

Significant exposures to isoeugenol derivatives in perfumes

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Background: Isoeugenol, an important fragrance allergen in consumers, has been restricted to 200 p.p.m. since 1998 according to guidelines issued by the fragrance industry. However, no decline in contact allergy to isoeugenol has been detected. It has been speculated that isoeugenol derivatives, especially isoeugenyl acetate, are used instead. Isoeugenyl acetate is probably metabolized in the skin to isoeugenol and gives positive patch test reactions in 1/3 of isoeugenol-sensitized individuals.

Objectives: To investigate the content of isoeugenol, isoeugenyl acetate, and two isoeugenol ethers in perfumes/aftershaves.

Materials and Methods: 29 international brand perfumes/aftershaves were analysed for the target fragrance ingredient by gas chromatography–mass spectrometry. All samples were analysed in duplicate at detection levels of 1–5 p.p.m.

Results: 16 products (55%) contained isoeugenol. The maximum concentration was 202 p.p.m. 10 products (34%) contained isoeugenyl acetate, which in 9 cases occurred together with isoeugenol. The concentrations of isoeugenyl acetate ranged from 20 to 4689 p.p.m. 13 products (44%) contained 64.9–1755.0 p.p.m. isoeugenyl methyl ether. Isoeugenyl benzyl ether was not detected in any of the investigated products.

Conclusions: Isoeugenyl acetate is present in perfumes/aftershaves, in some products in significant amounts. This may lead to elicitation of contact allergy in isoeugenol-sensitized individuals and may contribute to unchanged levels of isoeugenol sensitization.

Key words: allergy; fragrances; isoeugenol; isoeugenyl acetate; perfumes; risk; sensitization; skin metabolism. © Blackwell Munksgaard, 2008.

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Isoeugenol is used as a fragrance material in perfumes and perfumed products. It is a moderate to strong skin allergen and a frequent cause of contact allergy (1, 2). It is one of the eight substances in the fragrance mix I, which has been part of the European baseline series for more than 20 years (3) and it is also an important individual allergen.

From 1980, the International Fragrance Association (IFRA) recommended, in its Code and Standards, that isoeugenol be limited in finished cosmetic products to maximum 2000 p.p.m. (0.2%) due to its allergenic properties and from 1998 this limit was lowered to 200 p.p.m. (0.02%) (4). If this intervention had been effective, a decreased incidence of contact allergy to isoeugenol should be seen. However, a recent study from the

Department of Cutaneous Allergy, St. John's Institute of Dermatology, London showed the opposite. A statistically significant increasing trend was found in isoeugenol contact allergy over a 5-year period from 2001 to 2005 among 3636 tested patients (5). It was speculated that this could be due to substitution of isoeugenol with isoeugenol derivatives (5), some of which have been shown to give reactions in isoeugenol-sensitized individuals (6, 7), and for which no recommendations of limitation exist. It is, however, not known whether any of these isoeugenol substitutes are used at significant levels.

In the present study, we have investigated the contents of isoeugenol and 3 isoeugenol derivatives in 29 international brand perfumes/aftershaves.

Materials and Methods

Products

29 hydroalcoholic products of international brands were analysed in the present study: 2 aftershave lotions, 26 eau de toilette/eau de parfum (10 males and 16 females), and 1 parfum (F). The products were purchased from Danish retail outlets in the period April 2006 and April 2007.

Chemicals

Isoeugenol (CAS No. 97-54-1) and isoeugenyl benzyl ether (CAS No. 120-11-6, purity 98%) were purchased from Sigma-Aldrich, Denmark, and isoeugenol methyl ether (CAS No. 99-16-3) was purchased from TCI, Japan. Isoeugenyl acetate (CAS No. 93-29-8) was kindly provided by Firmenich, Switzerland. The purity of all chemicals was $\geq 98\%$.

Isoeugenyl benzoate was also considered, but it appeared that this substance is not used, and it is not available commercially. Furthermore, the obtained isoeugenyl phenylacetate (Sigma-Aldrich), CAS No. 120-24-1, appeared to be too impure (by gas chromatography analysis) to serve as an analytical standard and was hence excluded from the study.

Analysis

The analyses of isoeugenol and the 3 derivatives were performed by gas chromatography–mass spectrometry (8). The identification of the target fragrance compounds was performed in total scan mode (m/z 33–300), and quantification was performed in selective ion monitoring mode (Table 1). All samples were analysed in duplicate at detection levels of 1–5 p.p.m. The relative standard deviation of the method was below 10% for all target compounds.

Results

Among the 29 products analysed in the present investigation, 16 products (55%) contained 26.6–202.8 p.p.m. isoeugenol, 10 products (34%) con-

tained 20.1–4689.3 p.p.m. isoeugenyl acetate, and 13 products (44%) contained 64.9–1755.0 p.p.m. isoeugenyl methyl ether. Isoeugenyl benzyl ether was not detected in any of the investigated products.

Mean and median contents of isoeugenol and its derivatives in the investigated products are reported in Table 2. Neither isoeugenol nor its derivatives were present in 7/10 perfumes for men or in 4/16 women's perfumes. One of the two investigated aftershave lotions contained significant amounts of isoeugenol (202.8 p.p.m.), isoeugenyl acetate (4689.3 p.p.m.), and isoeugenyl methyl ether (222.5 p.p.m.), while another aftershave lotion contained only isoeugenyl methyl ether (1897.9 p.p.m.). 3 perfume products (2 males and 1 female) contained only isoeugenol. Isoeugenol together with isoeugenyl acetate or isoeugenyl methyl ether or with both these compounds was present in 13/29 products (44%). Isoeugenol together with isoeugenyl acetate was present in 9/29 (31%) investigated products, isoeugenyl methyl ether together with isoeugenol was present in 12/29 (41%) products, and all these three fragrance materials were present in 8/29 products (27%) (Table 3).

There was a significant relationship between the presence of isoeugenol and isoeugenyl acetate (odds ratio: 15, confidence interval: 1.6–149) and between isoeugenol and isoeugenyl methyl ether (odds ratio: 36, confidence interval: 3.4–371).

One of the investigated products (an aftershave lotion) contained only isoeugenyl methyl ether.

Discussion

In a small case series, it was previously found that some individuals allergic to isoeugenol could react to the isoeugenol derivative isoeugenyl acetate (6). In a later study, isoeugenol as well as its 5 derivatives were patch tested in equimolar concentration on 2261 consecutive eczema patients (7). Isoeugenol was positive in 1.7% and isoeugenyl acetate in 0.8% of patients. 1/3 of patients positive to isoeugenol had a concomitant positive reaction to isoeugenyl acetate (7). Isoeugenyl acetate is probably easily hydrolysed in the skin to produce isoeugenol and is a likely substitute for isoeugenol in perfumes and perfumed products as it has a similar smell (6). In the current investigation, isoeugenyl acetate was found in 34% of the products, in most cases together with isoeugenol (Table 3). Isoeugenol has been restricted to maximum 200 p.p.m. since 1998 according to the IFRA Code and Standards (4). This limit is under consideration for implementation in the Cosmetic Directive (9). In the

Table 1. Ions used for the quantification of target fragrance compounds in selective ion monitoring mode by gas chromatography–mass spectrometry

Fragrance compound	Qualifier ions (m/z)	Quantifier ion (m/z)
Isoeugenol	103, 131, 164	164
Isoeugenol acetate	107, 163, 178	178
Isoeugenol methyl ether	131, 164, 206	164
Isoeugenol benzyl ether	107, 163, 254	163

Table 2. Mean and median content of isoeugenol and its derivatives in the 29 perfume products investigated

Fragrance compound	Products containing target fragrance <i>n</i> (%)	Content (p.p.m.)			
		Range	Mean	Standard deviation	Median
Isoeugenol	16 (55)	26.6–202.8	70.5	54.2	45.2
Isoeugenyl acetate	10 (34)	20.1–4689.3	984.9	1569.5	166.0
Isoeugenyl methyl ether	13 (45)	64.9–1755.0	359.7	442.3	222.5

current investigation no product exceeded this limit. However, it seems that in some products isoeugenyl acetate is used to compensate for the restricted use of isoeugenol e.g. 1 aftershave contained 202 p.p.m. isoeugenol together with 4689 p.p.m. isoeugenyl acetate (Table 3). This is likely to pose a problem for those sensitized to isoeugenol and also contribute to the sensitizing potential of the products. It also provides a plausible explanation for the unchanged or even increased incidence of contact allergy to isoeugenol, which recently has been reported (5). It could be interesting to see the levels of isoeugenyl phenylacetate, which in a previous investigation gave concomitant positive reactions in 37.5% of individuals sensitized to isoeugenol; unfortunately, a pure form of this

substance could not be obtained for the current investigation.

The contents of 2 isoeugenyl ethers, isoeugenyl methyl ether and isoeugenyl benzyl ether, were examined. Isoeugenyl benzyl ether was not found in any product. This apparent rare use could explain that only 2 positive reactions were found in testing of 2261 patients (7). Isoeugenyl methyl ether is less likely to produce isoeugenol than isoeugenyl acetate, as it requires P450 oxidation to be cleaved (6). Isoeugenyl methyl ether occurs in small quantities in several essential oils (10), and it was present in 44% of the investigated products, but does not seem to cause problems in isoeugenol-sensitized individuals (7).

It may be difficult to predict which substance can be used as substitutes by the perfumer (11), but

Table 3. Content of isoeugenol and its derivatives in the investigated products

Sample No.	Product	Content in p.p.m. (µg/ml)			
		Isoeugenol	Isoeugenyl acetate	Isoeugenyl methyl ether	Isoeugenyl benzyl ether
1	EdP W	132.5	76.5	501.9	–
2	EdT W	28.0	–	–	–
3	Aftershave	202.8	4689.3	222.5	–
4	EdT W	88.1	–	516.3	–
5	EdP W	96.7	75.4	1755.0	–
6	EdT M	27.2	–	–	–
7	EdT M	26.6	–	–	–
8	EdP W	87.3	315.7	252.3	–
9	EdP W	60.8	2560.5	–	–
10	EdP W	37.8	62.0	309.9	–
11	EdT W	–	–	–	–
12	EdP W	161.5	1772.5	207.1	–
13	EdP W	–	–	–	–
14	EdT M	–	–	–	–
15	EdT M	–	–	–	–
16	EdP W	–	–	–	–
17	EdP W	–	–	–	–
18	EdT M	52.5	–	69.8	–
19	EdP W	27.8	21.2	292.4	–
20	EdT M	–	–	–	–
21	EdP W	32.9	20.1	99.2	–
22	EdT M	–	–	–	–
23	EdT W	–	255.5	–	–
EdT	EdT M	–	–	–	–
25	EdT M	–	–	–	–
26	Aftershave	–	–	187.9	–
27	EdP W	35.2	–	64.9	–
28	EdT M	–	–	–	–
29	Perfume W	30.2	–	196.8	–

M, product for men; W, product for women; EdT, eau de toilette; EdP, eau de parfum.

substances which are likely to be transformed into the sensitizer in question e.g. by skin metabolism, or which is an important cross-reacting substance should be taken into account. Otherwise, the considerable efforts made to change products in the marketplace are wasted and the problems of skin sensitization/elicitation continue.

In the case of isoeugenol, the same restriction should apply to esters of isoeugenol as for isoeugenol for effective prevention.

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