Fragrance Allergens, Overview with a Focus on Recent Developments and Understanding of Abiotic and Biotic Activation

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Abstract: Fragrances and fragranced formulated products are ubiquitous in society. Contact allergies to fragrance chemicals are among the most common findings when patch-testing patients with suspected allergic contact dermatitis, as well as in studies of contact allergy in the general population. The routine test materials for diagnosing fragrance allergy consist mainly of established mixes of fragrance compounds and natural extracts. The situation is more complex as several fragrance compounds have been shown to be transformed by activation inside or outside the skin via abiotic and/or biotic activation, thus increasing the risk of sensitization. For these fragrance chemicals, the parent compound is often non-allergenic or a very weak allergen, but potent sensitizers will be formed which can cause contact allergy. This review shows a series of fragrance chemicals with well-documented abiotic and/or biotic activation that are indicative and illustrative examples of the general problem. Other important aspects include new technologies such as ethosomes which may enhance both sensitization and elicitation, the effect on sensitization by the mixtures of fragrances found in commercial products and the effect of antioxidants. A contact allergy to fragrances may severely affect quality of life and many patients have multiple allergies which further impact their situation. Further experimental and clinical research is needed to increase the safety for the consumer.

Keywords: contact allergy; fragrance; abiotic and biotic activation; autoxidation; fragrance allergens; prohaptens; patch test

1. Introduction

Contact allergies to fragrances are among the most frequent findings when patch-testing patients with suspected allergic contact dermatitis, as well as in studies of contact allergy in the general population. As fragrances are ubiquitous in everyday products, such as perfumes, fragranced cosmetics and hygiene products, contact allergy can pose a serious impairment in daily life and can also constitute a significant worsening factor in clinical dermatitis.

Traditionally, the routine test materials for diagnosing fragrance allergy have consisted of two established mixes of fragrance chemicals and natural blends, sometimes supplemented with additional allergens. The situation has, however, been revealed to be more complex as several fragrance substances have been shown to form reactive compounds via abiotic (chemical and physical factors) and/or biotic activation inside or outside the skin. For such fragrance chemicals, the parent compounds are often non-allergenic or very weak sensitizers, but since potent sensitizers can be formed, the risk of sensitization increases. Standardized patch test materials for two oxidized fragrance materials, oxidized linalool (lavender scent) (Hydroperoxides of linalool®, Chemotechnique Diagnostics, Vellinge, Sweden)

and oxidized limonene (citrus scent) (Hydroperoxides of limonene®, Chemotechnique Diagnostics), have recently been made commercially available, and have, in some test centers, been incorporated into routine patch testing.

2. Fragrance Markers in Baseline Series

In most cases, patients with dermatitis who are patch tested for suspected contact allergy will be tested with a baseline patch test series which may vary to some extent by region or country. Most baseline series contain the standardized patch test materials Fragrance Mix I (FM I), Fragrance Mix II (FM II), Myroxylon Pereirae (MP), and sometimes separately also hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) for the detection of fragrance contact allergy. Colophonium, obtained from the oleoresin of pine trees or as a by-product in the pulp industry, is also presented with the fragrance markers in some studies.

The FM I consists of seven defined fragrance chemicals (cinnamyl alcohol, cinnamal, hydroxycitronellal, amyl cinnamal, geraniol, eugenol, isoeugenol) and one natural blend (*Evernia prunastri* (oakmoss absolute)). The constituents of FM I are widely used in perfumed products. The frequency of positive reactions to FM I varies between 5% and 14% in patch-tested dermatitis patients [1–3]. In a retrospective British study, 4.8% of 500 patch-tested children and adolescents 0–16 years old had allergic reactions to FM I [4]. This is in keeping with a European multicenter study where 4.7% of children showed a contact allergy to FM I [5]. Recent population estimates in Europe suggest that about 1.8%–2.6% of the general population are sensitized to one or more of the components of the FM I [6,7]. Among almost 2300 adolescents in a Swedish population birth cohort who were patch tested at 16 years old, 2.1% had positive patch test reactions to FM I [8]. Analyses of the frequencies of contact allergy to the individual components of the FM I over two decades show varying trends [2], while positive reactions were overall most frequently recorded in response to *Evernia prunastri*, isoeugenol, cinnamyl alcohol and eugenol.

FM II, a mix consisting of hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC), hexyl cinnamal, farnesol, coumarin, citronellol and citral, has been widely used for patch testing since 2005. The FM II has shown positive patch test reactions in 2%–8% of tested dermatitis patients [1]. Data from 1990 to 2011 with analyses of positive reactions to the individual components of the FM II show fluctuating trends analogous to the components of FM I [2,9], with HICC, farnesol and citral giving the most positive reactions among the tested components. One of the components in FM II, HICC, has routinely been tested separately in addition, giving 1%–3% positive patch test reactions in dermatitis patients [1,9,10]. In a recent population estimate in Europe, 1.9% of the general population was sensitized to ingredients of the FM II [6,7].

MP is a natural blend which itself is not permitted to be added to cosmetic products in the European Union (EU), but can be used as an extract or distillate [11]. It originates from a naturally found resinous material, and contains a mix of constituents that are generally related to fragrance substances from cinnamon, vanilla, and clove, among other materials. MP in the baseline patch test series is mostly regarded as a general screening agent for contact allergy to fragrances. The frequency of positive reactions varied between 3% and 13% in patch-tested dermatitis patients in a recent European multicenter overview [1]. In population studies, 0.7% of the adult population [7] and 0.35% of the adolescents in the birth cohort [8] showed positive patch test reactions to MP.

Colophonium has been included in the large European multicenter studies, giving 1%–6% positive reactions at the different test centers [6,7]. Although concomitant reactions to colophonium often are found in patients reacting to fragrance markers, it is important to remember that colophonium has a large usage in many products not related to fragrances [12].

Calculations are frequently presented for the overall rate of positive patch test reactions to one or more fragrance markers, as an estimate for fragrance allergy in the individual patient. In the British retrospective study of children aged 16 or below, 5.2% of the tested children reacted to at least one of FM I, FM II, HICC or MP [4]. On a similar note, of over 10,000 dermatitis patients patch tested for

suspected contact dermatitis over a 15-year period in Loewen, Belgium, 14.5% reacted positively to at least one of FM I, FM II, HICC or MP [13].

The focus in some clinical studies has been on the rates of allergy to the fragrances listed in the European Union (EU) Cosmetics Directive, *i.e.*, the 26 specific fragrance ingredients which must be declared on the ingredient lists of cosmetic products sold within the EU if the concentration of the fragrance exceeds 0.001% in leave-on products or 0.01% in rinse-off products [14–18]. These studies have all reported positive reactions not covered by the baseline fragrance markers. The list of fragrance allergens of which the consumer needs to be made aware was again reviewed by the Scientific Committee on Consumer Safety of the European Commission (SCCS). Based on this work, 54 individual chemicals and 28 natural extracts (essential oils), including the 26 substances previously identified as contact allergens, can be categorized as established contact allergens in humans [3]. A recent proposal for an amendment to the Cosmetics Regulation suggests that atranol and chloroatranol (components of oak moss and tree moss extracts) as well as HICC should be withdrawn from cosmetics with the ultimate goal that these fragrance materials should not be present in such products [19].

3. Activation of Fragrance Chemicals to Strong Sensitizers

Many of the most common fragrances used today belong to the chemical family of terpenes. Terpenes constitute a large and structurally diverse group of natural or industrially produced chemicals which share the common structural unit isoprene (C_5H_8). It has been repeatedly and consistently shown that many fragrance chemicals with low skin-sensitizing potencies can be activated via chemical reactions into compounds with high sensitizing potencies. The activation can either take place as abiotic activation by chemical and physical factors such as air oxidation, or by biotic activation through enzyme activity.
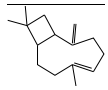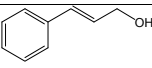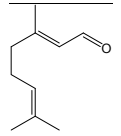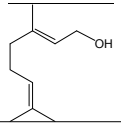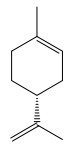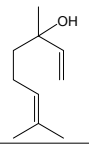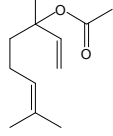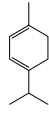
Compounds that can be activated to skin sensitizers by enzymatic activity in the skin are called prohaptens. The cytochrome P450 enzymes, catalyzing the oxidation of xenobiotica in the skin, have been the focus of skin metabolism studies. An array of cytochrome P450 enzymes are identified in human skin [20–22]. Other enzymes detected in human skin include the flavin-containing monooxygenases [23,24]. In the skin, expression of the studied enzymes is weaker than in the liver. However, considering that the skin is the largest organ in the body, its metabolic capacity cannot be overlooked. Patterns of metabolic transformation as well as structural alerts and Structure Activity Relationships (SARs) are discussed in review articles [25,26].

A prehapten is a chemical that in itself is non- or low-sensitizing, but which can be transformed into a hapten outside the skin, mainly by air oxidation. Prehaptens are activated to skin-sensitizing compounds via the spontaneous oxidation reaction known as autoxidation. This is a chain reaction involving radicals, which makes the reaction difficult to interrupt once it has started. In the autoxidation process of terpenes, hydroperoxides will be formed as primary oxidation products. The most stable hydroperoxides are stable enough to be detected and quantified and have been shown to be highly sensitizing in experimental studies [25]. In the autoxidation mixture, secondary oxidation products are also found, among which conjugated aldehydes and allylic epoxides are important sensitizers [25].

Experimental studies of the effect of autoxidation on the sensitization potential have been performed for a number of fragrance compounds (Table 1). In these experiments, fragrance chemicals have been exposed to air, and the oxidation processes have been followed over time. After autoxidation, investigations of sensitizing potencies of the oxidation mixtures containing the parent compound and its different oxidation products, as well as of individual oxidation products, have been performed using the murine local lymph node assay (LLNA), which up to now has been the dominating method for the determination of sensitization potency [27]. In the LLNA, an EC3 value (eliciting concentration to give a stimulation index of 3) is calculated and the lower the EC3 value, the more sensitizing the test material [28]. The sensitization potencies obtained for the air-exposed fragrance chemicals are five to 10 times lower compared to those of the corresponding pure fragrance chemicals (Table 1). Thus, much

stronger sensitization potencies are observed after air exposure for all the tested fragrance chemicals. Patterns of autoxidation as well as structural alerts and SARs are discussed in review articles [25,26].

Table 1. Local lymph node assay (LLNA) data on a studied fragrance compound and one essential oil before and after air exposure, comparing the sensitization potency of the pure (or not oxidized) compound with the potency of the oxidized fragrance compound.

Substance (INCI Name)	Chemical Structure	CAS No.	EC3 Value (% *w/v*) [a]			References
			Pure [b]	Oxidized [b]	Oxidation Time	
β-Caryophyllene		87-44-5	NT [c]	26.2	10 weeks	[29]
Cinnamyl alcohol		104-54-1	20.1 [d]	4.9	2 weeks	[30,31]
Geranial		141-27-5	6.8	1.3	5 weeks	[32,33]
Geraniol		106-24-1	22.4	4.4 / 5.8	10 weeks / 45 weeks	[33]
D-Limonene [e]		5989-27-5	30	3.0	10 weeks	[34]
Linalool		78-70-6	46.2	9.4 / 4.8	10 weeks / 45 weeks	[35,36]
Linalyl acetate		115-95-7	25	3.6	10 weeks	[37]
α-Terpinene		99-86-5	8.9	0.94	3 weeks	[38,39]
Lavender oil	Main components: Linalool, linalyl acetate, caryophyllene	8000-28-0	36 (Not oxidized)	11 / 4.4	10 weeks / 45 weeks	[40]

[a] Vehicle: acetone:olive oil (4:1); [b] Categories of purity or oxidation state: Pure: Purified before testing as most commercially available fragrance substances are not pure; Oxidized: oxidized by air exposure during *x* weeks; Not oxidized: Not purified but used as it was delivered as this is a complex mixture and not a specific substance; [c] NT = not tested in the LLNA. Pure β-caryophyllene was tested according to Freund's complete adjuvant test (FCAT) in guinea pigs. No reactions were obtained [29]; [d] As such according to literature; [e] D-Limonene according to the INCI name. It corresponds to d-limonene or *R*-limonene in the original papers.

As skin metabolism and autoxidation both involve oxidative reactions, it is not uncommon that these activation routes can result in the formation of identical compounds, for example aldehydes and epoxides. In experimental studies, some fragrance chemicals have been shown to be prone to both autoxidation and metabolic transformation, making the compound both a prehapten and a

prohapten. A well-studied example of this is geraniol (Table 1 and Figure 1). A highly sensitizing stable hydroperoxide of geraniol has been identified after autoxidation. However, the main products in common after autoxidation and bioactivation of geraniol are the sensitizing isomeric aldehydes geranial and neral, which constitute the fragrance chemical citral [33,41]. Support for a link between geraniol and citral was first described clinically in a German multicenter study where a high proportion of concomitant reactions for geraniol and citral was noticed [42]. Swedish clinical studies comparing autoxidized and pure geraniol show that the oxidized form of geraniol is more important as a cause of contact allergy than the pure compound (2.3% and 0.46% positive reactions, respectively, Table 2) [43]. Clinical studies with oxidized geraniol, pure geraniol and citral added to the baseline screening protocol, demonstrated a stronger correlation between positive patch test reactions to oxidized geraniol and citral than between reactions to pure geraniol and citral, supporting that the oxidation is more important than the metabolic transformation [44].

Geranial, the aldehyde formed from geraniol, has been shown to be susceptible to further oxidation to form sensitizing epoxides via either skin metabolism or autoxidation, as well as to form a sensitizing geranial hydroperoxide by autoxidation [32,41]. This illustrates that the oxidation products themselves may in turn oxidize, thus forming additional sensitizers, which complicates the picture even further (Figure 1).
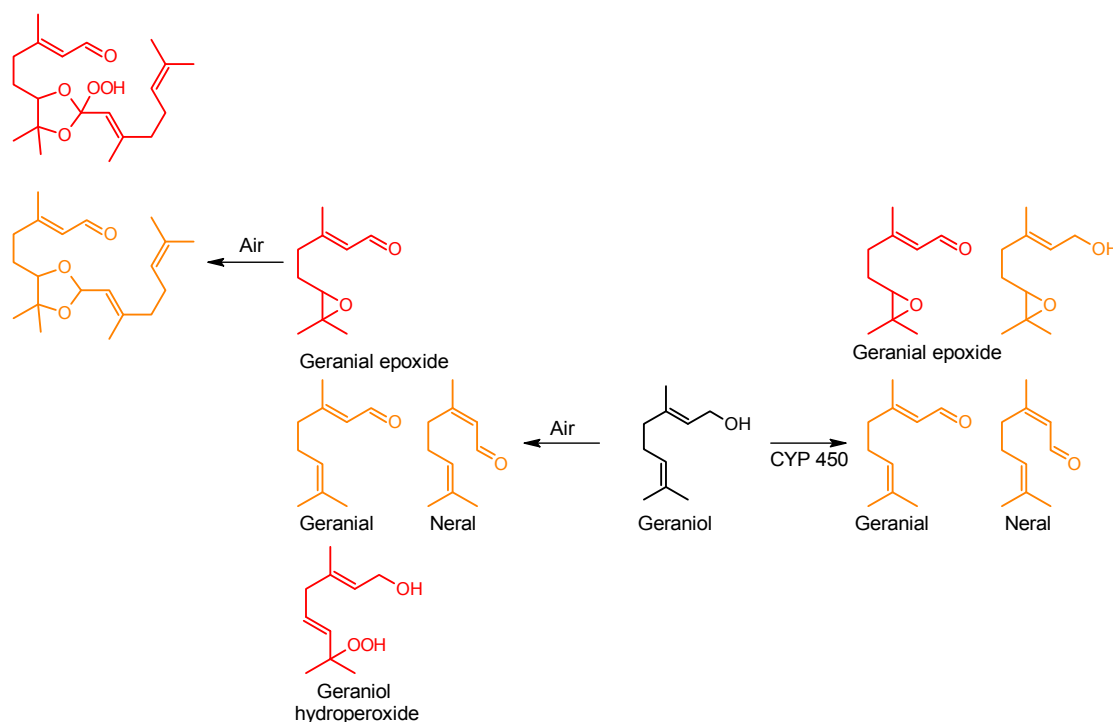
Figure 1. Biotic and abiotic activation of geraniol. Many of the same products are detected in both pathways, such as geranial and neral [33,41]. Also, the oxidation can continue in several steps, where new sensitizers are detected, as for geranial where a sensitizing dimeric dioxolane has been identified [32]. Hydroperoxides can be formed from several of the compounds involved, not only geraniol itself. Moderate sensitizers according to the LLNA are depicted in orange and strong sensitizers in red.

Another example of a terpene that has been shown to be activated to sensitizers through both biotic activation and autoxidation is α-terpinene (Table 1) [38,39]. Strongly sensitizing epoxides were detected as main products in both activation pathways. No clinical studies of contact allergy to oxidized α-terpinene are published.

Table 2. Allergic contact reactions in clinical patch test studies to pure or unintentionally oxidized fragrance preparations, as well as to oxidized patch test materials. In many cases, for the non-oxidized patch test materials, the purity or purification is not given but is postulated to be pure by the investigators. For more detailed information, see the original references. Note that for some fragrances, one can compare the outcome of concomitant patch testing with pure and oxidized fragrance preparations in the same group of patients, while other comparisons come from separate studies with different study populations and with different test concentrations. The studies performed on the same study population are shown by a darker background color.

Substance	Pure or Not Intentionally Oxidized				Oxidized, See Details in References Given			
	Test Conc. (% w/w) [a]	*n* Positive	*n* Tested (%)	Reference	*Test Conc. (% w/w)* [a]	*n* Positive	*n* Tested (%)	Reference
Geraniol	*2*	3/2227	0.13%	[45]	*2*	12/2179	0.55%	[45]
	4	1/655	0.15%		*4*	6/655	0.92%	
	6	3/649	0.46%	[43]	*6*	15/655	2.3%	[43]
	11	7/655	1.1%		*11*	30/653	4.6%	
D-Limonene [b]	*2*	0/1200	–	[46]	*3*	63/2273	2.8%	[47]
					3	49/1812	2.3%	
L-Limonene [c]	*2*	11/1241	0.88%	[49]	*3*	36/1812	2.0%	[48]
D- and/or L-Limonene	*2*	0/320	–	[18]	*3*	63/2411	2.6%	
D-Limonene	*2*	3/2396	0.1%	[42]	*3 (0.3% hydroperoxides)*	152/2900	5.2%	[50]
	10	11/4731	0.2%	[51]	*3 (0.3% hydroperoxides)*	237/4731	5.0%	[51]
Lavender oil	–	–	–	–	*6*	47/1693	2.8%	[52]
Linalool	*20*	3/1825	0.2%	[53]	*2*	14/1693	0.83%	
	10	2/320	0.6%	[18]	*4*	67/2075	3.2%	[54]
	10	4/792	0.5%	[55]	*6*	91/1725	5.3%	
	5 and 1	0/100	–	[56]	*11*	72/1004	7.2%	
	10	7/2401	0.3%	[42]	*3*	11/483	2.3%	[57]
	10	2/985	0.2%	[49]	*6 (1% hydroperoxides)*	200/2900	6.9%	[58]
	10	12/4731	0.3%	[51]	*6 (1% hydroperoxides)*	281/4731	5.9%	[51]
	30	0/179	–	[59]	*2*	20/1511	1.3%	
Caryophyllene	*5*	10/1606	0.6%	[61]	*3.9*	2/1511	0.1%	[60]
Myrcene	–	–	–	–	*3*	1/1511	0.1%	
Linalyl acetate	*1, 5*	0/100	–	[56]	*6*	37/1717	2.2%	[62]
	10	4/1855	0.2%	[63]				

[a] Vehicle: petrolatum; [b] D-Limonene is according to the INCI name. It corresponds to d-limonene or *R*-limonene in the original papers; [c] L-Limonene is according to the INCI name. It corresponds to l-limonene or *S*-limonene in the original papers.

Linalool and its corresponding ester, linalyl acetate, seldom cause positive patch test reactions as pure compounds [14,42,46], but they autoxidize upon contact with air, forming a wide range of oxidation products of which the hydroperoxides are the main sensitizers [35,36]. Linalool is also susceptible to biotic oxidation, and some low-sensitizing products have been identified via both pathways [64]. Autoxidized linalool has been studied clinically in several multicenter patch test studies (Table 2). In an early study, four concentrations (2%–11% in petrolatum) gave positive patch test reactions in 1%–7% of the tested patients, respectively [54]. A patch test concentration of oxidized linalool 6.0% in petrolatum (pet.) has been suggested, and two recent multicenter patch test studies gave 6% and 7% positive patch test reactions, respectively, among consecutive dermatitis patients [51,58]. At some test centers in both multicenter studies, high frequencies of doubtful or irritant reactions have been recorded to oxidized linalool, and interpretation of reactions has been difficult in these centers [51,58]. Linalyl acetate has not been studied as extensively in the clinic. However, the number of positive reactions to oxidized linalyl acetate is significantly higher compared to that of pure linalyl acetate when both are simultaneously tested in consecutive dermatitis patients (Table 2). The clinical study indicated that contact allergy to oxidized linalyl acetate is common [62].

Studies of air oxidation of the essential oil of lavender showed that air exposure of lavender oil increases its sensitization potential. Linalool, linalyl acetate and β-caryophyllene are the main components of lavender oil, and these terpenes autoxidized on air exposure in the same way in the natural essential oil as when the terpenes were air oxidized separately, and the same oxidation products were detected [40]. Although patch testing with the oxidized main components detected a number of patients allergic to the oxidized lavender oil, many reactions to the oxidized lavender oil were only detected using the oxidized essential oil (Table 2) [52]. This also indicates that other sensitizers are present in the oil and/or the uptake of the sensitizers is increased, as discussed below. The lesser component in lavender oil, the sesquiterpene β-caryophyllene, has been found to oxidize readily but with a low sensitizing potency after autoxidation [29], and oxidized β-caryophyllene gave comparably few positive patch test reactions in clinical studies (Table 2) [60].

D-Limonene has been shown to autoxidize upon contact with air, forming strongly sensitizing hydroperoxides and a moderately sensitizing aldehyde, carvone, as the main oxidation products [65–68]. Pure (non-oxidized) D-limonene seldom causes positive patch test reactions in dermatitis patients (Table 2) [14,42,46]. Clinical and experimental studies have shown that the individual limonene hydroperoxides (Limonene-1-hydroperoxide, Limonene-2-hydroperoxide) are important sensitizers and cause highly specific reactions in patients [34,69–71]. Oxidized D-limonene, as an oxidation mixture, tested at a concentration of 3.0% pet., has caused positive patch test reactions at a frequency of 2%–5% in consecutive dermatitis patients, and two recent international multicenter patch test studies using oxidized D-limonene 3.0% with limonene hydroperoxides at 0.33% (the commercial name is Hydroperoxides of limonene 0.3%®) gave 5% positive patch test reactions overall (Table 2) [50,51]. As for oxidized linalool, high frequencies of doubtful or irritant reactions have been recorded to oxidized D-limonene at some test centers in both multicenter studies, and interpretation of the reactions was considered difficult in these centers [50,51].

Eugenol and isoeugenol are present in FM I. Isoeugenol is the more potent sensitizer according to clinical experience [72,73] as well as experimental investigations [74]. According to SAR analyses, neither eugenol nor isoeugenol can act as a sensitizer without activation and studies show that both chemicals can act as prohaptens by metabolical demethylation followed by oxidation, while isoeugenol also can act as a prehapten via direct oxidation [75]. Investigations using a peroxidase/hydrogen peroxide activation system in the direct peptide reactivity assay (DPRA) showed that only hydrogen peroxide was necessary to activate isoeugenol, leading to reactions with the peptide. Also for eugenol, the enzymatic activity from peroxidase had to be included to get activation [76]. However, more studies are important to further elucidate the mechanisms.

One of the traditionally most well-known prohapten-hapten pairs is cinnamyl alcohol and cinnamal. Many patients show concomitant reactions to both compounds, which has been attributed

to oxidative metabolism of cinnamyl alcohol to cinnamal in the skin [77,78]. However, cinnamyl alcohol is also readily susceptible to autoxidation, forming the sensitizers cinnamal and cinnamic epoxyalcohol [30]. Cinnamyl alcohol and cinnamal are thus closely linked via both autoxidation and metabolism.

Overall, the frequencies of contact allergy to the oxidized fragrance compounds that have been studied so far are high and in most cases range between 2% and 7% of the tested patients (Table 2). These frequencies are in the same range as the rate of reactions observed for the established fragrance mixes (FM I, FM II) in the baseline series [1]. The majority of the contact allergic reactions to the discussed oxidized fragrance compounds are not detected in testing with the fragrance mixes, and *vice versa*. So far, only a limited number of compounds capable of oxidation have been experimentally and clinically investigated. However, structural alerts which can identify compounds that are prone to autoxidization were recognized in many of the frequently used fragrance substances screened by the Scientific Committee on Consumer Safety (SCCS) on fragrance allergens in cosmetic products [3].

4. Oxidation and Detection

Scented products frequently contain complex blends of various fragrance chemicals which are added to obtain the desired aroma. It can be expected that the resulting fragrance mixtures are even more complex after storage and handling if oxidation has occurred. The products may contain not only the specific low-molecular oxidation products that we expect when we discuss sensitizing oxidation products, but also a wide range of complex oxidation products obtained in interactions between compounds in the mixtures. Such compounds can be more or less sensitizing depending on chemical reactivity and skin penetration. It is obvious that large complexes formed at the end of the oxidation chain are of less interest since they do not penetrate the skin as easily. One illustrative example of this is the complex oxidation pattern of geraniol, shown in Figure 1 and described above.

When dealing with complex mixtures in formulated products, the separation and identification of individual components cannot yet be performed in a reliable and reproducible way. In general, identification and quantification of labile hydroperoxides in complex products is not yet possible for technical reasons. In the last years, methods for the detection of limonene, linalool and linalyl acetate hydroperoxides in essential oils and hydroalcohols have been developed [79–81]. In order to detect limonene-2-hydroperoxide, a gas chromatography/mass spectrometry (GC/MS) method with selective reduction of the hydroperoxide to carveol is described by the fragrance industry [82]. No evidence for significant concentrations of limonene-2-hydroperoxide in aged fine fragrances was found. Studies with regard to linalool hydroperoxides using liquid chromatography/tandem mass spectrometry (LC/MS/MS) without derivatization revealed low concentrations of linalool hydroperoxides in aged fine fragrances [79]. This study also showed linalool hydroperoxides in linalool of natural origin [79].

Hydroperoxides from natural limonene, linalyl actate, and linalool have been demonstrated in essential oils using LC/MS/MS. Limonene-2-hydroperoxide was detected in natural sweet orange oil and linalyl acetate hydroperoxides were identified in petigrain oil already at delivery from the producer [80,83]. After storage for one year in the refrigerator, the concentrations of hydroperoxides had increased in both oils, and in petitgrain oil, limonene hydroperoxides were also now detected. However, the quality of the oils still complied with the chromatographic profiles of the parent compounds in the natural oils in accordance with International Organization for Standardization (ISO) standards [83]. The example illustrates that it is important to develop new analytical ISO standard methods for the quality control of essential oils, which include the detection of hydroperoxides in the oils.

Taken together, oxidation products can be found in natural oils and in synthetically produced fragrance materials even before they are added to formulated products. Further work is needed to develop methods for quantification of hydroperoxides from other fragrance chemicals prone to autoxidation, and also to determine if the individual hydroperoxides are present in scented formulated

products such as creams and shampoos. This is a task within an ongoing joint project between academia, dermatologists and the fragrance industry [84].

To prevent air oxidation, antioxidants are often added. In a study using butylated hydroxytoluene (BHT) as an antioxidant, it was shown that the autoxidation was temporarily slowed down by addition of BHT, but when the antioxidant was consumed, the autoxidation continued at a high rate [85]. The study further demonstrated that the autoxidation rate depends not only on the compound itself, but also on its purity at the start of oxidation. An increased use of antioxidants due to awareness of the problem of autoxidation might cause problems with sensitization from antioxidants. BHT is one of the most used antioxidants and is considered safe with regard to sensitization, but with increased concentrations and high overall usage, a risk of sensitization should be considered. Furthermore, antioxidants might be activated to skin-sensitizing derivatives if they exert their function by being oxidized themselves, instead of oxidizing the compound that they protect. The compounds α-terpinene and its analogue γ-terpinene have been discussed as preserving agents for food, cosmetics and medicines [86–88]. As described above, α-terpinene is readily autoxidized and will thus form new sensitizing compounds (Table 1) [39]. Thus, the prevention of autoxidation using antioxidants should not be regarded as a simple solution for the problem of autoxidation but needs thorough investigation in the individual cases.

5. Exposure

Exposure to fragrances is widespread and a wide range of fragrances are found in products intended to be used in close contact with the skin. Up to 15%–30% of the total volume of perfumes can consist of fragrance materials, while liquid detergents and toilet soaps may contain fragrance materials in 0.1%–2% of the volume [89]. According to several studies, linalool, D-limonene, geraniol, citronellol, hexyl cinnamal and HICC are presently among the most common fragrance ingredients used in consumer products [90–94]. In a German survey, limonene and linalool were the most commonly identified fragrances, in addition to being the most frequent coupled (tandem) exposures to fragrance chemicals [95]. Calculations of estimated consumer exposure for fragrance chemicals showed that the maximum daily exposure to linalool exceeded other discussed fragrance chemicals for high-end users of cosmetic products, based on concentrations in 10 types of cosmetic products and on the mode of usage for the product [96]. A recent study has calculated an estimated risk of sensitization, the sensitization-exposure quotient (SEQ), based on the relative frequency of sensitization and the relative frequency of use/labeling of the individual fragrances studied. In this study, the SEQs indicated that oak moss, tree moss, farnesol, and isoeugenol gave high risks of sensitization [97].

Clinical relevance is a difficult assessment and it is often problematic to identify the culprit product containing a certain fragrance, and conversely, identifying the culprit fragrance in a complex product. This is especially true for ubiquitous chemicals present in many products at low concentrations. In addition, exposure to oxidation products is challenging to prove analytically, due to technical difficulties in identifying and quantifying hydroperoxides in formulated products, as discussed above. In spite of this, valuable case reports and reviews have been published in which important exposures to individual fragrances and scented formulated products are identified in allergic patients [18,45,51,58,98–102]. Besides assessing the clinical relevance by reading ingredient labels, patch tests using patients' products as well as use tests with suspected products may provide useful information, and should not be overlooked.

In a Danish study on contact allergy to the 26 specific fragrance ingredients listed in the EU Cosmetics Directive, it was found that 7.6% of the patients showed a contact allergy to at least one fragrance material. The majority (75.7%) of positive reactions to the 26 fragrances were judged to be of clinical relevance for the patients' dermatitis, based on identification of the culprit fragrance in the labeling of products used by the patient on dermatitis areas [16]. In a large Belgian study, geraniol, HICC and D-limonene were frequently found labeled on products brought in by patients who had been diagnosed with a contact allergy to the respective fragrance [102].

Clinical studies on oxidized fragrance terpenes show that contact allergy to oxidation products is common. Due to the lack of reliable chemical analytical methods for the detection of hydroperoxides in formulated products, assessments of exposure are based on exposure to the parent compounds in consumer products used on dermatitis areas. For oxidized D-limonene, 35%–40%, with some test centers reporting up to 70%, of the D-limonene–allergic patients had an exposure to limonene assessed as relevant for the dermatitis [51,98]. Similar figures have been found in patients reacting to oxidized linalool in multicenter settings [51,58].

To investigate if concentrations expected in common products can induce allergic contact dermatitis in allergic individuals, use tests and repeated open application tests (ROATs) have been performed for several fragrance materials, *i.e.*, HICC, eugenol, isoeugenol and oxidized linalool [103–111]. For HICC, investigations of a safe level, where sensitized subjects did not develop elicitation of their allergic eczema, showed that 90% of the allergic patients did not react to concentrations in the range of 0.009%–0.027% while 50% did react when the use concentrations were increased to 0.18%–0.34%, depending on the product type [103,104]. For HICC and isoeugenol, deodorants have been pointed out as a source of exposure, and use tests have shown that low concentrations caused eczema in allergic subjects with repeated use [10,108–110]. For oxidized linalool, a ROAT showed allergic reactions to a concentration corresponding to 0.056% (560 μg/g) linalool hydroperoxides [111]. This could be compared to a median concentration of 27 μg/g of linalool hydroperoxides detected in 40 fine fragrances recalled from the consumers in an investigation by the fragrance industry. Among these 40 perfumes, one product (corresponding to 2.5% of the tested fine fragrances) contained 132 μg/g linalool hydroperoxides [79].

Some important aspects of exposure need to be addressed. In recent years, formulations with ethosomes have been used to increase the uptake of active components in topical formulations. This can also increase the uptake of fragrance chemicals and other constituents in such products, as has been shown both experimentally and clinically [112–115]. In these studies, encapsulating contact allergens in ethosomes gave an increase in the challenge response compared to the same concentrations in an ethanol/water base without ethosomes. Another aspect is the enhancement of the sensitizing potency of mixtures of fragrance chemicals, compared to that of the corresponding single-fragrance chemicals. In murine experimental studies of cinnamal, isoeugenol and HICC, it was demonstrated that the mixtures of these non-cross-reacting compounds increased the potency both at sensitization and elicitation compared to when administered as isolated compounds [116]. The effect can be expected in fragrance mixtures in formulated products and it could further enhance the sensitizing effect from the constituents. A possible explanation for the enhanced effect is that a fragrance in a mixture may act as a penetration enhancer for other ingredients present [116]. This penetration enhancement effect has been shown for terpenes, and is used in the field of transdermal drug delivery [117,118]. This is also relevant for essential oils which are, in effect, complex mixtures of fragrance compounds.

It has also been demonstrated that reactivity to fragrances may be enhanced in combination with irritants, as shown for the allergen hydroxycitronellal in combination with the irritant sodium lauryl sulfate. This combination led to stronger reactions in allergic patients, indicating that reactivity to fragrances may be enhanced in common product types such as soaps and shampoos [119]. In aromatherapy and natural products, in home-made soaps and fragrances, as well as in massage oils, high exposure to fragrances as parts of essential oils may occur. Furthermore, it is important to also consider that part of the maturation of perfumes involves the formation of sweetly scented molecules such as aldehydes, which makes the oxidation a desirable event.

6. Multiple Reactions to Fragrances Are Common

Concomitant reactions between fragrance markers are described in many studies, and patients showing positive patch test reactions to a certain fragrance marker will in many cases also react to other fragrance markers [13,73,120]. Statistical associations for concomitant positive patch test reactions between fragrance markers have been demonstrated [13]. Around 40% of the patients reacting to

oxidized fragrance compounds have shown concomitant reactions to fragrance markers in the baseline series [48,50,54,58]. The general explanation for the commonly seen concomitant reactions between fragrance allergens is the frequent simultaneous exposure in everyday consumer products.

The high frequency of concomitant positive reactions to the fragrances in general and oxidation mixtures of linalool and D-limonene in particular has raised questions about the specificity of the reactions. Experimental studies on possible cross-reactivity [34,69] as well as clinical studies [34,69,71] using chemically related haptens have been performed, and all support that specific reactions are recorded. Even structurally closely-related haptens, such as limonene-1-hydroperoxide and limonene-2-hydroperoxide, have been shown to give specific reactions in clinical patch test studies [34,71]. Concomitant reactions between oxidized limonene and oxidized linalool have recently been studied and, although many allergic patients did react to both oxidation mixtures, 75% of the total number of patients reacting to any oxidation mixture were only positive to one of the tested oxidized patch test materials [51,121].

7. Quality of Life

As fragrance allergy is a lifelong condition which may cause permanent or recurrent contact dermatitis, it can affect the quality of life (QoL) of the allergic individuals. To investigate this, a fragrance quality of life instrument (FQL index) was recently developed in Denmark, and was shown to be a valuable and reliable tool [122]. Using this form, an impairment of QoL was identified among patients with fragrance allergy, especially among women of younger age and with multiple fragrance allergies. Recent diagnosis of the fragrance allergy as well as severe reactions at patch testing were further aggravating factors [123]. Thus, the effect on quality of life is an important aspect to consider.

8. Conclusions

The present review shows that contact allergy to fragrance chemicals still is an important problem, although efforts to reduce the exposure to known fragrance allergens have been made from both regulatory authorities and the fragrance industry. In the last 20 years it has become clear that many of our most common fragrance chemicals are activated both outside the skin by autoxidation and in the skin by metabolic activation, thereby forming new potent sensitizers. This review shows a series of fragrance chemicals with well-documented abiotic and/or biotic activation that are indicative and illustrative examples of the general problem. Due to their chemical properties, many fragrance compounds can be expected to autoxidize on air exposure. When fragrance chemicals with structural alerts for acting as prohaptens and/or prehaptens are identified, the possibility for activation to generate new potent sensitizers should be considered.

The majority of the contact allergic reactions to the autoxidized fragrance chemicals are not detected in testing with the established fragrance markers. Notably, the frequencies of positive reactions to the autoxidized fragrance chemicals tested so far are in the same range as the frequencies of reactions observed for routine test materials for diagnosing fragrance allergy. Thus, the actual frequency of contact allergy to fragrances is higher than earlier recognized. Contact allergy to fragrances may severely affect quality of life, and many patients have multiple allergies which further impact their situation.

Other important aspects include new technologies such as ethosomes which may enhance both sensitization and elicitation, as well as the effect on sensitization by the mixtures of fragrances found in commercial products. The effect of antioxidants needs to be investigated, as these added compounds may initially diminish oxidation but may also either oxidize themselves or influence oxidation pathways which may add new allergens to the picture.

Further experimental and clinical research is needed to increase the safety for the consumer. Investigations on the biotic mechanisms in skin should be performed with different enzyme systems and in various skin models. The studies should not only involve the area of abiotic and/or biotic activation of fragrance chemicals but should also include analytical development as well as

investigating factors such as volatility, photoactivation and skin penetration, which all impact the fate of the compounds applied on the skin.

Conflicts of Interest: Ann-Therese Karlberg worked with Chemotechnique Diagnostics to develop the patch test material for oxidized linalool and oxidized D-limonene. The other authors have no interests to declare.

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