

Selected important fragrance sensitizers in perfumes – current exposures

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Contact allergy to fragrance ingredients is frequent. Recommendations and regulations of some of the most frequent and potent fragrance allergens have recently been introduced. To investigate current exposures to 4 important fragrance allergens in hydroalcoholic cosmetic products. 25 popular perfume products of Danish as well as international brands were purchased from the Danish retail market. Contents of 4 important fragrance allergens, isoeugenol, hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC, Lyrall[®]), were determined by gas chromatography–mass spectrometry, and atranol and chloroatranol were determined by liquid chromatography–tandem mass spectrometry. Isoeugenol was found 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 the recommended maximum concentration of 0.02%. HICC reached a maximum of 0.2%, which is 10-fold higher than maximum tolerable concentration considered safe by the EU Scientific Committee. The median concentrations of atranol and chloroatranol in the investigated products were similar to those found in similar products in 2003. A significant decrease in the frequency of presence of chloroatranol in the products was observed. There is still a wide-spread exposure to potent fragrance allergens in perfumes.

Key words: after-shave lotion; atranol; chemical analysis; chloroatranol; eau de parfum; eau de toilette; fragrances; isoeugenol; Lyrall[®] (hydroxyisohexyl 3-cyclohexene carboxaldehyde).
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Isoeugenol, hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC; Lyrall[®]), and oak moss allergens atranol and chloroatranol are important fragrance sensitizers (1–4) present in many cosmetic products (5, 6). Because of these investigations, regulations such as labelling of specific fragrance allergens have been adopted by EU (7). The EU Scientific Committee on consumer products has also recommended that atranol and chloroatranol should not be present in cosmetic products (8) and that the previously unrestricted use of HICC should be limited to 0.02% (200 ppm) in cosmetic products (9).

In the present study we have investigated the content of isoeugenol, HICC, atranol, and chloroatranol in 25 perfume products, i.e. hydroalcoholic products to look at current consumer exposures.

Materials and Methods

Chemicals

Isoeugenol (98 %) was purchased from Sigma-Aldrich (Denmark). HICC (Lyrall[®]) was supplied

by Hermal, Germany by courtesy. Purity (>98.8%, w/w) was checked by gas chromatography (GC). Atranol and chloroatranol were as synthesized in an earlier study (10).

Products

25 perfume products for both men and women were purchased from Danish retail outlets in February 2006: 13 eau de parfum (12 F, 1 M), 8 eau de toilette (3 F, 5 M), one parfum spray (F), one parfum (F), and 2 after-shave lotion. The products were both of Danish and international brands. The 2 after-shave lotions as well as 2 eau de toilettes were of same brands as analysed for atranol and chloroatranol in a previous study (6); 8 eau de parfum/eau de toilette products were of the same brands of the perfumes analysed in the previous study but in a more diluted version (6). Other products were selected on the basis of the popularity indicated by the sales statistics in a major perfume retailer in Denmark.

Chemical analysis

The contents of isoeugenol and HICC were determined by GC–mass spectrometry (MS) (11), where identification was performed using total ion chromatogram and quantification was performed in selective ion mode. Atranol and chloroatranol concentrations were determined employing liquid chromatography–tandem MS as described before (12). The detection limits of the target compound in the present investigation were 1 ppm ($\mu\text{g/ml}$) for isoeugenol, 2 ppm for HICC, and 0.6 ppb (ng/ml) for both atranol and chloroatranol. The relative standard deviation (RSD) of isoeugenol and HICC determination at 10 $\mu\text{g/ml}$ (10 ppm) was <10%; and RSD of atranol and chloroatranol deter-

mination at a concentration of 10 ng/ml (10 ppb) was also <10%.

Results

The contents of the target compounds in the individual investigated products as well as median and mean values of the contents are described in Table 1. Isoeugenol (48–193 ppm or 0.0048–0.0193%) was present in 56% ($n = 14$) of the 25 investigated samples, and HICC (59–2058 ppm or 0.0058–0.2058%) was present in 72% ($n = 18$) of the products. 11 of the investigated products (44%) contained both isoeugenol and HICC, and 5 products (28%) contained neither isoeugenol nor HICC.

Table 1. Contents of target fragrance allergens in the investigated products

Product identification	Contents ($\mu\text{g/ml}$)*		Contents (ng/ml)*		Contents (ng/ml)* in the similar samples analysed in an earlier study (6)	
	Isoeugenol	HICC	Atranol	Chloroatranol	Atranol	Chloroatranol
Eau de parfum, F	93.4	1446.9	n.d.	n.d.	Perfume of the same brand n.d.	n.d.
Eau de toilette, F	48.2	1354.0	233	47	EdT of the same brand 791	175
Eau de parfum, F	101.3	126.4	880	208		
Eau de parfum, F	n.d.	n.d.	47	n.d.		
Eau de parfum, F	56.4	90.5	n.d.	n.d.		
Eau de parfum, F	n.d.	65.5	n.d.	n.d.		
Eau de parfum, M	n.d.	2058.2	n.d.	n.d.		
Eau de toilette, M	n.d.	n.d.	46	n.d.		
Eau de parfum, F	n.d.	182.6	29	n.d.		
Eau de parfum, F	71.0	68.3	78	10	Perfume of the same brand 231	235
Eau de toilette, M	63.5	n.d.	n.d.	n.d.		
Eau de toilette, F	66.9	407.0	n.d.	n.d.	Perfume of the same brand 1256	875
Eau de toilette, M	n.d.	264.4	90	22	Perfume of the same brand 3089	935
Eau de parfum, F	55.7	64.3	n.d.	n.d.	Perfume of the same brand 1647	268
Eau de toilette, M	n.d.	n.d.	3.5	n.d.		
Eau de parfum, F	n.d.	462.3	9.3	0.9		
After-shave lotion	79.8	59.4	1055	735	After-shave lotion of the same brand 1878	1016
Eau de parfum, F	n.d.	n.d.	0.6	n.d.		
Eau de toilette, F	n.d.	149.3	558	134	Perfume of the same brand 32111	8550
After-shave lotion	n.d.	n.d.	164	61	After-shave lotion of same brand 929	491
Eau de parfum, F	98.8	91.6	271	120	Perfume of the same brand 23600	8335
Eau de parfum, F	81.2	311.8	0.6	n.d.		
Parfum spray, F	192.9	174.2	43	11	Perfume of the same brand 502	51
Eau de toilette, M	71.7	n.d.	65	17	EdT of the same brand 74	10
Parfum, F	84.5	188.6	36	10		
Median	79.8	182.6	65.0	34.5		
Mean	70.7	441.2	209.5	114.7		
SD	36.7	589.8	319.3	205.6		

n.d.: not detected; SD, standard deviation; detection limits: 1 ppm isoeugenol, 2 ppm hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC; Lyrall[®]), 0.6 ppb atranol, 0.9 ppb chloroatranol.

*1 ppb (ng/ml): 0.0000001%.

Atranol (3.5–880 ppb) was present in 13 of the 22 (59%) products of eau de toilette/eau de parfum, and additionally 2 products contained atranol only at detection level (0.6 ppb). Chloroatranol (10–208 ppb) was present in 8 of the 22 (36%) products and additionally one product contained chloroatranol at detection level (0.9 ppb).

The 2 after shaves, which were investigated in this study, contained both atranol (1055 ppb and 164 ppb) and chloroatranol (735 ppb and 61ppb); and the one perfume analysed in this investigation contained both atranol (36 ppb) and chloroatranol (10 ppb).

2 of the investigated products contained only atranol (0.6 ppb and 3.5 ppb) and none of the other target fragrance compounds.

Discussion

In the present investigation, the concentration of atranol and chloroatranol in the 2 after-shave lotions were about half the concentrations found in the same brands analysed in an earlier study (6). However, this was not the case for the eau de toilette and eau de parfum. The maximum concentration of atranol (880 ppb) was similar to the atranol concentration (791 ppb) found in the earlier study (6). Similarly, the maximum chloroatranol level (208 ppb) found in eau de toilette and eau de parfum products analysed in the present investigation was not very different from the maximum chloroatranol concentration (175 ppb) found in such products in the previous study (6). It is, however, noted that chloroatranol and atranol contents in one eau de toilette and 2 eau de parfum analysed in the present investigation were lower than those in the respective products of same brand analysed in the earlier study (6). In the present investigation as well as the in the earlier study, we have not investigated other markers of oak moss. Therefore, it is not known whether the relative decrease in atranol and chloroatranol contents in these 3 products (as well as in above mention after-shave lotions) is due to use of refined oak moss, where atranol and chloroatranol have been extracted, or if it is due use of lesser amounts of oak moss extracts. It appears though the number of products containing chloroatranol has decreased significantly compared to the earlier investigation performed in 2003 (Table 2).

In 1998, the fragrance industry recommended that the usage concentration of isoeugenol in finished cosmetic products did not exceed 0.02% (200 ppm) due to its sensitizing properties (13, 14). The median concentration of isoeugenol in the investigated products was 80 ppm (mean 71 ppm), and in no products the limit of 0.02% was

Table 2. Atranol and chloroatranol content in eau de toilette/eau de parfum in the present study compared to those in a previous study (6)

	Present study	Previous study
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 $\pm$ SD	157 $\pm$ 249	97 $\pm$ 224
Median	47	20
Chloroatranol present in no. of samples	9 (41%)*	14 (82%)
Chloroatranol content	ppb (ng/ml)	ppb (ng/ml)
Range	0.9–208	1–175
Mean $\pm$ SD	63 $\pm$ 73	36 $\pm$ 51
Median	22	10

* $P < 0.05$ (chi-square test). SD, standard deviation.

exceeded (Table 1). In an investigation from 1996 the mean isoeugenol concentration, 0.17%, in fine fragrances ($n = 10$) was in the range 0.05–0.35% (15). The trend in the incidence of isoeugenol allergy has been monitored since then and contrary to expectations no sign of decline is seen (16). This may be due to the usage of esters of isoeugenol, which have not been restricted, but they have been shown to cause reactions in isoeugenol allergic patients (17). Exposure to these esters should be investigated.

HICC (Lyrall[®]) was found in 72% of the products in 0.0183% median concentration. The top concentration was 0.2% (2000 ppm), which is much less than historically has been reported (18) but 10 times more than recently recommended by the Scientific Committee on Cosmetics and Non-Food Products (SCCNFP) as safe for the consumer (9).

A check of labelling of target fragrances in the investigated products revealed that 6 products did not comply with the required labelling according to EU regulation, which came into effect the March 2005 (7). One product carried no labelling at all with regard to ingredients and 2 products were labelled just 'parfum', although these products contained at least 2 of the target fragrance substances. Furthermore, labelling of isoeugenol and HICC was required in 3 products each. Oak moss labelling, International Nomenclature of Cosmetic Ingredients (INC): *evernia prunastri*, was required in 5 products, unless atranol and/or chloroatranol found in these products were synthetic compounds added in the formulation and not due to addition of oak moss itself.

In conclusion, no decrease in the content of atranol in perfumes was seen, while fewer products contained chloroatranol. The content of isoeugenol was within the recommendations of fragrance industry, while HICC was found in

most perfumes and in maximum concentrations exceeding the recommendation for safe usage issued by the scientific committee advisory to the European Commission by a factor of 10.

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