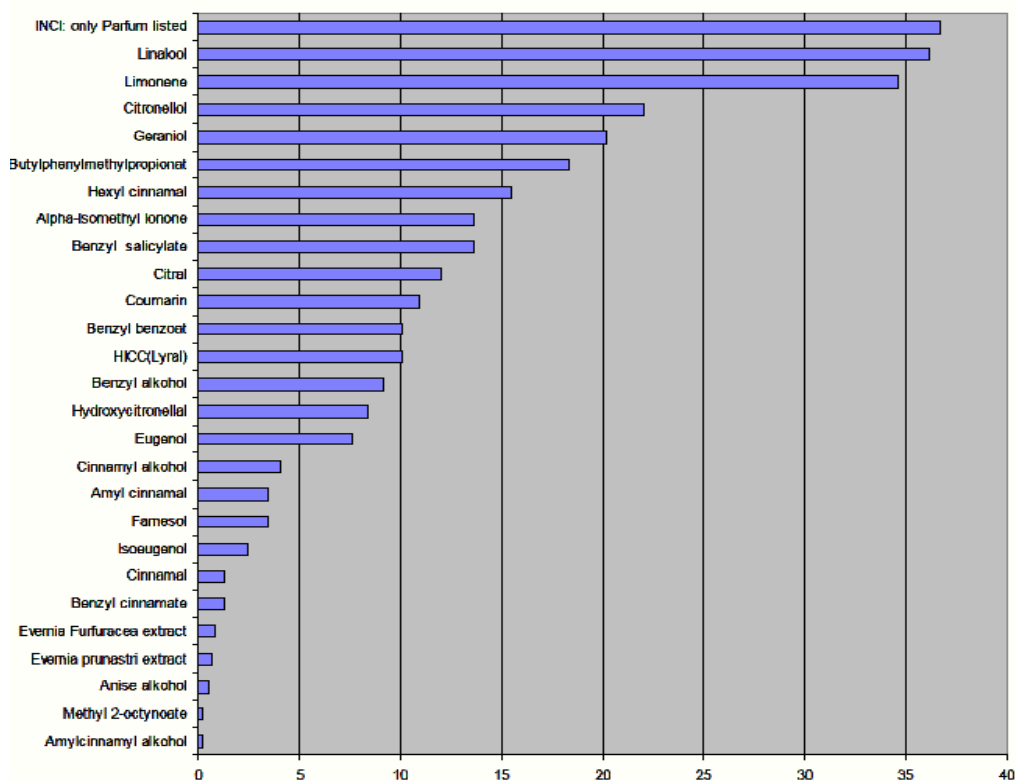
**Table 10-4:** Frequency in 516 consumer products of the 26 fragrance substances that are required to be labelled in cosmetics\* (114).

Fragrance substance INCI name	% Product containing the chemical
D-Limonene	48.3
Linalool	35.8
Butyl phenyl methyl propional	24.8
Geraniol	22.1
Alpha-isomethyl ionone	21.7
Hexyl cinnamal	21.3
Citronellol	21.1
Benzyl salicylate	18.6
Coumarin	17.0
Eugenol	15.7
Benzyl alcohol	15.3
Benzyl benzoate	14.7
Hydroxyisohexyl-3-cyclohexene carboxyaldehyde	12.8

## Opinion on fragrance allergens in cosmetic products

Fragrance substance INCI name	% Product containing the chemical
Citral	11.6
Hydroxycitronellal	10.8
Amyl Cinnamal	7.9
Anise alcohol	7.0
Cinnamyl alcohol	6.4
Farnesol	3.9
Isoeugenol	3.1
Cinnamal	2.5
Benzyl cinnamate	2.3
Amylcinnamyl alcohol	1.9
Methyl-2-octynoate	1.0
<i>Evernia prunastri</i> /oak moss	0.8
<i>Evernia furfuracea</i> /tree moss	0.4

Note: \* Consumer Products: Cosmetics and household products with labelling of the 26 fragrance allergens. The content of these fragrances was confirmed by chemical analysis.



**Figure 10-1:** Frequency of occurrence in 3,000 consumer products of the 26 fragrance allergens that are required to be labelled in cosmetics and detergents (CVUA Karlsruhe, Germany, 2006/2007), according to (104).

Contents of fragrance substances determined in cosmetic products have been described in several studies, both before and after the regulation of the 26 fragrance allergens. The studies prior to the regulation of the 26 fragrance allergens included many, but not all of these 26 allergens. On the other hand, these studies included some other possible fragrance allergens. The quantitative analysis of fragrance substances has been performed in prestige perfumes (5, 153, 217-219), deodorants (213, 216), children's cosmetics and cosmetic toys (114, 212, 220), products marketed as natural cosmetics (210) and in cosmetics used by patients with contact allergy to fragranced products (33, 68). Quantitative analyses have revealed that the consumer is exposed to most, but not all of the 26 fragrance allergens from the use of cosmetics. However, when fragrance exposure from other consumer products, for example detergents and other household products is also taken into consideration (Table 10-3, Table 10-4, Figure 10-1), (104, 114, 214, 221), exposure to all of the 26 allergens is foreseeable in daily life. Although from the data available, the exposure to  $\alpha$ -amylcinnamyl alcohol, cinnamal, methyl-2-octynoate, *Evernia prunastri* (oak moss) and tree moss may appear to be low, these are very strong allergens.

The changes in the use of fragrance chemicals in cosmetic formulations, during last 12 years, i.e. before and after the regulation of the 26 fragrance allergens, is reflected in the studies concerning contents of fragrances substances in popular perfumes (5, 217). As described in Table 10-5, the content of FM I allergens in prestige perfumes was significantly reduced from 1996 to 2003. Whether this is also the case for the perfumes sold as natural cosmetics (Table 10-6) has not yet been investigated.

**Table 10-5:** Concentration of Fragrance Mix I ingredients in five prestige perfumes before and after the regulation of the 26 fragrance allergens.

Fragrance INCI name	Concentration in the perfumes before regulation (5)			Concentration in the perfumes after regulation (217)		
	In no. of perfumes	Range % (w/w)	Mean % (w/w)	In no. of perfumes	Range % (w/w)	Mean % (w/w)
Geraniol*	5	0.072-0.432	0.340	5	0.090-0.236	0.156
Cinnamal	2	0.002-0.002	0.002	0	-	-
Hydroxy-citronellal	5	0.222-0.979	0.615	5	0.015-0.478	0.169
Cinnamyl alcohol	4	0.068-0.232	0.147	0	-	-
Eugenol	5	0.032-0.738	0.337	2	0.001, 0.001	0.001
Isoeugenol	3	0.026-0.249	0.119	2	0.001, 0.004	0.003
Amyl cinnamal	1	0.019	0.019	0	-	-

Note: \* Due to interference by linalyl acetate, concentration of geraniol+linalyl acetate is reported.



**Table 10-6:** Concentrations of Fragrance Mix I ingredients, hexyl cinnamal and coumarin in 22 perfumes marketed as natural cosmetics investigated in 1996.

<b>Fragrance</b>	<b>In no. of perfumes</b>	<b>Concentration % (w/w)</b>
Geraniol	14	1.191*
Cinnamal	3	0.089, 0.109, 2.101
Hydroxycitronellal	5	0.135-6.044
Cinnamyl alcohol	8	0.035-2.289
Eugenol	2	0.027, 0.139
Isoeugenol	8	0.194-3.039
Amyl cinnamal	9	0.105-7.706
Coumarin	11	0.046-6.043

Note: \* Quantification was performed in one sample only, due to interference by a very large amount of linalyl acetate in other samples.

The trend in the use of most of the fragrance allergens in deodorants before and after their regulation is reflected by the two studies performed by Rastogi et al. (213, 216). The results of these studies cannot be directly compared, because the study from 1998 included randomly selected deodorants, while selection of the deodorants for the 2007 study was based on the labelling of the presence of known strong fragrance allergens in these products. The number of products analysed in the 1998 study were three times more than those analysed in 2007, but not all of the 26 fragrance allergens were analysed in the 1997 study. However, an indication of the change in the use of the fragrance allergens during 1998-2007 may be obtained by reviewing the results of these two studies. Among the 17 common fragrance substances studied in the two studies, the frequency of use of 16 of these substances in deodorants was reduced in 2007 compared to that in 1998 (Table 10-2). The frequency of use of butyl phenyl methyl propional in deodorants appeared to be unchanged. The contents of benzyl alcohol, benzyl salicylate, cinnamal, cinnamyl alcohol, eugenol, geraniol, isoeugenol and linalool were found to be lower in the deodorants analysed in 2007 compared to those in 1998. Citronellol, coumarin and alpha-isomethylionone contents in the deodorants were similar in both studies, but concentrations of benzyl benzoate, butyl phenyl methyl propional, hexyl cinnamal, hydroxyisohexyl-3-cyclohexene carboxyaldehyde and linalool were much higher in deodorants in 2007 compared to those in 1998. This analysis of trend of use of fragrance allergens in cosmetic products indicates that the regulated fragrance allergens are used less frequently, but exposures from some of the regulated fragrance allergens may be much higher compared to those before regulation.

**Table 10-7:** Atranol and chloroatranol content in eau de toilette/eau de perfume, investigated in 2004 and in 2007.

	<b>2007 Study</b>	<b>2004 Study</b>
No. of samples	22	17
Atranol present in no. of samples	15 (68%)	12 (70%)
Atranol content	ppb (ng/ml)	ppb (ng/ml)
Range	n.d.-880	n.d.-791
Mean±SD	157±249	97±224
Median	47	20
Chloroatranol present in no. of samples	9 (41%)*	14 (82%)
Atranol content	ppb (ng/ml)	Ppb (ng/ml)
Range	0.9-208	1-175
Mean±SD	63±73	36±51
Median	22	10

Notes: n.d. Not detected.

\* $P < 0.05$  (chi-square test).

SD: Standard deviation.

Atranol (CAS no. 526-37-4) and chloroatranol (CAS no. 57074-21-2), constituents of oak moss and tree moss have been shown to be very potent fragrance allergens (222, 223). The EC Scientific Committee on Consumer Products (SCCP) recommended that atranol and chloroatranol should not be present in cosmetic products (224). Two other commonly used fragrance chemicals, isoeugenol (225) and hydroxyisohexyl-3-cyclohexene carboxyaldehyde (HICC) (68), have also been shown to be important contact allergens. The contents of atranol, chloroatranol, isoeugenol and hydroxyisohexyl-3-cyclohexene carboxyaldehyde in fine fragrances was determined for the exposure assessment of these fragrances (218). The results revealed that isoeugenol was present in 56%, HICC in 72%, atranol in 59%, and chloroatranol in 36% of the 22 eau de toilette/eau de parfum products. The concentrations of isoeugenol were, in all products, below 0.02% which is the maximum concentration recommended by the fragrance industry. HICC reached a maximum concentration of 0.2%, which is 10-fold higher than the maximum tolerable concentration considered safe by the EC Scientific Committee (226). The concentrations of atranol and chloroatranol in the products investigated in 2007 were comparable to those found in similar products in 2004 (Table 10-7, (218, 219). A significant decrease in the frequency of the presence of chloroatranol in the products was found in 2007 (Table 10-7).

## 10.2. Global exposure (household and occupational exposures)

Fragrances are used in cosmetics that the consumer applies to themselves, as described in the previous section. In addition, exposure to fragrance substances is possible by a number of other exposure routes briefly outlined in this section.

### *Topical pharmaceutical products*

In a study from Belgium, 370 of the 3,280 topical products marketed in Belgium have been found to contain one or more of 66 fragrance substances (227). This publication also contains a description of causative fragrance allergens in 127 patients reacting to 48 specific topical products. In a broader sense, exposure of the patient by extracts used in aromatherapy falls in this category as well.

### *Childrens products and toys*

Children's products may contain fragrance allergens and high levels may be present (220). It has been stated that children may become sensitised to fragrance chemicals used by their mothers (228).

### *Clothing*

Washed fabrics have been reported to contain fragrances (229). Odour-neutralising agents are sometimes used for shoe insoles. In one case, an insole containing cinnamon, has been reported to lead to plantar vesicular contact dermatitis due to contact sensitisation to FM I and, in the breakdown, to cinnamal and cinnamyl alcohol (230).

### *Cleaning agents and other household products*

Contact dermatitis from geraniol in washing-up liquid has been reported (231). Terpenes are used as solvents and cleansing agents (e.g. limonene) (232) and have been reported as cause of hand dermatitis (233, 234). In an analysis of 59 household products the most common fragrance allergens were limonene (78%), linalool (61%) and citronellol (47%) (235). In a review of 301 cosmetic and detergent consumer products in Sweden, in half of the cosmetics and one-third of the detergents, one or more of the 26 fragrances requiring labelling were identified (236). In the UK, a review of 300 consumer products showed that linalool and limonene were present in 63% of products. Dental products contained on average 1.1 fragrance substances that are presently required to be labelled and women's perfumes contained 12 of these fragrance substances (Table 4-1 and Table 4-3) (214).

### *Candles*

The dermal hand transfer of three fragrance materials (cinnamic aldehyde, d-limonene and eugenol) from scented candles was determined in ten subjects (i.e. 20 hands) after grasping scented candles for five consecutive 20 second exposures/grasps. The total mean residues of cinnamal and eugenol transferred per grasp from the candles to the hands were 0.255 µg/cm(2) and 0.279 µg/cm(2), respectively (237).

### *Food*

Food causing cheilitis or bullous stomatitis (e.g. due to cinnamal (238)) or lichen planus-like lesions (e.g. due to cinnamal (239)) or contact gingivitis (e.g. due to eugenol (240)) has been reported. Moreover, food containing fragrance allergens, e.g. citrus oil terpenes (241) may cause allergic contact dermatitis by handling this food.

### *Occupational exposure*

In a number of occupations, contact allergy to fragrances is more common than in others, including geriatric nurses, masseurs and physiotherapists, metal furnace operators and potters/glass makers, according to a multifactorial analysis (88). Moreover, hairdressers, beauty therapists and aroma therapists are examples of occupations where there is occupational exposure to fragrance-containing cosmetic and other products. Cleaners are exposed to fragrance-containing household products (e.g. detergents). Cooks and bakers are exposed to flavour chemicals and spices. Healthcare workers are also at risk of acquiring fragrance contact allergy. "Odour maskers" may contain important fragrance allergens (87, 88, 242-244). Occupational exposure and occupational ACD to fragrances have been described in perfume bottlers (245). Industrial

use of a powder masking the vinyl smell of car seats, containing cinnamal, causing occupational ACD has been reported (244).

A number of fragrance chemicals are also used as biocides (see Commission Regulation (EC) No 1451/2007 of 4 December 2007 on the second phase of the 10-year work programme referred to in Article 16(2) of Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market, published 11.12.2007 EN Official Journal of the European Union L 325/3 –L325/65), see Table 10-8 below.

**Table 10-8:** Parts of Annex I to (EC) No 1451/2007 (see above): "Active substances identified as existing".

<b>Biocide</b>	<b>EINECS</b>	<b>CAS number</b>	<b>Biocidal product group</b>
Linalool	201-134-4	78-70-6	19
Geraniol	203-377-1	106-24-1	18, 19
Benzyl benzoate	204-402-9	120-51-4	2, 18
Eugenol	202-589-1	97-53-0	Not given
Farnesol	225-004-1	4602-84-0	Not given
(R)-p-mentha-1,8-diene	227-813-5	5989-27-5	12
Citriodiol/mixture of cis- and trans-p-menthane-3,8 diol	255-953-7	42822-86-6	1, 2, 19
Citral	226-394-6	5392-40-5	Not given
Margosa ext.	283-644-7	84696-25-3	18, 19
Pine ext.	304-455-9	94266-48-5	10
Chrysanthemum vulgare	310-127-6	natural oil	Not given
Chrysanthemum cinerariaefolium, ext.	289-699-3	89997-63-7	18
Citrus oils (main component: limonene)	several	various	
Clove oil (main component: eugenol (83.8 %), caryophyllene (12.4 %))	/	8000-34-8	

**Product groups(According to Biocide Directive 98/8/EC)**

- 1 Human hygiene biocidal products
- 2 Private area and public health area disinfectants and other biocidal products
- 3 Veterinary hygiene biocidal products
- 10 Masonry preservatives
- 12 Slimicides
- 18 Insecticides, acaricides and products to control other arthropods
- 19 Repellents and attractants

The above illustrates that the consumer is exposed to fragrance substances from a wide variety of cosmetic products, other consumer products, pharmaceuticals and occupational exposures.

All these exposures are of importance in the context of contact allergy as it is not the source of exposure that is critical for both induction and elicitation, but the cumulative dose per unit area.

### **10.3. Exposures related to particular anatomical sites**

Contact allergy to fragrances most often causes dermatitis of the hands, face and axillae. Axillary involvement has been shown to be statistically related to fragrance allergy (9). It is recognised that the axillary skin is a problematic area as it is moist, occluded and is easily irritated. Moreover, facial eczema is a common manifestation of fragrance allergy (3, 45). There is an association between fragrance allergy and hand eczema or aggravation of hand eczema (13-15). Vehicles may influence elicitation capacity of an allergen and the presence of detergents (surfactants) as in hand cleaning products may increase the clinical response by a factor of 4-6 (246). Men using wet shaving as opposed to electric razors have an increased risk of being fragrance allergic (17), most likely due to microtraumata and to the presence of surface active substances in shaving foam.

In use tests, the upper arm has been shown to be more sensitive than the forehead and lower arm (247). The axillae, neck and face are more sensitive than the upper arms (10). The threshold of elicitation may vary depending on the volatility of the substance (248). A cumulative effect of exposures occurs so that repeating exposures cause elicitation in more individuals (249).

Patients appear to become sensitised to fragrances primarily from deodorants and perfumes and to a lesser extent from other cosmetic types (72). Allergic contact dermatitis may develop where a perfume has been applied (behind ears, neck, upper chest, antecubital fossae, wrists and the axillae bilaterally (250). Following this, eczema may appear, or be worsened by, the use of a variety of product types including other cosmetics, household products, industrial products and flavours.

The association between contact allergy to fragrance ingredients and certain anatomical sites, which mirrors exposure to fragrance-containing products on these anatomical sites, has been described in several publications (251, 252), see above. However, due to the potential confounding effect of other factors, at least on some anatomical sites, an adjusted analysis will provide a more valid impression of the association between certain anatomical sites and contact allergy to fragrance ingredients. As an adjusted, multifactorial analysis relies on: (i) a substantial number of observations (patients tested); and (ii) an outcome prevalence not too close to 0%, such an approach has, hitherto, been limited to FM I.

In a paper published 2001, data from the IVDK in terms of patch test reactions to FM I and relevant clinical and demographic information of the patients tested (n=57,779) was studied by Poisson regression analysis (88). Risk was quantified by the prevalence ratio, which can be interpreted as an estimate of relative risk, i.e. the factor by which the risk of being sensitised to FM I (in this example) is to be multiplied (RR > 1: elevated risk; or RR < 1: reduced risk) if a certain "risk factor" is present, compared to those patients in whom this risk factor is not present (the reference category) (general aspects of such analyses are discussed in (253)). In the analysis, potential risk factors and confounders, respectively, including occupation, year of patch testing (to address a possible time trend), sex, age, past or current atopic dermatitis, in addition to anatomical site. The relevant part of Table 3 of (88) is reproduced below.

**Table 10-9:** Result of a Poisson regression analysis of patients tested with the Fragrance Mix between January 1992 and December 1998, considering two alternative outcomes – part I: non-occupational factors

Attribute	Prevalence (%)	At least + (11.5%)		At least ++ (4.0%)	
		PR	95% CI	PR	95% CI
Age:					
≤30	26.7	1.00	Reference	1.00	Reference
>30–44	23.8	1.42	1.31 to 1.53	1.61	1.40 to 1.84
>44–58	25.6	1.67	1.55 to 1.80	1.90	1.66 to 2.16
>58	23.9	1.93	1.77 to 2.10	2.07	1.79 to 2.39
Sex (female)	64.5	1.29	1.21 to 1.37	1.18	1.07 to 1.31
Main site:*					
Trunk	2.9	1.00	Reference	1.00	Reference
Hands	29.9	1.24	1.07 to 1.46	1.28	0.98 to 1.67
Arm	3.8	1.23	1.01 to 1.49	1.19	0.86 to 1.65
Face	15.2	1.20	1.03 to 1.42	1.13	0.86 to 1.48
Neck	1.4	1.39	1.10 to 1.75	1.31	0.88 to 1.94
Feet	2.8	1.26	1.02 to 1.55	1.19	0.84 to 1.68
Leg	8.7	1.59	1.36 to 1.89	1.50	1.14 to 1.99
Axilla	0.9	2.77	2.20 to 3.46	2.73	1.87 to 4.00
Other site	8.9	0.66	0.55 to 0.80	0.48	0.35 to 0.67

\*Additionally controlled for several more sites—none of these associated with a significantly increased or decreased risk.

Compared to the trunk, which was arbitrarily chosen as the reference category, all other anatomical sites are associated with an increased risk of being sensitised to FM I (significantly if the lower limit of 95% CI is > 1). Most evidently, dermatitis of the axilla(e) is strongly associated with contact allergy to FM I, presumably due to the application of deodorants. Furthermore, the part of the table shown above illustrates a strong, positive age gradient, i.e. the older patients are, the more likely they are to be sensitised to FM I, the risk being almost double when comparing the oldest with the youngest age group. This observation is in concordance with a bivariate (unadjusted) association between age and contact allergy to FM I found in another study (87). This association is presumably the result of life long exposures and cumulative risk.

In a similar analysis of *Myroxylon pereirae* resin, published in 2002 (254): (i) an even stronger age gradient; and (ii) no particular association to axillary dermatitis (included in the “other” category) was found (Table 10-10).

Table 10-10: Association between selected risk factors and positive patch test to *Myroxylon pereirae* resin. For full model see (254). Risk quantified with the prevalence ratio (PR) with accompanying 95% confidence interval (CI).

<b>Factor</b>	<b>PR</b>	<b>95% CI</b>
Atopic dermatitis, past or present	1.02	(0.95-1.10)
Female sex	1.13	(1.06-1.20)
<i>Site</i>		
Trunk	1.00	(reference)
Hand or Arm	1.03	(0.94-1.12)
Foot or Leg	1.76	(1.61-1.92)
Head or Neck	0.94	(0.86-1.03)
"Other" site	0.72	(0.64-0.81)
Missing site	1.07	(0.97-1.19)
<i>Age</i>		
30 years and younger	1.00	(reference)
31 to 44	1.92	(1.73-2.12)
45 to 58	2.87	(2.61-3.16)
58 or older	3.85	(3.49-4.25)

#### 10.4. Conclusion

There are various modes of exposure to fragrances, including not only products used for their scent, such as perfumes and eau de toilette, after shaves, and deodorants, but also types of products where scent is an added feature, such as other cosmetic categories (including wipes), topical pharmaceuticals, household products, and products encountered in the occupational setting.

Consumer exposure can change over time, both qualitatively and quantitatively.

Different routes of exposure are reflected by certain anatomical sites affected: deodorants are associated with axillary dermatitis, the axillary skin being particularly vulnerable to sensitisation due to occlusion, maceration and irritation. However, while sensitisation and initial disease may follow a distinct pattern, later less specific exposures, e.g. via hand creams, cleaning lotions etc. may be sufficient to cause allergic contact dermatitis.



## 11. Dose-response relationships and thresholds

The dose-response relationship between exposure to contact allergens and induction of allergy, i.e. sensitisation, is well established in animal models and by experiments in healthy volunteers (255). It seems that not only the dose per unit area of allergen, but also the number of exposures, i.e. the accumulated dose, is of importance for the risk of induction of contact allergy (256). The induction of contact allergy is an immunological process (type IV-allergy), which is without any clinical symptoms. In the case of continued exposure or re-exposure with a sufficient dose of allergen, elicitation will occur. Elicitation is an inflammatory response (eczema) with clinical symptoms of erythema, induration and in some cases vesicles. Studies of the elicitation response are normally done in patients with an allergy to the substance in question. Different provocation models exist (see chapter 11.2.1). Elicitation experiments in healthy human volunteers following the induction have only rarely been performed (257, 258) and may be considered a less valid model than patient studies. The reason is that following experimental induction, the level of sensitivity may not be at the same level as in a real life situation and that individuals who have actually acquired the disease are a more relevant endpoint to study.

Knowledge of the dose-response relationship provides an opportunity to establish levels of exposure which are safe for the majority of individuals. In the following chapter, the use of different data and models for the establishment of such safe levels in relation to fragrance ingredients are explored. The focus will be on those chemicals, which have been identified in chapter 7.1 as established contact allergens in humans and which have already given rise to a significant number of published cases (category 3 or more): cinnamal, cinnamyl alcohol, citral, coumarin, eugenol, farnesol, geraniol, hydroxycitronellal, isoeugenol. Limonene and linalool are considered in chapter 5 as their ability to cause sensitisation depends on air oxidation, and hydroxyisohexyl 3-cyclohexene carboxaldehyde is considered in chapter 4.2.2 and 11.4.

### 11.1. Induction

A model for dermal sensitisation quantitative risk assessment (QRA) has been developed and implemented by the fragrance industry. This model relies on thresholds, no effect or low-effect levels, established in healthy human volunteers and/or in animal experiments, mainly the local lymph node assay (LLNA) (see chapter 8.1). A set of safety factors are applied for inter-individual differences, for vehicle effects and for use considerations, stated to give rise to a safety margin from 10 to 1000 (259). In this way, a so-called "acceptable exposure level" is derived. The exposure to an allergen in different types of products should be below this level. The restrictions, which have been introduced by the fragrance industry based on the QRA model, are given in



Table 11-1 for some important product categories.

The IFRA guidelines give concentration limits for 11 product categories ([http://www.ifraorg.org/en-us/standards\\_1](http://www.ifraorg.org/en-us/standards_1), last accessed 2011-11-02), three of which are mentioned in

Table 11-1. These three products have the lowest concentrations except for lip products, which give a slightly lower concentration limit.

**Table 11-1:** Current IFRA restrictions based on induction experiments.

Fragrance chemicals	IFRA guideline <sup>1</sup>		
	Deodorant (%)	Hand cream (%)	Perfume (%)
Cinnamal	0.02	0.05	0.05
Cinnamyl alcohol	0.1	0.4	0.4
Citral	0.05	0.3	0.6
Coumarin	0.13	0.8	1.6
Eugenol	0.2	0.5	0.5
Farnesol	0.11	0.6	1.2
Geraniol	0.4	2.8	5.3
Hydroxycitronellal <sup>2</sup>	0.2	1.0	1.0
Isoeugenol <sup>2</sup>	0.01	0.02	0.02

Notes: 1) Exposure per mg/cm<sup>2</sup>/day is based on 8.5 mg/cm<sup>2</sup>/day for deodorants, 2.2 for perfumes and 4.2 for hand creams as it is these exposure levels that are used by the IFRA.

2) Cosmetic Directive Annex III: Hydroxycitronellal restricted to 1% in all products and isoeugenol to 0.02% in all products.

The SCCP evaluated this methodology (260) as well as its application to three model fragrance substances.

It was, among other things, concluded that:

“The data provided show that the application of the dermal sensitisation QRA approach would allow increased exposures to allergens already known to cause allergic contact dermatitis in consumers. The model has not been validated and no strategy of validation has been suggested. There is no confidence that the levels of skin sensitisers identified by the dermal sensitisation QRA are safe for the consumer.”

and that:

“Identification of safe levels of exposure to existing substances known to cause allergic contact dermatitis in the consumer should be based on clinical data and/or elicitation low-effect levels. Currently, these are the only methods which have proven efficient in reducing/preventing existing problems of sensitisation/allergic contact dermatitis in the consumer.”

## 11.2. Elicitation

### 11.2.1. General considerations

A response in terms of elicitation of allergic contact dermatitis by application of the (suspected) allergen under standardised conditions is the outcome of interest of the routine diagnostic procedure for suspected contact allergy, the patch test. While the patch test procedure is largely standardised, exposure conditions are not comparable to actual exposures occurring in the daily life or working environment of the patient, which often involve long-term, repeated and low-dose contact with the allergen. Here, procedures such as the repeated open application test (ROAT) or provocative use test are often used, because they much better reflect actual exposure and can be used, for instance, to validate the current clinical relevance of a positive PT reaction.

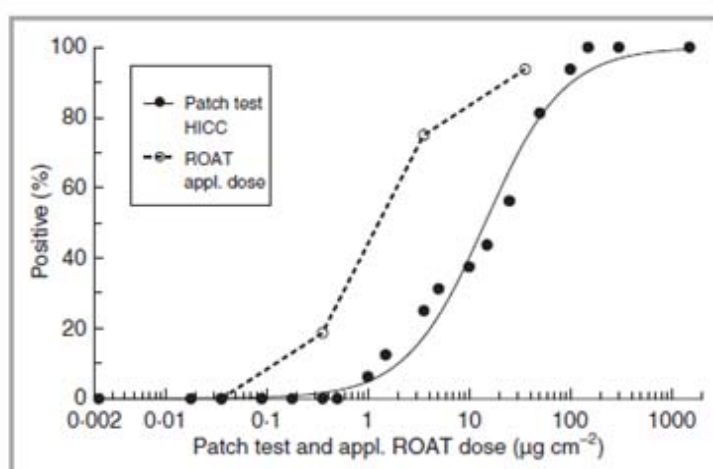
Generally, exposure of a sensitised patient to a set of graded doses (quantity/area) of the suspected allergen, i.e. threshold testing, will allow not only quantitative diagnosis of the presence or absence of specific contact sensitisation but will additionally provide evidence on the intensity (degree) of sensitisation. This may have important individual

consequences in terms of everyday or occupational exposures being capable (or not) of eliciting allergic contact dermatitis. However, beyond the individual perspective, clinical dose-response data collected from sensitised individuals provide a valuable estimate of the usual doses/unit area resulting in a positive, allergic response in a certain proportion of sensitised persons, e.g. 10, 50 or 90%. Maximum concentration levels can be derived, which are safe in terms of eliciting allergic reactions in only a defined low percentage of sensitised persons. As such data will always be based on small samples, the precision of the estimate should be considered, and therefore results are preferably given with confidence intervals.

A statistically significant relationship between threshold concentrations in the ROAT and patch test has been found, on analysing results from different allergens (see Table 11-2) (261), but the dose of allergen per unit area per application needed to elicit a reaction in the two study methods is not the same. A translation factor between the two methods has been suggested for non-volatile substances:  $ED_{xx}(ROAT) = 0.0296 * ED_{xx}(\text{patch test})$  based on testing nickel and methyl dibromo glutaronitrile (261). Based on this the eliciting dose per application in an open test is 33 times lower than in the patch test. In practice it means that the cumulative dose in a ROAT (in  $\mu\text{g}/\text{cm}^2$ ) in two weeks with two applications per day (total 28 applications) will be almost identical to the eliciting patch test dose (in  $\mu\text{g}/\text{cm}^2$ ) for a given number of responders (see Figure 11-1). For a given cut-off point the elicitation dose determined by patch testing will be higher than determined by ROATs.

**Table 11-2:** Spearman's rank correlation between the threshold concentration in the patch test and the repeated open application test for three allergens.

Allergen	Number of patients	Correlation coefficient	P-value
Nickel	18	0.45	0.033
MDBGN	15	0.76	0.0021
HICC	16	0.59	0.011



**Figure 11-1:** The fitted dose-response curve for patch test (solid line) is seen to be displaced to the right compared to the observed response from repeated open applications of the same allergen (HICC). It means that a smaller dose per application is needed to elicit a response than by one single occluded application as in the patch test.

In the translation between methods, evaporation needs to be taken into consideration for volatile substances. The experience, based on a study of the fragrance ingredient HICC and using the results from the literature on isoeugenol, is that if the same equation is used as for non-volatile substances, the response in the ROAT will be overestimated by a factor 3 to 4. Thus, the translation factor would be 0.1060 instead of 0.0296, but this needs to be confirmed by other fragrance allergens. This implies that for the fragrance ingredients tested, the eliciting dose per application in a ROAT was 9.4 times lower than the patch test compared to a 33 times lower dose for non-volatile substances (261). This needs to be confirmed by studying other fragrance allergens. Thus, according to these experiments, the dose ( $\mu\text{g}/\text{cm}^2$ ) eliciting a response in threshold patch testing will be at most 33 times higher than established in the ROAT if an identical vehicle is used.

### Volatility effects in skin sensitisation

The potency of volatile skin sensitisers can be underestimated, to an extent depending on how rapidly it evaporates, by assays such as the LLNA in which the test substance is applied topically to exposed healthy skin without occlusion. Such sensitisers present a greater sensitisation risk to consumers when the skin is occluded by clothing and/or compromised, than when healthy non-occluded skin is exposed.

Volatility at physiological temperature, say  $40^\circ\text{C}$ , is represented by the vapour pressure  $p_{40}$  at that temperature. This is related to the boiling point  $T_B$  by the Clapeyron-Clausius equation, which can be written (262):

$$\text{Log}(p_{40}) = - (T_B - 40)\text{Tr}/2.303RT$$

Where  $p$  is in atmospheres,  $T_B$  is in  $^\circ\text{C}$ ,  $R$  is the gas constant,  $\text{Tr}$  is the Trouton constant (also defined as the molar entropy of vaporisation, and equal to  $22 \text{ cal}\cdot\text{deg}^{-1}$  for many organic compounds) and  $T$  is physiological temperature in degrees absolute (= 313 for  $40^\circ\text{C}$ ).

It has been shown, in experiments where evaporation from a glass slide is measured under simulated LLNA conditions, that 2-hexenal ( $T_B = 146\text{-}149^\circ\text{C}$ ,  $p_{40} = 17 \text{ mmHg}$ ) evaporates rapidly, less than 20% remaining after 5 minutes, whereas with cinnamal ( $T_B = 248^\circ\text{C}$ ,  $p_{40} = 0.5 \text{ mmHg}$ ), more than 90% remains after 1 hour (263). In agreement with these findings, cinnamal fits a QSAR relating LLNA EC3 to reactivity, whereas the EC3 for 2-hexenal is higher (lower potency) than predicted from its reactivity.

The above is only a partial rationalisation, since different solubilities in different vehicles will influence the tendency to evaporate, according to Henry's law.

#### 11.2.2. Studies on specific fragrance ingredients

Studies concerning chloroatranol/atranol, cinnamal, hydroxycitronellal, hydroxyisohexyl 3-cyclohexenecarboxaldehyde and isoeugenol have been identified. These are summarised in Annex III.

### Overview of results

In four studies dummy deodorants spiked with a single fragrance allergen in realistic use concentrations have been used to study elicitation responses, unscented deodorants were used as control products in paired designs. The deodorants were used by patients sensitised to the fragrance allergen in question as well as a healthy control group (without fragrance allergy) (95-97, 243). Between 76 and 100% of the sensitised individuals reacted to the deodorants spiked with allergen, isoeugenol, cinnamal, hydroxycitronellal and hydroxyisohexyl 3-cyclohexene carboxaldehyde, and none of the controls (Table 11-4).

## Opinion on fragrance allergens in cosmetic products

**Table 11-3:** Overview of results of deodorant provocation investigations with different allergens. Frequency in % of test groups, which reacted at different doses of allergen applied in a roll-on deodorant in the axilla, is given in the table.

Dose in ppm in deodorant	Isoeugenol	Cinnamal (1)	Cinnamal (2)	Hydroxycitronellal	HICC
0	0	0	0	0	0
63	23				
100			11		
200	69				64
320		25	55	57	
600					85
630	76				
1000		75	88	71	
1800					100
3200		100		100	
No. test persons	13	8	9	7	14
No. of control persons	10	20		7	10
% control persons, who reacted	0	0		0	0
<b>Exposure according to study should be:</b>	<b>&lt; 63 ppm</b>	<b>&lt;100 ppm</b>		<b>&lt;320 ppm</b>	<b>&lt; 200 ppm</b>
Reference	(264)	(102)		(103)	(101)

Note: HICC hydroxyisohexyl 3-cyclohexene carboxaldehyde.

Eleven studies concerning dose-response results of the five allergens listed above were identified, including the above mentioned studies of deodorants. An overview of the results of the studies concerning thresholds is given in Table 11-4. In Annex III the details of each study are given.

**Table 11-4:** Overview of threshold results from clinical studies.

“Observed” means that the proportion was actually observed in the study while “estimated” means that the value is derived from a fitted curve, i.e. is interpolated.

<b>Chloroatranol</b>			
<b>ROAT</b>			<b>Ref.</b>
In ethanol 92 % positive	0.025 µg/cm <sup>2</sup>	observed	(223)
In ethanol 100% positive	0.125 µg/cm <sup>2</sup>	observed	(223)
<b>PATCH TEST</b>			
ED10%	0.0004 µg/cm <sup>2</sup>	estimated	(223)
ED50%	0.0045 µg/cm <sup>2</sup>	estimated	(223)
<b>Cinnamal</b>			

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<b>ROAT</b>			
In ethanol no effect	0.02%	observed	(100)
In ethanol 44 % positive	0.1%	observed	(100)
In ethanol 72 % positive	0.8%	observed	(100)
Deodorant matrix 11% positive	0.26 µg/cm <sup>2</sup> (0.01%)	observed	(102)
Deodorant matrix 41% positive	0.84 µg/cm <sup>2</sup> (0.032%)	observed	(102)
Deodorant matrix 82% positive	2.63 µg/cm <sup>2</sup> (0.1%)	observed	(102)
<b>PATCH TEST</b>			
ED50%	96 µg/cm <sup>2</sup>	estimated	(100)
No effect level	0.4 µg/cm <sup>2</sup> (0.01%)	observed	(100)
No effect level	NG (0.002%)	observed	(102)
<b>HICC</b>			
<b>ROAT</b>			
In a cream base ED10%	4.9 µg/cm <sup>2</sup>	interpolated	(104)
In a perfume (ethanol) ED10%	1.2 µg/cm <sup>2</sup>	interpolated	(104)
In ethanol 61% positive	15.3 µg/cm <sup>2</sup> (3.4-22.2)	observed	(209)
In ethanol 89% positive	126.2 µg/cm <sup>2</sup> (40.5-226.2)	observed	(209)
In ethanol/water no response	0.0357 µg/cm <sup>2</sup>	observed	(248)
In ethanol/water ED10%	0.064 µg/cm <sup>2</sup>	estimated	(248)
In deodorant matrix between 64% to 100% positive	0.79 µg/cm <sup>2</sup> (median)	observed	(101)
<b>PATCH TEST</b>			
ED10% (95% CI)	0.662 µg/cm <sup>2</sup> (0.052-2.35)	estimated	(248)
ED10%	0.75 µg/cm <sup>2</sup>	estimated	(101)
ED10%	0.9 µg/cm <sup>2</sup> 29 (7-69) ppm	estimated	(209)
ED50% (95% CI)	11.1 µg/cm <sup>2</sup> (3.41- 33.1)	estimated	(248)
ED50% (95% CI)	18.3 µg/cm <sup>2</sup> (3.41- 33.1)	estimated	(101)
ED50% (95% CI)	20 µg/cm <sup>2</sup> 662 (350-1250) ppm	estimated	(209)
No effect level	<0.0022 µg/cm <sup>2</sup>	observed	(248)
<b>Hydroxycitronellal</b>			
<b>ROAT</b>			
Deodorant matrix 57 % positive	0.94 µg/cm <sup>2</sup> (0.032%)	observed	(103)
Deodorant matrix 71 % positive	2.94 µg/cm <sup>2</sup> (0.1%)	observed	(103)
Deodorant matrix 100 % positive	9.40 µg/cm <sup>2</sup> (0.32%)	observed	(103)
<b>PATCH TEST</b>			
No effect level	<0.00012 % (=0.036 µg/cm <sup>2</sup> )* (*calculated)	observed	(103)
<b>Isoeugenol</b>			

<b>ROAT</b>			
in ethanol 63% positive	5.6 µg/cm <sup>2</sup>	observed	(99)
in ethanol 42% positive	2.2 µg/cm <sup>2</sup>	observed	(249)
in ethanol 67% positive	9.0 µg/cm <sup>2</sup>	observed	(249)
Deodorant matrix 23 % positive	0.167 µg/cm <sup>2</sup>	observed	(264)
Deodorant matrix 69 % positive	0.53 µg/cm <sup>2</sup>	observed	(264)
Deodorant matrix 77 % positive	1.67 µg/cm <sup>2</sup>	observed	(264)
<b>PATCH TEST</b>			
ED50% (in petrolatum)	32 µg/cm <sup>2</sup>	estimated	(99)
No effect (in ethanol)	<0.0005% (0.15 µg/cm <sup>2</sup> )	observed	(249)
No effect (in petrolatum)	<0.4 µg/cm <sup>2</sup>	observed	(99)

### **Summary of results for specific fragrance ingredients**

#### **Chloroatranol (constituent of *Evernia prunastri*)**

In ROAT a dose of 0.025 µg/cm<sup>2</sup> to 0.125 µg/cm<sup>2</sup> in ethanol elicited reactions in 92% to 100% of sensitised subjects.

In patch testing the ED10% was 0.0004 µg/cm<sup>2</sup>.

#### **Cinnamal**

In ROAT a dose of 0.26 µg/cm<sup>2</sup> gave a response in 11% when applied as deodorant in the axilla and 82% responded to 2.63 µg/cm<sup>2</sup>.

The ED50 in patch testing was 96 µg/cm<sup>2</sup>.

#### **HICC**

In ROAT a dose of 0.0357 µg/cm<sup>2</sup> gave no response, while the dose that elicited a reaction in 10% of the sensitised test group (in ethanol) ranged from 0.064 µg/cm<sup>2</sup> to 1.2 µg/cm<sup>2</sup>. The dose in a cream base was 4.9 µg/cm<sup>2</sup>.

In ROAT a dose of 15.3 µg/cm<sup>2</sup> to 126.2 µg/cm<sup>2</sup> in ethanol elicited reactions in 61% to 89% of sensitised subjects.

The ED10 in patch testing ranged from 0.66-0.9 µg/cm<sup>2</sup>.

#### **Hydroxycitronellal**

In ROAT a dose of 0.94 µg/cm<sup>2</sup> gave a response in 57% when applied in a deodorant in the axilla and 100% responded to 9.40 µg/cm<sup>2</sup>.

The no-effect level in patch testing was below 0.036 µg/cm<sup>2</sup>.

#### **Isoeugenol**

In ROAT a dose of 2.2 µg/cm<sup>2</sup> a response in 42% and 9.0 µg/cm<sup>2</sup> in 67%, when applied in ethanol on the arm. With a deodorant applied to the skin of the axillary, a dose of 0.167 µg/cm<sup>2</sup> caused a response in 23% and 77% reacted to 1.67 µg/cm<sup>2</sup>.

The ED50 in patch testing was 32 µg/cm<sup>2</sup>.

The no-effect in patch testing was below 0.15 µg/cm<sup>2</sup>.

Elicitation levels have been studied for cinnamal, isoeugenol and hydroxycitronellal which are established contact allergens in humans and which already have given rise to a significant number of cases (> 100, see chapter 7). Further HICC has been studied extensively, but is considered in a separate section (chapter 11.3) of this opinion. It is



however not possible to derive a safe threshold directly from the data of cinnamal, isoeugenol and hydroxycitronellal. The main reasons are that many of the test subjects reacted to all the tested doses in ROAT, which is a simulation of every day exposures. Thus it was not possible to determine the dose only eliciting responses in a few, e.g. 10% of the subjects and that only a limited number of exposure scenarios were studied.

The studies have covered few product types: hydro-alcoholic products, e.g. perfumes and deodorant roll-on matrix. The vehicle is one of many factors which influence the thresholds of allergic reactions. Also the presence of irritants and other allergens can influence the elicitation level. This means that the currently available studies do not cover all the relevant exposure scenarios. However, taking into account that dose-response investigations in sensitised patients are very complex to perform, it is not likely that much more data will become available in the near future. It is therefore necessary to exploit the full pool of elicitation data, also covering chemicals other than fragrance ingredients, to derive a more general threshold which could be used when no or insufficient data exist to set a specific threshold for a substance of concern.

### General thresholds

The methodology of the different experiments has varied to some extent as different anatomical sites of exposure have been employed, different vehicles, exposure periods and cut-off points. The reason is that the studies have been performed to investigate various clinical and scientific aspects of allergic contact reactions and not for formal regulatory requirements. Some studies are small and for this reason the precision of the estimates of thresholds is limited. In spite of this, the results of the various experiments are reasonably uniform, except for chloroatranol which had very low threshold reactions, and show that low concentrations may elicit allergic reactions.

The reasonably uniform data generated on the above fragrance ingredients are in agreement with a recent "meta-analysis" of dose-response data of different allergens, incorporating some of the same studies as mentioned above, but also other allergens, such as preservatives and metals. The ED10 at patch testing varied by a factor of 7 from the lowest to the highest value and the median was  $0.82 \mu\text{g}/\text{cm}^2$  if the three outliers formaldehyde (1997), nickel (1999) and methylidibromo glutaronitrile (2004) were left out and  $0.84 \mu\text{g}/\text{cm}^2$  if included (see Table 11-6 and Figure 11-2 below: (265)). An explanation of these results could be that thresholds in elicitation is less dependent on the antigenic properties of the individual substance (inherent potency) than thresholds of induction and more on the level of sensitivity of the individual, i.e. the level of T-cell clones able to recognise the antigen, which is not present in naïve not-sensitised, individuals. This seems plausible, based on both the recent clinical evidence (265) and guinea pig QSAR evidence (266). It provides the basis for a general approach in establishing safe thresholds for substances of concern.

The consequences of a limit of  $0.8 \mu\text{g}/\text{cm}^2$  for the product types most important for fragrance allergy are calculated below.

The calculation is based on:

- The generally safe exposure level, which is the median ED10 value (the dose which will elicit allergic contact dermatitis in 10% of sensitised eczema patients) under patch test conditions:  $0.8 \mu\text{g}/\text{cm}^2$  (265).
- Exposure doses and exposure areas from SCCS notes of guidance 7<sup>th</sup> revision (267) [Tables 2 and 3] and Technical dossier Quantitative Risk Assessment from RIFM (259).

## Opinion on fragrance allergens in cosmetic products

Equation:

Safe concentration in product = (Generally safe exposure level ( $0.8 \mu\text{g}/\text{cm}^2$ )/daily exposure to product ( $\mu\text{g}/\text{cm}^2/\text{day}$ )) x 100 (for %).

**Table 11-5:** Concentration limits in different product types based on  $0.8 \mu\text{g}/\text{cm}^2$  allergen as a 'generally safe exposure level', if specific dose-response data are unavailable.

	<b>Estimated daily exposure level (g) (Table 3 SCCS NoG)</b>	<b>Mean exposed skin surface (<math>\text{cm}^2</math>) (Table 2 SCCS NoG)</b>	<b>Exposure /<math>\text{cm}^2/\text{day}</math> in grams</b>	<b>Exposure /<math>\text{cm}^2/\text{day}</math> in <math>\mu\text{g}</math> (<math>1\text{g} = 1 \times 10^6 \mu\text{g}</math>)</b>	<b>Concentration limit in product % in product: (GEL/daily exposure) x 100</b>
Body lotion	7.82 g	15,670 $\text{cm}^2$	0.000499	499	0.16%
Face cream	1.54 g	565 $\text{cm}^2$	0.002725	2725	0.03%
Hand cream	2.16 g	860	0.002511	2511	0.03%
Deodorant aerosol spray ethanol based	1.43 g	200 $\text{cm}^2$	0.007150	7150	0.01%
Perfume spray	not given	?	0.00221 <sup>1)</sup>	2210	0.04%

Note: 1)  $2.21 \text{ mg}/\text{cm}^2/\text{day}$  from Technical dossier Quantitative Risk Assessment.

The estimated daily use of the various product categories in Table 11-5 are based on the SCCS Notes of Guidance (see above), except for perfume, for which no value is given. This value is taken from the Technical Dossier on Quantitative Risk Assessment from RIFM.

Generally the estimated use of different products is higher in the IFRA/RIFM assessments than in SCCS Notes of Guidance.

**Table 11-6:** Overview of dose-response studies and thresholds for eight allergens, after (265).

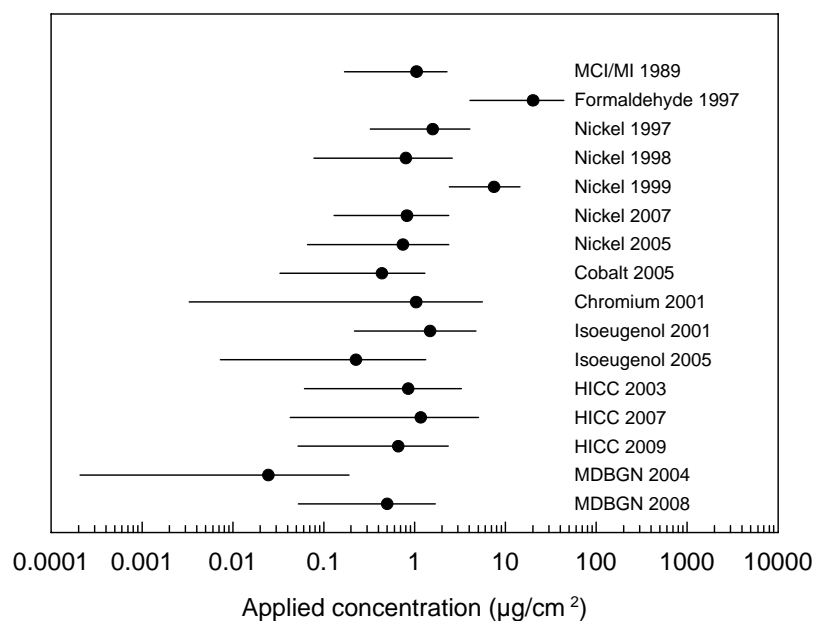
ED<sub>10</sub> patch test values from each of the 16 selected studies with 95 % confidence intervals with the allergens chromium (268), MCI/MI (Kathon™ CG) (269), nickel (270), methylidibromo glutaronitrile (MDBGN) (271), hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) (101, 209, 248), isoeugenol (249, 264) and formaldehyde (272). The shaded values were considered as outliers.

<b>Study</b>	<b>Number of patients</b>	<b>ED<sub>10</sub> (<math>\mu\text{g}/\text{cm}^2</math>)</b>	<b>95 % interval</b>
MCI/MI	12	1.05	0.17–2.27
Formaldehyde	20	20.1	4.09–43.9
Nickel 1997	24	1.58	0.32–4.04
Nickel 1998	19	0.8	0.078–2.59
Nickel 1999	26	7.49	2.42–14.5
Nickel 2005	13	0.74	0.066–2.38
Nickel 2007	20	0.82	0.13–2.37
Cobalt 2005	11	0.44	0.033–1.3

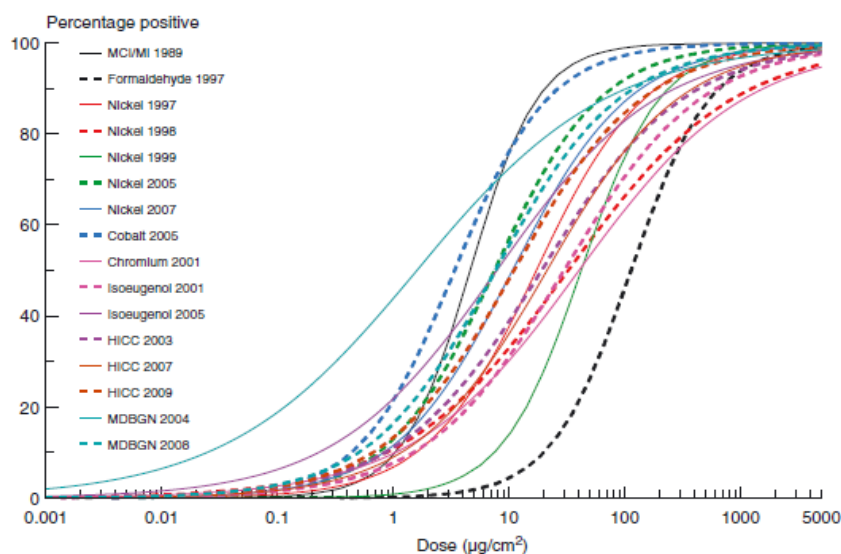
Study	Number of patients	ED <sub>10</sub> (µg/cm <sup>2</sup> )	95 % interval
Chromium	17	1.04	0.0033–5.55
Isoeugenol 2001	24	1.48	0.22–4.74
Isoeugenol 2005	13	0.23	0.0073–1.32
HICC 2003	18	0.85	0.062–3.26
HICC 2007	14	1.17	0.043–5.05
HICC 2009	17	0.66	0.052–2.35
MDBGN 2004	19	0.025	0.00021–0.19
MDBGN 2008	18	0.50	0.052–1.69

Note: The ED<sub>10</sub> value is the concentration which elicits an allergic reaction in 10% of a group of sensitised individuals under patch test conditions.

ED<sub>10</sub> with 95 % confidence limits



**Figure 11-2:** The threshold data with 95% confidence intervals from Table 11-6 presented graphically, after (265).



**Fig. 1.** Logistic dose–response curve for 16 patch test elicitation dose–response studies with methylchloroisothiazolinone/methyl isothiazolinone (MCI/MI) (8), formaldehyde (9), nickel (10–14), cobalt (14), chromium (15), isoeugenol (16, 17), hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) (18–20), and methyldibromo glutaronitrile (MDBGN) (21, 22).

**Figure 11-3:** The fitted dose-response curves from the studies in Table 11-6, which are the basis for estimation of the ED10 value, after (265).

The meta-analysis above has shown that the median elicitation dose by patch testing for 10% of sensitised individuals was  $0.8 \mu\text{g}/\text{cm}^2$ . In the model data for the fragrance substances isoeugenol and HICC was included. The two studies on isoeugenol and the three studies on HICC gave an average ED10 value of  $0.85 \mu\text{g}/\text{cm}^2$  and  $0.89 \mu\text{g}/\text{cm}^2$  with a range 0.23-1.48. This means that even if the model was used for these substances individually the result would be very similar to the general threshold value.

The data from cinnamal and hydroxycitronellal studies was not incorporated in the model because: (i) serial dilution patch testing was done in petrolatum for cinnamal, making the dosing less exact; (ii) and only seven patients participated in the hydroxycitronellal study, while a criteria for inclusion in the model was ten participants (265).

According to the above calculations, a limit of  $0.8 \mu\text{g}/\text{cm}^2$  for the product types of most importance for fragrance allergy corresponds to concentrations of 100 to 400 ppm (0.01-0.04%) for deodorants, perfume spray, hand and face lotions. For body lotion the general threshold was 0.16%. However, it does not seem meaningful in the context of contact allergy to distinguish between different types of creams, as a body cream would be applied with the hands and the relevant parameter in contact allergy is dose per area skin and not total dose.

A general threshold would have to take into consideration the uncertainties in quantification of exposure and safe thresholds as well as the possibilities of aggregate exposures and exposure to chemically similar substances. Therefore in setting one general threshold the product category carrying the highest risk of sensitisation and elicitation, which is deodorants, was chosen to drive the generation of the threshold. This means that a threshold of  $0.8 \mu\text{g}/\text{cm}^2$  is equal to 0.01% or 100 ppm (see Table

Table 11-1 and the related text), the lowest of the threshold values derived.

The general threshold is indicative of a safe level for the majority of sensitised individuals, but does not preclude that the most sensitive subset of the population may react upon exposure to the allergen. These levels are based on patch tests and take no account of anatomical sites of exposure, frequency of exposure or vehicle effects. Therefore, any limitations in exposures are not substitutes for providing information to the consumer about the presence of a substance in a product as a certain fraction of sensitised individuals will still need to avoid specific exposures.

Based on experience, limitations in exposure based on elicitation thresholds will, apart from helping the sensitised consumer, also significantly reduce the risk of induction. This is the case for nickel allergy, where the restrictions in the EU nickel directive are based on elicitation threshold, leading to a significant reduction in new cases of sensitisation in young women (273) and in a reduction in morbidity, i.e. elicitation (274). Another example is restriction of chromium VI in cement (275).

It is not possible to provide a safe threshold for natural extracts of concern, as no specific investigations exist, and the model providing the general use concentration limit (0.01%) has been based on chemicals only.

The SCCP concluded in 2004 that Chloroatranol and atranol, the main allergenic constituents of *Evernia prunastri* and *Evernia furfuracea*, should not be present in consumer products because they are extremely potent allergens (224). The persistently high frequency of contact allergy to *Evernia prunastri* and *Evernia furfuracea* noted in eczema patients does point to a persisting problem with exposure to the allergenic constituents.

### **11.3. Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)**

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) has been the most frequently reported individual fragrance chemical causing allergy since the 1999 opinion on fragrance allergy. In total, reports of about 1500 cases have been published in the scientific literature (see chapter 7.1 and Annex I to this opinion), while the second most frequently reported individual chemical was cinnamal with around 350 published cases. Only a minority of the cases seen by clinicians is published and only a (small) proportion of those with allergic contact dermatitis seeks or has the possibility to seek medical attention.

Natural extracts such as *Myroxylon pereirae* and turpentine (oil) have been more frequently reported, but while HICC is a synthetic fragrance chemical, where the only source of exposure is fragrances, the natural extracts are used in many other contexts than fragrances/cosmetics.

Of patients tested by the Danish monitoring network of dermatologists 2.4% were found to be allergic to HICC in 2005-2008 (with no decreasing trend from 2003 to 2007 (276)) (for more studies see chapter 4.2.2); in 70% of the cases the reaction was of current relevance, i.e. causing disease (66). This is in agreement with the results of a recent German study with HICC, where 48 out of 51 patients (94.1%) with a positive patch test reaction to HICC also reacted in a repeated open application test, simulating normal use conditions of cosmetics containing HICC (104). In a Danish study 69% of 14 HICC allergic individuals developed allergic contact dermatitis from use of cosmetics containing HICC in realistic amounts (101).

On the basis of the high frequency of allergy to HICC, in 2003 the Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP) recommended 0.02% (200 ppm) as maximum amount of HICC in cosmetic products (277). This has not been implemented and no restrictions apply in the Cosmetic Directive.

The fragrance industry, via the International Fragrance Association (IFRA), has its own safety guidelines. Up until 2003 HICC was used without any restriction; in 2003 a limit of

1.5% HICC in any kind of product was introduced. In 2008 this was changed according to the new risk assessment model (QRA) applied by the fragrance industry to different levels in 11 different product types derived from the QRA (see 11.1). Limits from 0.11% in lip products to 1.5% in hair styling products were set. In 2009 a further lowering was made of the limits by industry with the following reasoning: "The industry firmly believes and continues to support thresholds based on induction rather than elicitation. However, given the exceptional situation in Europe, the fragrance industry elected to take further restrictive action on this material" (278). An overview of the IFRA restrictions is given in the table below.

**Table 11-7:** Restriction for HICC independent of the QRA according to (278).

IFRA QRA Category	Product type that drives the category	Consumer exposure level 2003–2008 (%)	IFRA Standard July 2008 (%)	IFRA Standard July 2009 (%)
Category 1	Lip products	1.5	0.11	0.02
Category 2	Deodorants/antiperspirants	1.5	0.15	0.02
Category 3	Hydroalcoholics for shaved skin	1.5	0.60	0.2
Category 4	Hydroalcoholics for unshaved skin	1.5	1.5	0.2
Category 5	Hand cream	1.5	1.0	0.2
Category 6	Mouthwash	1.5	1.5	Not applicable*
Category 7	Intimate wipes	1.5	0.3	0.02
Category 8	Hair styling aids	1.5	1.5	0.2
Category 9	Rinse-off hair conditioners	1.5	1.5%	0.2%
Category 10	Hard surface cleaners	1.5	1.5%	0.2%
Category 11	Incidental or non-skin contact	15	Not restricted	Not restricted

Note: HICC Hydroxyisohexyl 3-cyclohexene carboxaldehyde.  
QRA Quantitative risk assessment.

\* Not applicable because HICC is not approved for flavour use.

#### 11.4. Conclusion

- A dose-response relationship between exposure to contact allergens and induction of allergy (sensitisation) as well as elicitation is well established. This means that in principle, thresholds can be identified which are safe for the consumer.
- A model for dermal sensitisation quantitative risk assessment has been developed (QRA) and implemented by the fragrance industry. This model relies on thresholds, no effect or low-effect levels, established in healthy human volunteers and/or in animal experiments. The SCCP has previously reviewed this methodology and concluded that: "There is no confidence that the levels of skin sensitisers identified by the dermal sensitisation QRA are safe for the consumer."

- Elicitation data can provide thresholds indicative for the safe use of those substances which have already caused significant problems in the consumer. In this context, "safe use" means that the thresholds will protect the majority of consumers from allergic contact dermatitis, but does not preclude that the most sensitive subset of the population may react upon exposure to the allergen.
- Furthermore, based on experience from intervention studies, such thresholds will also be sufficiently low to protect (most of) the non-sensitised consumers from developing contact allergy.
- Elicitation levels have been studied specifically for the three fragrance chemicals cinnamal, hydroxycitronellal and isoeugenol. These studies, however, are not adequate to derive safe thresholds for the individual substances directly from the data.
- In the absence of adequate substance specific data it is possible to use a general threshold. Based on a statistical analysis of the available data in the scientific literature, a threshold of 0.8 µg/cm<sup>2</sup> was derived. This corresponds to 0.01% (100 ppm) limit in cosmetic products indicative for safe use.
- It is not possible to provide a safe threshold for natural extracts of concern, as no specific investigations exist and the model providing the general threshold (0.01%) has been based on individual chemicals only. However the maximum use concentration applies to the identified chemicals both if added as chemicals or as an identified constituent of a natural ingredient. This will also reduce the risk of sensitisation and elicitation from natural extracts.
- For substances for which there are no clinical data of concern, models such as the dermal sensitisation QRA approach may, after refinement and validation, be used to suggest a safe level of exposure prior to incorporation into products. However, aggregated exposures must be incorporated in the dermal sensitisation QRA model.
- HICC has for more than 10 years been recognized as an important allergen with more cases documented in the scientific literature than for any other fragrance chemical in this period. HICC has been shown to be a significant cause of disease as many of those with contact allergy to HICC had also reactions to cosmetics, which contained or were likely to contain HICC. Since 2003 attempts have been made by the fragrance industry to contain the outbreak of HICC allergy, but with no convincing success so far. Recent voluntary restrictions (recommendations to lower use concentrations, at least for some product types, to the level recommended by the SCCS in 2003) are not reflected in available evidence and are considered insufficient. The SCCS considers that the number of cases of HICC allergy documented over the last decade is exceptionally high and that continued exposure to HICC by the consumer is not considered safe, even at concentrations as low as 200 ppm. Therefore, HICC should not be used in consumer products in order to prevent further cases of contact allergy to HICC and to limit the consequences to those who already have become sensitized.
- The SCCP concluded in 2004 that chloroatranol and atranol, the main allergenic constituents of *Evernia prunastri* and *Evernia furfuracea*, should not be present in consumer products because they are extremely potent allergens. The persistently high frequency of contact allergy to *Evernia prunastri* and *Evernia furfuracea* noted in eczema patients does point to a persisting problem with exposure to the allergenic constituents.



## 12. Data gaps and research needed

In the course of working on this opinion, the following points are highlighted as important data gaps, ordered by research area:

### 12.1. Clinical and epidemiological research

- Clinical data on more fragrance substances are needed to assess more fully the epidemiology of fragrance contact allergy and pin-point the culprit substances for induction and elicitation of contact allergy in man.
- Data from a broader range of EU countries on the clinical and epidemiological picture of fragrance contact allergy is needed, as difference in exposure and use habits are expected across Europe.
- A co-ordinated strategy for data collection should be developed.
- Very little is known about susceptible groups of the population, e.g. up to 10% of the European population carry mutations, which impair the skin barrier and which seem to increase the risk of fragrance allergy. Data are needed to qualify and quantify the increase in risk of susceptible groups in order to provide a better protection of all consumers.
- Aberrant enzyme activity in certain individuals, often related to genetic enzyme polymorphisms, may give an increased or reduced risk of sensitisation to prohaptens (that need enzymatic activation) in certain individuals or populations. More research into the role of relevant traits is needed.
- Dose-response data from clinical studies are available for only a few allergens. To establish individual safe levels such data are required for all established allergens of concern and covering an appropriate range of product types. This would also consolidate the basis of the use of a general threshold for safe use of fragrance allergens.
- Data on human exposure to fragrances from the use of different product categories is very scarce and therefore does not provide an optimal basis of risk assessment, e.g. exposure data on use for perfume/eau de cologne are lacking.
- Most experimental studies are done on individual fragrance ingredients, while exposure to allergens in cosmetic products is usually to mixtures of allergens. The risk of sensitisation and elicitation may depend on the mixture of substances, but very few studies on this exist. It is necessary to improve the knowledge base on cocktail effects on sensitisation/elicitation to improve the basis of risk assessment and management.
- Screening in dermatitis patients should be performed with air exposed samples of such fragrance substances that in experimental studies have been demonstrated to act as pre-haptens, i.e. autoxidise and form oxidation mixtures containing allergenic oxidation products.
- Patch testing should if possible, be performed with the isolated true haptens formed from pre-haptens and pro-haptens to increase the possibility to diagnose allergy from these type of substances.
- There is a need for more experimental research to further establish the impact of the behaviour of fragrance substances when applied on the skin (including factors such as volatility, autoxidation, skin penetration, reactivity in skin and bioactivation).

### 12.2. Non-human studies

- Several studies in the industry submission (159) were of insufficient quality, not following the OECD guidelines.



- In some cases it was found that either very few concentrations points had been used in LLNAs, or concentrations were insufficient for achieving a 3-fold increase of the SI.

A sufficient number of doses (concentrations) should be applied in LLNAs (at least 5) so that interpolation (for deriving an EC3 value) can rely on more than two or three actual data points to be more reliable. SCCS therefore suggests a change in the OECD guideline 429. (It is important to remember that the production of unreliable data is a waste of animals.) Moreover, the maximum concentration should be high enough to achieve a > 3-fold increase in SI, as far as this is possible with the substance/vehicle combination chosen.

- Data on experimental results are often not published, but available only on file in the companies having performed the tests. Access to such results would be important for the scientific community, e.g. in the context of REACH, or independently, either to the public domain, or to a Public Trustee.
- The OECD guideline 429 recommends several vehicles. It is well known that a difference in the EC3 value can be obtained for the same substance depending on which vehicle is used in the LLNA. Thus, as an additional control, supplementary to the guideline based LLNA control, a clinically relevant solvent or the commercial formulation in which the test substance is marketed may be used.
- As long as no validated *in vitro* method exists, more research is needed. Until one or more method(s) have been decided to fulfil the requirements for substituting *in vivo* testing, the *in vivo* testing for prediction of skin sensitisation has to be used.
- Applying only mechanism-based QSAR (QMM) as a tool in non-animal based risk assessment for skin sensitisation is of limited value for fragrance substances. This is due to major information gaps in the present model when addressing substances that act via abiotic or metabolic activation, and the high incidence of such substances in fragrances. Therefore, further experimental and clinical research in the area of abiotic and/or metabolic activation of fragrance substances is needed to increase the safety for the consumer, i.e. experimental studies which include air oxidation and bioactivation.
- Further experimental investigations of the sensitisation potential of fragrance substances are needed to determine the impact of the volatility of the substance as well as the effect of the vehicle on skin penetration/absorption and reactivity.
- From a clinical perspective it is important for the individual who is sensitised to one fragrance substance to know if they must also avoid other fragrance substances that can cause allergic contact dermatitis due to cross-reactivity with the original sensitiser. Prediction of risks for cross-reactivity requires sound application of theoretical principles in combination with well-designed experimental studies. This is a field that has not been studied very much so far and needs to be focused on much more in the future.
- Quantitative structure activity relationship (QSAR) models should be further developed, combining, as appropriate, information from *in silico*, *in chemico* and *in vitro* methods as possible. Prediction of different activation pathways should be included.
- Effect estimates such as proportions of sensitised humans or animals, or mean stimulation indices, EC3 values and other derivations should ideally be accompanied by an interval estimate (confidence interval) to address precision (279).

### 13. Opinion

Contact allergy to fragrances is a common, significant and relevant problem in Europe. The studies since the SCCNFP opinion on fragrance allergy in consumers in 1999 (SCCNFP/0017/98) (SCCNFP 1999) have confirmed that the 26 fragrance allergens, identified by the SCCNFP, are still relevant fragrance allergens for consumers because of their exposure from cosmetic products. Additional exposure to many of these 26 fragrance allergens also occurs from the use of other consumer products, such as detergents, toys, etc. Some of these fragrance substances are also used as preservatives.

The overall trend of fragrance contact allergy appears to have been stable for the last 10 years, as some causes of fragrance allergy have decreased and others increased. From the few population-based studies, it can be estimated that the frequency of contact allergy to fragrance ingredients in the general population in Europe is 1-3%. This is based on the limited testing with eight common fragrance allergens (FM I) out of the approximately 2500 fragrance ingredients listed in CosIng and indicative of the substances that may be present in fragrance compounds. However, the real prevalence of contact allergy to fragrance substances may be higher if the testing were to be performed with the full spectrum of fragrance allergens, including oxidised substances, where relevant.

Among eczema patients in the European population, around 16% are sensitised to fragrance ingredients. The disease can be severe and generalised, with a significant impairment of quality of life and potential consequences for fitness for work.

Contact sensitisation, and its clinical manifestation, allergic contact dermatitis, can be prevented if the exposure to known contact allergens is reduced or abolished (primary prevention). Experiences so far, have indicated that not all substances that later turned out to be significant contact allergens after human exposure, were predicted by experimental studies, e.g. the preservative methyldibromo glutaronitrile and the fragrance chemical HICC. Thus, a significant exposure of the population may occur before a substance is established as an important contact allergen in man.

Elicitation of allergic contact dermatitis occurs when a consumer sensitised to a certain substance is re-exposed to the substance in question. Prevention at this stage, termed secondary prevention, can be achieved if use of the allergen in products is eliminated or reduced to a tolerable level (general prevention), or if the patients succeed in avoiding all sources of exposure (individual prevention). Ingredient listing of individual fragrance allergens has been shown to be an important tool to enable consumers with an identified allergy to reduce/avoid relevant exposures. Moreover, ingredient listing is also of great importance to ensure that an adequate diagnosis of fragrance contact allergy can be made without undue delay. If the information given on the presence of fragrance allergens is incomplete, diagnosis of fragrance contact allergy may be missed.

The SCCNFP, in its 1999 opinion, identified 26 fragrance allergens for which information should be provided to consumers concerning their presence in cosmetic products. This was implemented in the European Cosmetics legislation (280) as ingredient labelling of these 26 fragrance substances (Annex III, entries 67-92). However, safe use concentrations for these substances in cosmetic products have not yet been determined and much new evidence concerning fragrance allergy has been published since 1999. The present opinion updates the SCCNFP opinion with a systematic and critical review of the scientific literature up to October 2010. This review addresses the issue of contact allergy to fragrance substances, including natural extracts and updates the list of fragrance allergens relevant to consumers. Clinical, epidemiological and experimental studies were evaluated, as well as modelling studies performed, to establish lists of: (i) established fragrance allergens; (ii) likely fragrance allergens; and (iii) possible fragrance allergens. The review also includes fragrances, which on modification by oxidation or by enzyme mediated processes, can produce allergens. Available dose-response data have been

examined to answer whether safe thresholds can be established for the most frequent fragrance allergens.

### 13.1. Question 1

***Does the SCCS still consider that the fragrance allergens currently listed in Annex III, entries 67-92, for labelling purposes represent those fragrance ingredients that the consumer needs to be made aware of when present in cosmetic products?***

In order to answer this question, the SCCS has used clinical and epidemiological data to identify known fragrance allergens. These were categorised as *established contact allergens in humans* (see Table 13-1).

Where sufficient animal evidence was present, these substances were categorised as established contact allergens in animals (Table 13-2). For a number of other fragrance substances, combinations of limited clinical data together with SAR considerations have been applied to indicate likely fragrance allergens in man (Table 13-3). Finally, SAR has also been applied to substances that lack human data to identify fragrances that have the structural potential to be contact allergens. Substances with insufficient human data were also considered as possible fragrance allergens. For these further tests (experimental/clinical data) are required (Table 13-4).

**Table 13-1:** Established contact allergens in humans.

For categorisation of importance (+ to +++) see chapter 7.1. Allergens of special concern are substances where between 100 and 1,000 cases (+++) and more than 1,000 (++++) have been published. These are set in bold. Fragrance substances identified as allergens in the 1999 opinion of SCCNFP (1) are marked with an asterisk.

<b>INCI name (or, if none exists, perfuming name according to CosIng)</b>	<b>CAS number</b>	<b>Human evidence: see text</b>
<b>Individual chemicals</b>		
ACETYLCEDRENE	32388-55-9	+
AMYL CINNAMAL*	122-40-7	+
AMYL CINNAMYL ALCOHOL*	101-85-9	+
AMYL SALICYLATE	2050-08-0	+
trans-ANETHOLE	4180-23-8	+ (r.t.)
ANISE ALCOHOL*	105-13-5	+
BENZALDEHYDE	100-52-7	+
BENZYL ALCOHOL*	100-51-6	+
BENZYL BENZOATE*	120-51-4	++
BENZYL CINNAMATE*	103-41-3	++
BENZYL SALICYLATE*	118-58-1	+
BUTYLPHENYL METHYLPROPIONAL (Lilial®)*	80-54-6	++
CAMPHOR	76-22-2 / 464-49-3	+ (r.t.)
beta-CARYOPHYLLENE (ox.)	87-44-5	Non-ox.: +, ox.: +
CARVONE	99-49-0 / 6485-40-1 / 2244-16-	+ (r.t.)

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INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text
	8	
<b>CINNAMAL*</b>	<b>104-55-2</b>	<b>+++</b>
<b>CINNAMYL ALCOHOL*</b>	<b>104-54-1</b>	<b>+++</b>
<b>CITRAL*</b>	<b>5392-40-5</b>	<b>+++</b>
CITRONELLOL*	106-22-9 / 1117-61-9 / 7540-51-4	++
<b>COUMARIN*</b>	<b>91-64-5</b>	<b>+++</b>
(DAMASCENONE ) ROSE KETONE-4	23696-85-7	+ (r.t.)
alpha-DAMASCONE (TMCHB)	43052-87-5 / 23726-94-5	++
cis-beta-DAMASCONE	23726-92-3	+
delta-DAMASCONE	57378-68-4	+
DIMETHYLBENZYL CARBINYL ACETATE (DMBCA)	151-05-3	+
<b>EUGENOL*</b>	<b>97-53-0</b>	<b>+++</b>
<b>FARNESOL*</b>	<b>4602-84-0</b>	<b>++ - +++</b>
<b>GERANIOL*</b>	<b>106-24-1</b>	<b>+++</b>
HEXADECANOLACTONE	109-29-5	+ (r.t.)
HEXAMETHYLINDANOPYRAN	1222-05-5	++
HEXYL CINNAMAL*	101-86-0	++
<b>HYDROXYISOHEXYL CARBOXALDEHYDE (HICC)*</b>	<b>3-CYCLOHEXENE</b> <b>31906-04-4 /</b> <b>51414-25-6</b>	<b>++++</b>
<b>HYDROXYCITRONELLAL*</b>	<b>107-75-5</b>	<b>+++</b>
<b>ISOEUGENOL*</b>	<b>97-54-1</b>	<b>+++</b>
alpha-ISOMETHYL IONONE*	127-51-5	++
<b>(DL)-LIMONENE*</b>	<b>138-86-3</b>	<b>++ (non-ox.);</b> <b>+++ (ox.)</b>
<b>LINALOOL*</b>	<b>78-70-6</b>	<b>++ (non-ox.)</b> <b>+++ (ox.)</b>
LINALYL ACETATE	115-95-7	+ (non-ox.) ++ (ox.)
MENTHOL	1490-04-6 / 89- 78-1 / 2216-51- 5	++
6-METHYL COUMARIN	92-48-8	++
METHYL 2-OCTYNOATE*	111-12-6	++
METHYL SALICYLATE	119-36-8	+
3-METHYL-5-(2,2,3-TRIMETHYL-3-CYCLOPENTENYL)PENT-4-EN-2-OL	67801-20-1	++ (r.t.)

## Opinion on fragrance allergens in cosmetic products

<b>INCI name (or, if none exists, perfuming name according to CosIng)</b>	<b>CAS number</b>	<b>Human evidence: see text</b>
alpha-PINENE and beta-PINENE	80-56-8 and 127-91-3, resp.	++
PROPYLIDENE PHTHALIDE	17369-59-4	+ (r.t.)
SALICYLALDEHYDE	90-02-8	++
alpha-SANTALOL and beta-SANTALOL	115-71-9 and 77-42-9, resp.	++
SCLAREOL	515-03-7	+
TERPINEOL (mixture of isomers)	8000-41-7	+
alpha-TERPINEOL	10482-56-1 / 98-55-5	
Terpinolene	586-62-9	+
TETRAMETHYL ACETYLOCTAHYDRONAPHTHALENES	54464-57-2 / 54464-59-4 / 68155-66-8 / 68155-67-9	+
TRIMETHYL-BENZENEPROPANOL (Majantol)	103694-68-4	++
VANILLIN	121-33-5	++
<b>Natural extracts</b>		
<b><i>CANANGA ODORATA and Ylang-ylang oil</i></b>	<b>83863-30-3; 8006-81-3</b>	<b>+++</b>
<i>CEDRUS ATLANTICA BARK OIL</i>	92201-55-3; 8000-27-9	++
<i>CINNAMOMUM CASSIA LEAF OIL</i> <i>CINNAMOMUM ZEYLANICUM BARK OIL</i>	8007-80-5 84649-98-9	++ (r.t.)
<i>CITRUS AURANTIUM AMARA FLOWER / PEEL OIL</i>	8016-38-4; 72968-50-4	++
<i>CITRUS BERGAMIA PEEL OIL EXPRESSED</i>	89957-91-5	+ (r.t.)
<i>CITRUS LIMONUM PEEL OIL EXPRESSED</i>	84929-31-7	++
<i>CITRUS SINENSIS (syn.: AURANTIUM DULCIS) PEEL OIL EXPRESSED</i>	97766-30-8; 8028-48-6	++
<i>CYMOPOGON CITRATUS / SCHOENANTHUS OILS</i>	89998-14-1; 8007-02-1; 89998-16-3	++
<i>EUCALYPTUS SPP. LEAF OIL</i>	92502-70-0; 8000-48-4	++
<b><i>EUGENIA CARYOPHYLLUS LEAF / FLOWER OIL</i></b>	<b>8000-34-8</b>	<b>+++</b>
<b><i>EVERNIA FURFURACEA LICHEN EXTRACT*</i></b>	<b>90028-67-4</b>	<b>+++</b>
<b><i>EVERNIA PRUNASTRI*</i></b>	<b>90028-68-5</b>	<b>+++</b>
<b><i>JASMINUM GRANDIFLORUM / OFFICINALE</i></b>	<b>84776-64-7; 90045-94-6; 8022-96-6</b>	<b>+++</b>
<i>JUNIPERUS VIRGINIANA</i>	8000-27-9; 85085-41-2	++

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<b>INCI name (or, if none exists, perfuming name according to CosIng)</b>	<b>CAS number</b>	<b>Human evidence: see text</b>
<i>LAURUS NOBILIS</i>	8002-41-3; 8007-48-5; 84603-73-6	++
<i>LAVANDULA HYBRIDA</i>	91722-69-9	+ (r.t.)
<i>LAVANDULA OFFICINALIS</i>	84776-65-8	++
<i>MENTHA PIPERITA</i>	8006-90-4; 84082-70-2	++
<i>MENTHA SPICATA</i>	84696-51-5	++
<b>MYROXYLON PEREIRAE</b>	<b>8007-00-9;</b>	<b>++++</b>
<i>NARCISSUS SPP.</i>	diverse	++
<i>PELARGONIUM GRAVEOLENS</i>	90082-51-2; 8000-46-2	++
<i>Pinus mugo</i>	90082-72-7; 97676-05-6	++
<i>POGOSTEMON CABLIN</i>	8014-09-3; 84238-39-1	++
<i>ROSE FLOWER OIL (ROSA SPP.)</i>	Diverse	++
<b>SANTALUM ALBUM</b>	<b>84787-70-2;</b> <b>8006-87-9</b>	<b>+++</b>
<b>TURPENTINE (oil)</b>	<b>8006-64-2;</b> <b>9005-90-7;</b> <b>8052-14-0</b>	<b>++++</b>
<b>Verbena absolute</b> ( <i>Lippia citriodora</i> Kunth.)	8024-12-2	++

**Table 13-2:** Fragrance substances categorised as established contact allergens in animals.

<b>INCI name (or, if none exists, perfuming name according to CosIng)</b>	<b>CAS number</b>	<b>Human evidence: see text</b>	<b>EC 3 value (min; %)</b>
<b>Individual chemicals</b>			
Allyl phenoxyacetate	7493-74-5	none	3.1
p-tert. -Butyldihydrocinnamaldehyde	18127-01-0	none	4.3
Cinnamyl nitrile	1885-38-7	none	> 10
CYCLAMEN ALDEHYDE	103-95-7	none	22
Dibenzyl ether	103-50-4	none	6.3
2,3-DIHYDRO-2,2,6-TRIMETHYLBENZALDEHYDE	116-26-7	limited	7.5
trans-2-Hexenal	6728-26-3	none	2.6
2-Hexylidene cyclopentanone	17373-89-6	none	2.4
HEXYL SALICYLATE	6259-76-3	negative	0.18
p-Isobutyl- $\alpha$ -methyl hydrocinnamaldehyde	6658-48-6	none	9.5
Isocyclocitral	1335-66-6	none	7.3

## Opinion on fragrance allergens in cosmetic products

<b>INCI name (or, if none exists, perfuming name according to CosIng)</b>	<b>CAS number</b>	<b>Human evidence: see text</b>	<b>EC 3 value (min; %)</b>
Isocyclogeraniol	68527-77-5	none	> 25
$\alpha$ -Methyl cinnamic aldehyde	101-39-3	none	4.5
METHYLENEDIOXYPHENYL METHYLPROPANAL	1205-17-0	none	16.4
6-Methyl-3,5-heptadien-2-one	1604-28-0	none	> 5
METHYLUNDECANAL	110-41-8	none	10
2-Methoxy-4-methylphenol	93-51-6	none	5.8
4-Methoxy- $\alpha$ -methyl benzenpropanal	5462-06-6	none	23.6
METHYL OCTINE CARBONATE	111-80-8	limited	2.5
1-Octen-3-yl acetate	2442-10-6	none	> 30
Perillaldehyde p-Mentha-1,8-dien-7-al	2111-75-3	none	8.1
PHENYLACETALDEHYDE	122-78-1	limited	3
<b>Natural extracts</b>			
Camellia sinensis leaf <i>Tea Leaf Absolute</i>	84650-60-2	none	> 5
Jasminum Sambac Flower CERA / Extract / Water	91770-14-8	none	35.4

**Table 13-3:** Fragrance substances categorised as likely contact allergens by combination of evidence.

<b>INCI name (or, if none exists, perfuming name according to CosIng)</b>	<b>CAS number</b>	<b>Human evidence: see text</b>	<b>EC 3 value (min; %)</b>	<b>SAR</b>
AMBRETTOLIDE	7779-50-2	limited	none	+
CARVACROL	499-75-2	limited	none	+
CUMINALDEHYDE	122-03-2	limited	none	+
CYCLOPENTADECANONE	502-72-7	limited	none	+
trans-trans-delta-DAMASCONE	71048-82-3	limited	none	+
DIMETHYLTETRAHYDRO BENZALDEHYDE	68737-61-1	limited	none	+
ETHYL VANILLIN	121-32-4	limited	none	+
HELIOTROPINE	120-57-0	limited	none	+
ISOAMYL SALICYLATE	87-20-7	limited	none	++
ISOLONGIFOLENEKETONE	33407-62-4	limited	none	+
METHOXYCITRONELLAL	3613-30-7	limited	none	+
METHYL CINNAMATE	103-26-4	limited	none	++
METHYL EUGENOL	93-15-2	limited	none	++
METHYLIONANTHEME	55599-63-8	limited	none	+
5-METHYL- $\alpha$ -IONONE	79-69-6	limited	none	+
MYRCENE	123-35-3	limited	none	++

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INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text	EC 3 value (min; %)	SAR
MYRTENOL	515-00-4	limited	none	+
NEROL	106-25-2	limited	none	++
Nerolidol (isomer not specified)	7212-44-4	limited	none	++
NOPYL ACETATE	128-51-8	limited	none	+
PHYTOL	150-86-7	limited	none	+
RHODINOL	6812-78-8	limited	none	+
trans-ROSE KETONE-5	39872-57-6	limited	none	++

**Table 13-4:** Fragrance substances categorised as possible contact allergens.

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text	EC 3 value (min; %)	SAR
<b>Individual chemicals</b>				
CYCLOHEXYL ACETATE	622-45-7	limited	none	0
ETHYLENE DODECANEDIOATE	54982-83-1	limited	none	0
HYDROXYCITRONELLOL	107-74-4	limited	none	0
METHOXYTRIMETHYLHEPTANO L	41890-92-0	limited	none	0
METHYL p-ANISATE	121-98-2	limited	none	0
METHYL DIHYDROJASMONATE	24851-98-7	limited	none	0
PHENETHYL ALCOHOL	60-12-8	limited	none	0
PHENYLPROPANOL	122-97-4	limited	none	0
AMYL CYCLOPENTANONE	4819-67-4	negative	none	+
BENZYL ACETATE	140-11-4	negative	none	+
6-ETHYLIDENEOCTAHYDRO-5,8-METHANO-2H-BENZO-1-PYRAN	93939-86-7	negative	none	+
3 $\alpha$ ,4,5,6,7,7 $\alpha$ -HEXAHYDRO-4,7-METHANO-1H-INDEN-5(OR 6)-YL ACETATE	54830-99-8	negative	none	+
alpha-IONONE	127-41-3	negative	none	+
beta-IONONE	79-77-6	negative	none	+
METHYL IONONE (mixture of isomers)	1335-46-2	negative	none	+
TERPINEOL ACETATE (Isomer mixture)	8007-35-0	negative	none	+
alpha-TERPINYL ACETATE	80-26-2	negative	none	+
CITRONELLYL NITRILE	51566-62-2	none	none	++



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<b>INCI name (or, if none exists, perfuming name according to CosIng)</b>	<b>CAS number</b>	<b>Human evidence: see text</b>	<b>EC 3 value (min; %)</b>	<b>SAR</b>
alpha-CYCLOHEXYLIDENE BENZENEACETONITRILE	10461-98-0	none	none	+
DECANAL	112-31-2	none	none	++
DIHYDROMYRCENOL	18479-58-8	none	none	+
2,4-DIMETHYL-3-CYCLOHEXEN-1-CARBOXALDEHYDE	68039-49-6	none	none	+
3,7-DIMETHYL-1,6-NONADIEN-3-OL	10339-55-6	none	none	++
2-ETHYL-4-(2,2,3-TRIMETHYL-3-CYCLOPENTEN-1-YL)-2-BUTEN-1-OL	28219-61-6	none	none	+
GERANYL ACETATE	105-87-3	none	none	++
HEXAHYDRO-METHANOINDENYL PROPIONATE	68912-13-0	none	none	+
IONONE isomeric mixture	8013-90-9	none	none	+
ISOBERGAMATE	68683-20-5	none	none	+
Longifolene	475-20-7	none	none	+
METHYL DECENOL	81782-77-6	none	none	+
TRICYCLODECENYL PROPIONATE	17511-60-3	none	none	+
OXACYCLOHEXADECENONE	34902-57-3	none	none	++
VERDYL ACETATE	2500-83-6/ 5413-60-5	none	none	+
trans-beta-Damascone	23726-91-2	none	none	+
gamma-Damascone	35087-49-1	none	none	+
Citronellal	106-23-0	none	none	++
Phenethyl salicylate	87-22-9	none	none	++
<b>Natural extracts</b>				
ACORUS CALAMUS ROOT OIL	84775-39-3	Limited	none	
CEDRUS DEODARA WOOD OIL	91771-47-0	Limited	none	
CITRUS AURANTIUM AMARA LEAF OIL	72968-50-4	Limited	none	
CITRUS TANGERINA ...	223748-44-5	Limited	none	
CYMBOPOGON NARDUS / WINTERIANUS HERB OIL	89998-15-2; 91771-61-8	Limited	none	
ILLICIAM VERUM FRUIT OIL	84650-59-9	Limited	none	
LAVANDULA SPICA	97722-12-8	Limited	none	
LITSEA CUBEBA	90063-59-5	Limited	none	
PELARGONIUM ROSEUM	90082-55-6	Limited	none	

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INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text	EC 3 value (min; %)	SAR
SALVIA spp.	Diverse	Limited	none	
TAGETES PATULA	91722-29-1	Limited	none	
THYMUS spp.	84929-51-1	Limited	none	
VETIVERIA ZIZANOIDES	8016-96-4; 84238-29-9	Limited	none	

Regarding the above categorisation of fragrance substances, the following aspects need to be considered when interpreting an outcome other than established contact allergen in humans:

- If human evidence is negative, there is still a potential sensitisation risk, as in this set of substances the number of (consecutive) patients tested was low, i.e. up to a few hundred.
- If EC3 values are given as higher (>) than a certain value, an exact EC3 could not be established, as the substance had been tested in too low concentration(s).
- Two single substances; 2,4-dimethyl-3-cyclohexen-1-carboxaldehyde (CAS no. 68039-49-6) and longifolene (CAS no. 475-20-7), and two natural extracts *Citrus paradisi* (CAS no. 8016-20-4) and *Mentha arvensis* (CAS no. 68917-18-0) were classified as R43, according to the submission by IFRA. The evidence on which this classification was based was not available to the SCCS, so the validity of classification cannot be assessed. Nevertheless, following a precautionary approach, the four substances/substance mixtures should be treated as *likely contact allergens*.
- For SAR, the categories of prediction are: non-sensitiser (0); possible-sensitiser (+); predicted sensitiser (++); and not predictable (n.p.). (For details see Table 9-3 and Table 9-4). SAR predictions are only considered when human and animal data are limited or missing.
- Several substances are currently banned from the use in cosmetic products by Annex II of the Cosmetics Directive, based on concerns regarding one or more toxicological endpoints. While available clinical evidence regarding this set of substances is listed in Annex I to this opinion, these substances have not further been evaluated.

Fragrance ingredients listed in Table 13-1 clearly have caused disease in man, and based on the clinical experience alone, these 82 substances were classified as established contact allergens in humans, 54 individual chemicals and 28 natural extracts (mixtures of chemicals), including all 26 fragrance allergens identified by SCCNFP in 1999. Of those, 12 chemicals and eight natural extracts are considered of special concern as they have given rise to at least 100 reported cases (listed in Table 13-5). These substances pose a particularly high risk of sensitisation to the consumer and are further considered in the answer of question 2. One substance, hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC), was shown to be the cause of allergic contact dermatitis in more than 1500 reported cases since 1999. The number of cases is only those reported in scientific publications, and therefore the actual number of cases is severely under-estimated.

**Table 13-5:** Established fragrance contact allergens of special concern (single chemicals only).

Cinnamal,
Cinnamyl Alcohol
Citral
Coumarin
Eugenol
Farnesol
Geraniol
Hydroxycitronellal
Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)
Isoeugenol
Limonene (oxidised)
Linalool (oxidised)

The established contact allergens in animals (Table 13-2) and the likely contact allergens, identified based on a combination of limited evidence from man together with positive SAR predictions (Table 13-3), are predicted to cause disease in man given sufficient exposure.

Information on the presence of all the substances given in Table 13-1, Table 13-2 and Table 13-3 in cosmetic products is important in order to enable aimed testing of patients with contact dermatitis and to diagnose fragrance allergy without delay. Further, this information is important to the sensitised consumer as it will enable them to avoid cosmetic products, which they may not tolerate.

Substances given in Table 13-4 are possible contact allergens and further data are required to judge if these are contact allergens in humans and give rise to contact allergy in consumers.

### Conclusions - Question 1

The studies since the SCCNFP Opinion on fragrance allergy in consumers (1) have confirmed that the fragrance allergens currently listed in Annex III, entries 67-92 are still relevant fragrance allergens for the consumers from their exposure to cosmetic products.

The review of the clinical and experimental data shows that many more fragrance substances than those identified in the SCCNFP opinion of 1999 have been shown to be sensitisers in humans. A comprehensive list of established contact allergens in humans is given in Table 13-1.

Moreover, animal experiments indicate that additional fragrance substances can be expected to be contact allergens in humans, although human evidence is currently lacking.

Additionally, limited human and/or animal evidence together with structure activity relationship analysis suggests that other fragrance ingredients may be a cause of concern with regard to their potential of causing contact allergy in humans.

Ingredient listing is important in clinical practice for the management of patients who are allergic to one or more of the listed fragrance chemicals. It is also important for the patients in order to avoid future exposure to fragrance contact allergens which they may not tolerate.

The SCCS considers that those substances itemised in Table 13-1, Table 13-2 and Table 13-3 represent those fragrance ingredients that the consumer should be made aware of when present in cosmetic products.

Substances known to be transformed (e.g. hydrolysis of esters) to known contact allergens should be treated as equivalent to these known contact allergens. Important indicative, but not exhaustive, examples include isoeugenol and its esters, geraniol and its esters, eugenol and its esters, and linalool and its esters.

Substances known to be transformed (e.g. by oxidation either via air oxidation or via bioactivation) to known contact allergens should be treated as equivalent to these known contact allergens. Important indicative examples include limonene, linalool, linalyl acetate, geraniol, geranial, alpha-terpinene, eugenol, isoeugenol and cinnamyl alcohol.

### 13.2. Question 2

***Can the SCCS establish any threshold for their safe use based on the available scientific data?***

Dose-response relationships exist between exposure to contact allergens and the proportion of consumers who will become sensitised to an allergen (i.e. induction), as well as the proportion who will suffer from allergic contact dermatitis (elicitation). For a number of recognised contact allergens in man, dose-elicitation studies on sensitised individuals are available. These studies indicate that it is in principle possible to derive exposure levels that the majority of sensitised individuals will tolerate. The SCCS considers that thresholds based on elicitation levels in sensitised individuals will be sufficiently low to protect both sensitised individuals as well as most of the non-sensitised consumers from developing contact allergy and limit the risk of induction.

Among the established chemical fragrance allergens, 12 were identified as posing a high risk of sensitisation to the consumer (Table 13-5), i.e. more than 100 reported cases. For these substances, limitation of exposure would help to protect sensitised consumers from developing allergic contact dermatitis.

Dose-response studies have been performed with only four of these fragrance substances (HICC, isoeugenol, cinnamal and hydroxycitronellal). In addition, such a study has also been performed on chloroatranol, a potent allergen in *Evernia prunastri* and *Evernia furfuracea*. These studies, however, are not adequate to derive safe thresholds for the individual substances directly from the data.

If no such data are available, for substances posing a high risk to the consumer (like the 12 listed in Table 13-5), the use of a general threshold may be considered. A threshold of 0.8 µg/cm<sup>2</sup> has been derived based on a statistical analysis of the available data in the scientific literature, including two fragrance allergens. This corresponds to 0.01% (100 ppm) limit in cosmetic products indicative for safe use. This approximation may hold for weak to strong allergens. However, some strong and extreme sensitisers may require lower individual thresholds. As an example, chloroatranol, present in the natural product *Evernia prunastri* and in *Evernia furfuracea*, has been shown to have an elicitation threshold of 0.0004 µg/cm<sup>2</sup> under experimental conditions similar to those yielding above results. On the other hand, for very weak sensitisers, this generic threshold may be too conservative.

In cases where specific data of sufficient quality on threshold levels for a particular allergen are available, these data should be used to set an individual safe threshold. However, when such quality data are not available and a substance has been identified to pose a high risk of sensitisation to the consumer, the general threshold limit (100 ppm in cosmetic products) can be applied.

The model providing the general threshold of 100 ppm has been based on single substances only and no general safe level for the natural extracts of concern can be

identified, but the maximum use concentration applies to the identified fragrance allergens also when present in the natural extract.

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) has been the most frequently reported chemical causing fragrance allergy since the 1999 opinion on fragrance allergy. In total, reports of more than 1500 cases have been published in the scientific literature (see chapter 7.1 and Annex I), which will severely underestimate the actual prevalence in the population. HICC has been shown to be a significant cause of disease as many of those with contact allergy to HICC had also reactions to cosmetics, which contained or were likely to contain HICC. The SCCP concluded in 2003 that 200 ppm of HICC would be tolerated by the majority of sensitised individuals and this level of exposure would have a low potential to induce sensitisation (226). Since 2003 attempts have been made by the fragrance industry to contain the outbreak of HICC allergy, but with no convincing success so far. Recent voluntary restrictions (recommendations to lower use concentrations, at least for some product types, to the level recommended by the SCCS in 2003) are not reflected in available evidence and are considered insufficient. The SCCS considers that the number of cases of HICC allergy documented over the last decade is exceptionally high and that continued exposure to HICC by the consumer is not considered safe, even at concentrations as low as 200 ppm. Chloroatranol and atranol are the main allergenic components of *Evernia prunastri* and *Evernia furfuracea*. The SCCS concluded in 2004 (224) that these should not be present in cosmetic products, due to their exceptionally high sensitisation potential). Attempts to effectively reduce the content of these compounds in "oak moss abs." (281) have largely failed to reduce contact allergy to *Evernia prunastri* and *Evernia furfuracea* and the data presented in this opinion show that the number of cases remains high.

## Conclusions - Question 2

There are two components to the safety of fragrance ingredients in terms of contact allergy. First, the need to eliminate or reduce induction of contact allergy (primary prevention), which, when it occurs, is life long. Secondly, the need to eliminate or reduce elicitation reactions (secondary prevention) on the skin of those individuals who are already sensitised. Human dose elicitation experiments have hitherto been performed only for a very small number of substances. It is unlikely that more of these studies will be performed due to experimental and subject recruitment difficulties.

For individual substances, no levels that could be considered safe for the majority of consumers could be established from the available data.

The dose elicitation studies available indicate that a general level of exposure of up to 0.8 µg/cm<sup>2</sup> (0.01%) may be tolerated by most consumers with contact allergy to fragrance allergens. The SCCS considers that this level of exposure could be efficient in limiting elicitation unless there is substance specific data, either experimental or clinical, to the contrary.

Such a thresholds based on elicitation levels in sensitised individuals will be sufficiently low to protect both sensitised individuals as well as most of the non-sensitised consumers from developing contact allergy.

The SCCS is of the opinion that for substances identified as posing a high risk to the consumer and for which no individual thresholds could be derived (Table 13-5), the general threshold of 0.01% would limit the problem of fragrance allergy in the consumer significantly.

It was not possible to provide a safe threshold for natural extracts of concern, as no specific investigations exist and the model providing the general threshold (0.01%) has been based on individual chemicals only. However the SCCS considers that the maximum use concentration applies to the above identified fragrance allergens also when present in the natural extract. This will also reduce the risk of sensitisation and elicitation from natural extracts.

It is important to stress that this general threshold, although limiting the problem, does not preclude that the most sensitive segment of the population may react upon exposure to these levels. Hence, this threshold does not remove the necessity for providing information to the consumer concerning the presence of the fragrance substance in cosmetics.

In the case of hydroxyisohexyl 3-cyclohexene carboxaldehyde, in 2003 the SCCP suggested that levels of up to 200 ppm would be tolerated by the majority of sensitised individuals. Recent voluntary restrictions (recommendations to lower use concentrations, at least for some product types, to the level recommended by the SCCS in 2003) are not reflected in available evidence and are considered insufficient. The SCCS considers that the number of cases of HICC allergy documented over the last decade is exceptionally high and that continued exposure to HICC by the consumer is not considered safe, even at concentrations as low as 200 ppm. Therefore, HICC should not be used in consumer products in order to prevent further cases of contact allergy to HICC and to limit the consequences to those who already have become sensitized. The SCCP concluded in 2004 that chloroatranol and atranol, the main allergenic constituents of *Evernia prunastri* and *Evernia furfuracea*, should not be present in products for the consumer. The persistently high frequency of contact allergy to *Evernia prunastri* and *Evernia furfuracea* noted in eczema patients does point to a persisting problem with exposure to allergenic constituents. The SCCS is of the opinion that the presence of the two constituents, chloroatranol and atranol, in cosmetic products are not safe.

### 13.3. Question 3

***Can the SCCS identify substances where processes (e.g. metabolism, oxidation and hydrolysis) may lead to cross-reactivity and new allergens which are relevant for the protection of the consumer?***

Many fragrance substances can act as prehapten or prohapten, forming potent allergens by abiotic and/or metabolic activation, and thus increasing the risk of sensitisation.

Experimental and clinical studies have shown that there are fragrance substances that act as prehapten, i.e. their sensitisation potency is markedly increased by air exposure due to oxidation (autoxidation). Non/low-sensitising compounds are thereby transformed into potent sensitisers.

Limonene, linalool, linalyl acetate, alpha-terpinene and geraniol have all been identified as prehapten. These fragrance substances are common in scented cosmetics as well as in household products. The clinical studies show that the exposure to allergens formed due to autoxidation causes significant contact allergy in consumers. Patch testing with oxidised limonene and oxidised linalool shows that these substances rank among the most common contact allergens.

In the SAR analyses performed in this work by the SCCS, fragrance compounds with structural alerts that indicate that they are possible prehapten have been identified (Table 9-1, Table 9-2). In such cases further thorough investigations are needed. It is also important to investigate the stability of the primary oxidation products (the hydroperoxides) formed from various structures of fragrance compounds. The stability of these compounds can have great impact on the sensitisation potency of the oxidised compound as they are strong sensitisers. However, the secondary oxidation products (aldehydes and epoxides) can also be important sensitisers depending on the overall structure of the compound as was demonstrated for oxidised geraniol.

Air oxidation of prehapten can be prevented to a certain extent by measures during handling and storage of the ingredients and final products to avoid air exposure, and/or by addition of suitable antioxidants. The autoxidation rate depends not only on the compound itself, but also on its purity. The prevention of autoxidation using antioxidants

needs thorough investigation because antioxidants can exert their function by being activated instead of the compound that they protect and might act themselves as skin sensitisers after oxidation. This is the case for alpha-terpinene which is described as the antioxidant in tea tree oil (Rudbäck J, Karlberg A-T et al, Chem Res Toxicol, manuscript submitted). As antioxidants are now frequently used at elevated concentrations in scented products due to a growing awareness of the problem of autoxidation, there is a risk that sensitisation caused by the antioxidants will rise. One of the most used antioxidants is butylated hydroxytoluene (BHT) which is considered a minimal risk for sensitisation in the concentrations used but nevertheless, with increased concentrations and usage, the risk of sensitisation could increase.

It should be noted that, to decrease the risk for sensitisation in the population, the possibility to reduce the sensitisation potency by preventing autoxidation is important also for a direct acting hapten or prohaptent, if a further activation by air oxidation to more allergenic compounds has been shown.

Based on the clinical data, oxidised limonene and oxidised linalool are allergens of high concern (Table 13-5) which pose a high risk of sensitisation to the consumer. For these substances the presence of the oxidised fraction represented by the peroxide content should not be higher than 10 ppm. Alternatively, the suggested general threshold dose/area of 0.8 µg/cm<sup>2</sup> (100 ppm in cosmetic products) could be applicable to the total oxidised fraction, i.e. not only peroxides but also secondary oxidation products such as aldehydes and epoxides.

Compounds that are bioactivated by metabolising enzymes to haptens are referred to as prohaptens. Established prohaptens of clinical importance are cinnamyl alcohol, geranial, geraniol, eugenol, isoeugenol and alpha-terpinene.

**Table 13-6:** Known prehaptens and prohaptens.

<b>Fragrance substance</b>	<b>Activation by air oxidation</b>	<b>Bioactivation (oxidation)</b>	<b>Bioactivation (hydrolysis)</b>
Cinnamyl alcohol		x	
Eugenol		x	
Eugenyl acetate		x	x
Geranial	x	x	
Geraniol	x	x	
Geranyl acetate	x	x	x
Isoeugenol		x	
Isoeugenol acetate		x	x
Limonene	x		
Linalool	x		
Linalyl acetate	x		
alpha-terpinene.	x	x	

When bioactivation occurs, the risk of cross-reactivity should be considered. An increased complexity in the cross-reactivity pattern is obtained when a compound could act both as a prehaptent and a prohaptent.

In case derivatives of a fragrance substance are used, it must be taken into account that the derivative could be transformed into the parent or a cross-reacting compound. For such derivatives the same rules as for the corresponding parents should apply, unless the



stability of the derivative has been demonstrated. In particular, hydrolysis of esters to the corresponding alcohols can cause cross-reactions. Acetate esters of eugenol, isoeugenol and geraniol are frequently used in cosmetics.

To be able to predict the sensitisation potency of prohaptens, steps of bioactivation have to be included in the predictive tests.

Activation of individual compounds to various haptens increases the risks of cross-reactivity between chemicals and also causes difficulties in prediction of these risks. Prediction of risks requires sound application of theoretical principles in combination with well designed experimental studies. Based on the acquired knowledge, qualified suggestions using structure activity relationship (SAR) regarding many fragrance substances have been made (Table 9-1 to Table 9-3). However, as the stability of formed oxidation products (mainly hydroperoxides) is important for the sensitisation potency, the SAR hypothesis must be followed by experimental investigations for the actual compounds.

### **Conclusions - Question 3**

Many fragrance substances can act as prehaptens or prohaptens, forming potent allergens by abiotic and/or metabolic activation. Activation can thus increase the risk of sensitisation. Fragrances with published data showing the formation of sensitising compounds by autoxidation, bioactivation or both are the following (see also Table 13-6).

Fragrance substances of clinical importance known to be prehaptens and to form sensitising compounds by air oxidation are limonene, linalool, and linalyl acetate.

Fragrance substances of clinical importance known to be prohaptens and to form sensitising compounds by metabolic transformation are cinnamyl alcohol, eugenol, isoeugenol and isoeugenyl acetate.

Fragrance substances of clinical importance with published data known to be both prehaptens and prohaptens and to form sensitising compounds by air oxidation (prehaptens) and by metabolic transformation are geraniol and alpha -terpinene.

A fragrance substance that sensitises without activation but forms more potent sensitising compounds by air oxidation and also by metabolic transformation is geraniol (one isomer of citral).

In the case of prehaptens, it is possible to prevent activation outside the body to a certain extent by different measures, e.g. prevention of air exposure during handling and storage of the ingredients and the final product and by the addition of suitable antioxidants. When antioxidants are used, care should be taken that they will not be activated themselves and thereby form new sensitisers.

The possibility to reduce the sensitisation potency by preventing air oxidation is important also for a direct acting hapten or prohaptens, if a further activation by air oxidation to more allergenic compounds has been shown.

In the case of prohaptens, the possibility to become activated is inherent to the molecule and activation cannot be avoided by extrinsic measures. Activation processes increase the risk for cross-reactivity between fragrance substances. Cross-reactivity has been shown for certain alcohols and their corresponding aldehydes, i.e. between geraniol and geraniol (citral) and between cinnamyl alcohol and cinnamal.

Cross-reactivity is also expected between ester derivatives and their parent alcohols, as the esters will be hydrolysed by esterases in the skin. Esters of important contact allergens that can be activated by hydrolysis in the skin are isoeugenyl acetate, eugenyl acetate and geranyl acetate which all are known to be used as fragrance ingredients.

The substances presented above are based on current knowledge and should be seen as indicative and illustrative of the general problem. As substances with structural alerts for acting as pro- and or prehaptens are quite common among the fragrance substances



listed (see Tables 9-1 and 9-2), the possibility for activation to generate new potent allergens should be considered.

The SCCS is of the opinion that substances known to be transformed to known contact allergens should be treated as equivalent to these contact allergens, i.e the same restrictions and other regulatory requirements should apply.

**List of abbreviations**

ACD	Allergic contact dermatitis
alc.	Alcohol (as vehicle)
CI	Confidence interval
CLP	Classification, labelling and packaging
coloph.	Colophonium
DCs	Dendritic cells
EC	European Commission
ESSCA	European Surveillance System on Contact Allergies
EDT	Eau de toilette
EDP	Eau de perfume
EU	European Union
FM	Fragrance mix
GC	Gas chromatography
GPMT	Guinea pig maximisation test
HICC	Hydroxyisohexyl 3-cyclohexene carboxaldehyde
HRIPT	Human repeat insult patch test
IFRA	International Fragrance Association ( <a href="http://www.ifraorg.org">www.ifraorg.org</a> )
IVDK	Information Network of Departments of Dermatology ( <a href="http://www.ivdk.gwdg.de">www.ivdk.gwdg.de</a> )
INCI	International Nomenclature on Cosmetic Ingredients
LCs	Langerhans cells
LLNA	Local lymph node assay
MPR	<i>Myroxylon pereirae</i> resin
NACDG	North American Contact Dermatitis Group
OECD	Organization of Economic Co-operation and Development
pet.	Petrolatum (as vehicle)
ppm	parts per million (1000 ppm = 1%)
PPV	Positive predictive value
PR	Prevalence ratio
PT(ed)(ing)	Patch test(ed) (ing)
QMM	Quantitative mechanistic model
QRA	Quantitative risk assessment
(Q)SAR	(Quantitative) structure activity relationship
REACH	Registration, Evaluation, Authorisation and restriction of CHemicals
RIFM	Research Institute for Fragrance Materials ( <a href="http://www.rifm.org/">www.rifm.org/</a> )
ROAT	Repeated open application test

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SC	Single constituents (of one of the fragrance mixes)
SCCS	Scientific Committee on Consumer Safety
SCCNFP	Scientific Committee on Cosmetic Products and Non-Food Products
SCCP	Scientific Committee on Consumer Products
UK	United Kingdom
US(A)	United States (of America)
UV	Ultraviolet

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