



Determination of fragrance allergens and their dermal sensitization quantitative risk assessment (QRA) in 107 spray perfumes

Duck Soo Lim, Seul Min Choi, Kyu-Bong Kim, Kyungsil Yoon, Sam Kacew, Hyung Sik Kim & Byung-Mu Lee

To cite this article: Duck Soo Lim, Seul Min Choi, Kyu-Bong Kim, Kyungsil Yoon, Sam Kacew, Hyung Sik Kim & Byung-Mu Lee (2018) Determination of fragrance allergens and their dermal sensitization quantitative risk assessment (QRA) in 107 spray perfumes, Journal of Toxicology and Environmental Health, Part A, 81:22, 1173-1185, DOI: [10.1080/15287394.2018.1543232](https://doi.org/10.1080/15287394.2018.1543232)

To link to this article: <https://doi.org/10.1080/15287394.2018.1543232>



Published online: 12 Nov 2018.



Submit your article to this journal [↗](#)



Article views: 164



View related articles [↗](#)



View Crossmark data [↗](#)



Citing articles: 4 View citing articles [↗](#)



Determination of fragrance allergens and their dermal sensitization quantitative risk assessment (QRA) in 107 spray perfumes

Duck Soo Lim^a, Seul Min Choi^a, Kyu-Bong Kim^b, Kyungsil Yoon^c, Sam Kacew^d, Hyung Sik Kim^a, and Byung-Mu Lee^a

^aDivision of Toxicology, College of Pharmacy, Sungkyunkwan University, Suwon, South Korea; ^bCollege of Pharmacy, Dankook University, Cheonan, South Korea; ^cLung Cancer Branch, Research Institute, National Cancer Center, Goyang, South Korea; ^dMcLaughlin Centre for Population Health Risk Assessment, University of Ottawa, Ottawa, ON, Canada

ABSTRACT

Cutaneous allergy occurs primarily as a result of using cosmetic, household, and laundry products available on the market that contain fragrances. The aim of this study was to develop a rapid and specific high-performance liquid chromatography with ultraviolet detection (HPLC-UV) method for quantification of 25 fragrance allergens (amyl cinnamyl alcohol, benzyl alcohol, benzyl benzoate, benzyl cinnamate, benzyl salicylate, citronellol, cinnamyl alcohol, citral, coumarin, eugenol, farnesol, geraniol, hydroxycitronellal, HICC (4-(4-hydroxy-4-methylpentyl)-3-cyclohexene-1-carbaldehyde), isoeugenol, isoeugenyl acetate, linal, linalyl acetate, linalyl propionate, limonene, linalool, methyl 2-octynoate, etc.). In addition, an exposure-based quantitative risk assessment (QRA) was performed to determine safe levels of fragrance ingredients in 107 perfumes. In 76 women's and 31 men's fragrances, 25 allergens were identified at concentrations ranging from undetectable (N.D.) to 8,997.68 mg/kg, and from N.D. to 17,352.34 mg/kg, respectively. An exposure-based sensitization QRA revealed that the ratios of acceptable exposure level (AEL) to consumer exposure level (CEL) of fragrance ingredients were greater than 1, suggesting an absence of skin sensitizing potential. However, the maximum level used in the exposure scenario was determined by the product purpose and application type, and AEL/CEL ratios of linal, HICC, citral, isoeugenol, and methyl 2-octynoate analyzed in women's perfume were 0.53, 0.67, 0.19, 0.13, and 0.57, respectively. As the ratios of AEL:CEL of these fragrance ingredients were below 1, the utilization of these potential skin sensitizers is not considered safe. Our findings indicate that the sensitization risk of allergens with AEL:CEL ratios below 1 detected in fragrances needs to be reduced to the appropriate human safety level for risk management.

Introduction

Products containing fragrances are widely used and are in direct contact with human skin. Today, more than 5,000 fragrance agents are employed as mixtures, particularly in cosmetics and household products including perfumes, shampoos, deodorants, air fresheners, and scented toys (2018b; Kim et al. 2018a; Loretz et al. 2006; Masuck, Hutzler, and Luch 2011; Rastogi, Johansen, and Bossi 2007). Perfumes are commonly utilized to provide the consumer with a feeling of pleasure and to mask body odor. Approximately 3,000 chemical substances, essential oils, fixatives, and solvents are commonly used in perfume formulations (Pybus and Shell 1999). The widespread utilization of products that contain

fragrances may be attributed to consequent development of allergic contact dermatitis (Cheng and Zug 2014; European Commission (EC) 1999; Nardelli et al. 2008). Therefore, risk assessments for various substances, including fragrance ingredients, are performed for safety management (Choi et al. 2018; Kim et al. 2017, 2018a; Lim et al. 2018a, 2018b). Schnuch et al. (2007) identified 26 fragrance ingredients that potentially might result in contact allergy. Consumer products that fall under this regulation need to undergo a risk assessment to evaluate the health hazards that may be caused by product use. Although some chemicals employed in cosmetics may exhibit the potential to induce dermal sensitization, several studies previously reported dermal

CONTACT Byung-Mu Lee  bmllee@skku.edu  Division of Toxicology, College of Pharmacy, Sungkyunkwan University, Seobu-ro 2066, 16419 Suwon, South Korea

Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/uteh.

© 2018 Taylor & Francis

sensitization risk assessments (Gerberick et al. 2001; Lim et al. 2018a; 2018b).

Safety standards need to be set for use of hazardous ingredients in consumer products based upon risk assessment data (Choi et al. 2018; Kim et al. 2018b, 2018c). Certain ingredients present in perfumes may contain compounds that are responsible for cosmetic-related allergic contact dermatitis (allergens), and legal restrictions imposed by the European Commission Scientific Committee on Cosmetics and Other Non-food Products (SCCNFP) limit the use of 24 fragrance agents suspected of producing cutaneous contact allergy (EC 2003; Frosch et al. 1995; Larsen et al. 2001, 2002; Rastogi, Menne, and Johansen 2003; Scheinman 1996, 2002). These regulations require an indication of the presence of potential fragrance allergens in cosmetic products with a limit of 0.01% (100 mg/kg) in products that are designed to be applied to the body and then rinsed off, such as soaps, and 0.001% (10 mg/kg) in products that are designed to remain on the body throughout the day, such as perfumes (EC (European Commission) 2003; SCCNFP 2003).

The ingestion of benzyl cinnamate is estimated to be 44 µg/person/day (0.7 µg/kg body weight (bw)/day) in Europe and 69 µg/person/day (1 µg/kg bw/day) in the USA. In addition, the no-observed-effect level (NOEL) is greater than 500 mg/kg bw for benzyl cinnamate (Bhatia et al. 2007; Mattia 2005). The median lethal doses (LD₅₀) of cinnamal via oral administration in rats and mice were found to be 2,200–3,400 mg/kg bw and 3,400 mg/kg bw, respectively (EC 2000). Cinnamal administration displayed no marked carcinogenic effects in *in vivo* animal studies (Bickers et al. 2005).

α-Butyl cinnamaldehyde underwent various toxicity assessments (Api et al. 2015a). Repeated administration of α-butyl cinnamaldehyde displayed an estimated NOEL of 29.9 mg/kg/day and a margin of exposure (MOE) of 3,784,810 for 14-week sub-chronic toxicity. National Toxicology Program (NTP), (1989) reported that the acute dermal toxicity of cinnamaldehyde was calculated as LD₅₀ = 0.42–0.84 mg/kg in rabbits. The maximum tolerated dose (MTD) of cinnamaldehyde was determined to be 0.25 g/kg (NTP 1989). Isoeugenol is used in various household

and personal hygiene products including perfumes, creams, lotions, soaps, and detergents (Api et al. 2016a). Isoeugenol is not mutagenic. However, an increasing trend and a 3-fold increase in the 600 mg/kg-treated group indicated a positive result for this test in female mice. Exposure to isoeugenol resulted in non-neoplastic lesions of the nose in male and female rats, of the nose, forestomach, and glandular stomach in male and female mice, and of the kidney in female mice (Api et al. 2016a). Citral possesses a potent lemon fragrance. The intake of citral is estimated to be 6,849 µg/person/day (114 µg/kg bw/day) in Europe and 6,990 µg/person/day (117 µg/kg bw/day) in the USA (Bickers et al. 2005; Lalko and Api 2008). The Joint Expert Committee for Food Additives (JECFA) calculated an acceptable daily intake (ADI) through foodstuffs for citral, citronellol, geranyl acetate, linalool, and linalyl acetate of 0–0.5 mg/kg bw (calculated as citral) (JECFA 2010; The Organisation for Economic Co-operation and Development (OECD) 2001; Pronk. 2004). The intake of citronellol is estimated to be 370 µg/person/day (6.2 µg/kg bw/day) in Europe and 771 µg/person/day (13 µg/kg bw/day) in the USA (Bickers et al. 2005).

Skin sensitization is an important consumer problem. It is mandatory to ensure that chemicals, ingredients, and products do not produce an allergic response in consumers. Quantitative risk assessments (QRA) for sensitizing allergies have only recently attracted attention in the context of risk assessment methods (Api et al. 2008; World Health Organization International Programme on Chemical Safety (WHO/IPCS) 2012); are still under development. Thus, an evaluation of cosmetic safety is of the utmost importance. Although certain chemicals in cosmetics may exhibit the potential to induce dermal sensitization, few studies have reported dermal sensitization risk assessments (Gerberick et al. 2001).

First, a QRA of the dermal sensitizing properties of fragrances was developed to assess the risk of induction in the general population. It was the first structured approach to quantitatively assess exposure to sensitizing allergens (Basketter et al. 2008; Nohynek et al. 2010). A QRA includes determination of the no expected sensitization induction level (NESIL), which is equivalent to

the point of departure (POD) in the hazard characterization of general toxic effects, and the application of sensitization assessment factors (SAF) (Api et al. 2008; Api and Vey 2008; Basketter et al. 2008; Nohynek et al. 2010; WHO/IPCS 2012). The SAF account for differences between experimental and real-life populations as well as between experimental and real-life exposure scenarios. With these parameters, the acceptable exposure level (AEL) is calculated. Subsequently, the AEL is compared with the consumer exposure level (CEL). In cases where the CEL is lower than the AEL, it is presumed that there is no appreciable risk of induction of sensitization for consumers (Api et al. 2008; Api and Vey 2008; WHO/IPCS 2012).

The purpose of this study was to determine the concentrations of 25 fragrance allergens in perfumes using high-performance liquid chromatography (HPLC). This study assessed the risk for dermal exposure based on a “worst-case scenario” related to 107 perfumes. This investigation also examined the human health risk of skin exposure to a fragrance present in consumer products on the Korean market.

Materials and methods

Chemicals and reagents

Acetonitrile (MeCN, HPLC gradient grade) for HPLC analysis was purchased from J&T Baker (St. Louis, MO, USA). Amyl cinnamyl alcohol, anise alcohol, benzyl alcohol, benzyl benzoate, benzyl cinnamate, benzyl salicylate, citronellol, cinnamyl alcohol, citral, coumarin, eugenol, farnesol, geraniol, hexyl cinnamaldehyde, hydroxycitronellal, HICC (4-(4-hydroxy-4-methylpentyl)-3-cyclohexene-1-carbaldehyde), isoeugenol, isoeugenyl acetate, isoeugenyl ethyl ether, α -isomethyl ionone (3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one), lilial (butyl phenyl methyl propional), limonene, linalool, methyl 2-octynoate, and methyl isoeugenol standards were obtained from Sigma (St. Louis, MO, USA). Deionized water was distilled and filtered using Millipore filters (Millipore S.A., Molsheim, France). All other chemicals utilized were of analytical grade. Perfumes were purchased from retail stores.

Apparatus and chromatographic conditions

Chromatographic experiments were performed using an Agilent HP1100 (Palo Alto, CA) HPLC system. Chromatographic separation was performed on a LiChrospher 100 C18 column (250–4 mm I.D., 5 μ m particle size), and the mobile phases comprised a 0.01% acetic acid aqueous solution (A) and methanol (B), at a flow rate of 1 ml/min. The following elution gradient was used: solvent B started at 60% and was maintained for 5 min, then reduced to 50% at 7 min, increased to 100% at 14 min, maintained for 4 min, and decreased to 60% at 19 min. The column was re-equilibrated for 5 min before the next injection. The eluate was continuously monitored by an ultraviolet (UV) detector at a wavelength of 270 nm.

Stock and working standard solutions

Individual stock solutions of the 25 selected allergens and internal standard (Table 2) were prepared by dissolving each standard in acetonitrile (accurately weighed) in volumetric flasks at concentrations of approximately 10 mg/ml, except for hydroxycitronellal, which was made up to a concentration of 100 mg/ml. The solutions were stored at 4°C. Fresh working solutions, prepared daily by appropriate dilutions of stock solutions, were processed individually and in a mixture to (1) optimize operative conditions, (2) record spectral data, and (3) obtain calibration curves. The standard mixture solution of all tested allergens and internal standard was prepared by diluting a known volume (10 to 50 μ l) of each stock solution in volumetric flasks with acetonitrile.

Sample preparation

A total of 107 perfumes (76 women's perfumes, 31 men's perfumes) were purchased from department stores in Anyang City, Korea. Approximately 100 μ l of each product was added to an appropriate volume of the internal standard stock solution (100 μ l), diluted with acetonitrile, and injected directly into the HPLC system.

Validation of the method

The analytical method developed was tested with regard to linearity, limits of detection (LOD), accuracy, and precision. The linearity of the HPLC method was examined after preparation of the calibration curves by analyzing 5 concentrations of each fragrance allergen, ranging from 0.1 to 500 µl/ml for all compounds except hydroxycitronellal (20–5000 µl/ml), in triplicate. Linear regression analyses were performed using the internal standard method. Linearity for each compound was established by plotting the peak area ratio (standard to internal standard) versus standard concentrations. A validation protocol was designed and applied to characterize the following performance parameters of the method: confirmation of identity, selectivity, specificity, LOD, limit of quantitation (LOQ), linearity (working and linear range), reproducibility, precision, and accuracy.

QRA methodology for fragrance ingredients

For the QRA, a NESIL for induction of skin sensitization was determined for fragrance ingredients following a weight of evidence (WoE)-based approach. A conservative WoE NESIL is shown in Table 1. In dermal sensitization risk assessments, an uncertainty factor (UF) is applied for extrapolation from experimental to real-life exposure scenarios. This is achieved by the application of a SAF, which takes into account three parameters: inter-individual variability, product matrix effect, and use consideration. Perfumes (hydroalcoholic products applied to unshaved skin) provide an example of a widely used rinse-off cosmetic product where exposure to a given chemical would be expected to be low (SAF = 100).

There are two key elements involved in risk characterization for dermal sensitization risk assessments. These are the AEL, and the

Table 1. Categorization of fragrance allergens labeled according to EU regulation and no expected sensitization induction level (NESIL) for fragrance ingredients derived by application of weight of evidence (WoE).

Category ¹⁾	Allergen	Uses	CAS No.	Potency	LLNA-EC3 ²⁾ (µg/cm ²)	WoE NESIL ²⁾ (µg/cm ²)
Group I (important sensitizers)	Farnesol	Deodorant, solvent, perfume	4602–84-0	Weak	1,200	2,700
	Hydroxycitronellal	Perfume	107–75-5	Weak	5,612	5,000
	HICC	Flavoring, perfume	31906–04-4	Weak	4,275	4,000
Group II (weak sensitizers)	Isoeugenol	Flavoring, perfume	97–54-1	Moderate	498	250
	Citronellol	Perfume	106–22-9	Extremely weak	10,875	29,500
	Amyl cinnamyl alcohol	Perfume	101–85-9	Weak	> 6,250	3,500
	Benzyl cinnamate	Perfume	103–41-3	Weak	4,600	4,700
	Geraniol	Tonic, perfume	106–24-1	Weak	3,525	11,800
	Lilial	Flavoring, perfume	80–54-6	Weak	2,372	4,100
	Cinnamyl alcohol	Flavoring, perfume	104–54-1	Weak	5,250	3,000
	Citral	Flavoring, perfume	5392–40-5	Weak	1,414	1,400
	Coumarin	Perfume	91–64-5	Weak	> 12,500	3,500
	Eugenol	Denaturant, perfume	97–53-0	Weak	2,703	5,900
	Amyl cinnamal	Perfume	122–40-7	Weak	2,942	23,600
	Benzyl benzoate	Antimicrobial, perfume	120–51-4	Extremely weak	> 12,500	59,000
Group III (rare sensitizers)	Hexyl cinnamal	Perfume	101–86-0	Weak	2,372	23,600
	Anise alcohol	Perfume	105–13-5	Weak	1,475	1,500
	Benzyl alcohol	Preservative, perfume	100–51-6	Weak	> 1,2500	5,900
	Benzyl salicylate	UV absorber, perfume	118–58-1	Weak	725	17,700
	α-isomethyl ionone	Flavoring, perfume	127–51-5	Weak	5,450	71,000
	Limonene	Flavoring, Perfume	5989–27-5	Extremely Weak	10,075	10,000
	Linalool	Flavoring, perfume	78–70-6	Extremely weak	15,000	15,000
	Methyl 2-octynoate	Perfume	111–12-6	Strong	< 125	120

Abbreviations: LLNA, local lymph node assay; EC3, estimated concentration required for a 3-fold stimulation index. Data from ¹⁾(Schnuch et al. 2007);

²⁾(Api et al. 2008).

Table 2. Linear regression data, limit of detection (LOD), accuracy, and precision related to the 25 fragrance allergens.

Allergen	Correlation coefficient (r^2)	Linear range ($\mu\text{g/ml}$)	LOD ($\mu\text{g/ml}$)	Accuracy (%)	% RSD
Amyl cinnamyl alcohol	0.995	0.5–500	0.2	100.6	1.7
Anise alcohol	0.990	0.5–500	0.13	101.6	1.7
Benzyl alcohol	0.995	0.5–500	0.43	93.9	3.7
Benzyl benzoate	0.993	0.5–500	0.33	103.6	2.2
Benzyl cinnamate	0.993	0.5–500	0.53	104.6	1.8
Benzyl salicylate	0.996	0.5–500	0.63	94.9	2.6
Citronellol	0.993	0.5–500	0.13	95.9	0.7
Cinnamyl alcohol	0.998	0.5–500	0.13	97.9	0.8
Citral	0.997	0.5–500	0.23	102.1	1.8
Coumarin	0.995	0.5–500	0.46	98.9	0.9
Eugenol	0.994	0.5–500	0.33	102.6	1.7
Farnesol	0.993	0.5–500	0.66	95.7	2.7
Geraniol	0.994	0.5–500	0.23	102.8	1.3
Hexyl cinnamaldehyde	0.994	0.5–500	0.33	90.9	2.3
Hydroxycitronellal	0.997	10–5000	10.88	95.8	3.4
HICC	0.998	0.5–500	0.73	97.9	1.9
Isoeugenol	0.996	0.5–500	0.13	101.3	0.8
Isoeugenyl acetate	0.993	0.5–500	0.66	97.7	2.7
Isoeugenyl ethyl ether	0.993	0.5–500	0.66	97.7	2.7
α -isomethyl ionone	0.992	0.5–500	0.67	98.9	1.7
Lilial	0.997	0.5–500	0.26	103.6	1.7
Limonene	0.995	0.5–500	0.47	101.2	2.8
Linalool	0.994	0.5–500	0.19	96.8	1.7
Methyl 2-octynoate	0.993	0.5–500	0.53	102.4	3.2
Methyl isoeugenol	0.993	0.5–500	0.66	99.7	1.7

Abbreviations: Lilial, butyl phenyl methyl propional; HICC, 4-(4-hydroxy-4-methylpentyl)-3-cyclohexene-1-carbaldehyde; α -isomethyl ionone, 3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one; RSD, relative standard deviation.

comparison of the AEL to the CEL. The CEL is a measure of exposure under intended foreseeable conditions of frequency of use, duration of utilization, and amount of product used per application/use. The calculation of consumer exposure established for perfumes (hydroalcoholic products for shaved skin) was 2.21 mg/cm²/day (Ryan et al. 2002).

The AEL is determined by dividing the NESIL by the product type SAF using the following equation:

$$AEL(\mu\text{g}/\text{cm}^2) = \frac{\text{WoE NESIL}(\mu\text{g}/\text{cm}^2)}{\text{SAF}}$$

The AEL is expressed in terms of dose/unit area/day. Finally, to establish the acceptability of consumer exposure to fragrance ingredients in a given product, the ratio of AEL to CEL is determined by dividing these two values (AEL/CEL). The % concentration of a fragrance ingredient in a product type is acceptable if the AEL is greater than or equal to the CEL (AEL $\geq$ CEL or $\geq$ 1). Conversely, cases where the CEL exceeds the AEL would require re-evaluation of risk management and may lead to a decrease in the concentration of fragrance ingredients in that product type (AEL < CEL or <1).

Results

Quantitative analysis of men's and women's perfumes

HPLC conditions were considered suitable to modulate the retention of all fragrance ingredients. Under the chromatographic conditions employed, the 25 analytes exhibited good linearity ($r^2 > 0.99$) over a relatively wide range of concentrations. The results of method validation related to all 25 allergens are presented in Table 2.

In women's perfumes ($n = 76$), the concentrations of the 25 allergens ranged from undetectable (N.D.) to 8,997.68 mg/kg (Table 3). Lilial showed the highest mean content ($4,026.84 \pm 7,138.12$ mg/kg), while amyl cinnamyl alcohol displayed the lowest content (2.39 ± 10.65 mg/kg).

In men's perfumes ($n = 31$), potential allergens were detected at concentrations ranging from N. D. to 17,352.34 mg/kg (Table 3). Limonene exhibited the highest mean content ($5,008.18 \pm 4,315.20$ mg/kg), while amyl cinnamyl alcohol showed the lowest (1.59 ± 10.95 mg/kg).

Table 3. Quantitative analysis of fragrance allergens in 107 commercial perfumes via HPLC-UV.

Allergen	Women's perfumes (n = 76)			Men's perfumes (n = 31)		
	Mean $\pm$ S.D. (mg/kg)	Median (mg/kg)	Range (min-max, mg/kg)	Mean (mg/kg)	Median (mg/kg)	Range (min-max, mg/kg)
Lilial	4,026.84 $\pm$ 7,138.12	2,108.08	N.D.-3,4945.26	1,129.32 $\pm$ 1,688.20	387.05	N.D.-6,867.86
Limonene	3,863.32 $\pm$ 4,922.01	2,494.12	N.D. -2,6196.84	5,008.18 $\pm$ 4,315.20	4,462.09	N.D.-17,352.34
HICC	2,768.27 $\pm$ 3,012.51	N.D.	N.D. -2,7106.25	1,924.16 $\pm$ 5,752.49	644.51	N.D.-9,315.32
Linalool	2,262.72 $\pm$ 3,210.98	1,553.69	N.D. -2,1845.38	2,616.97 $\pm$ 2,234.81	1,647.20	N.D.-8,314.67
α -isomethyl ionone	1,859.52 $\pm$ 1,821.12	99.12	N.D. -1,5102.7	2,168.85 $\pm$ 1,090.64	N.D.	N.D.-1,1105.20
Geraniol	1,630.49 $\pm$ 1,730.74	253.84	N.D. -8,997.68	1,146.85 $\pm$ 3,276.54	22.66	N.D.-8,458.32
Benzyl salicylate	1,128.03 $\pm$ 1,192.20	N.D.	N.D. -6,473.74	889.56 $\pm$ 1,141.68	N.D.	N.D.-8,152.90
Citronellol	1,005.3 $\pm$ 1,078.20	253.37	N.D. -7,897.15	547.36 $\pm$ 919.15	270.31	N.D.-4,253.99
Citral	811.79 $\pm$ 1,120.20	31.85	N.D. -3,4215.24	164.73 $\pm$ 166.55	118.46	N.D.-803.19
Hexyl cinnamaldehyde	671.12 $\pm$ 1,210.21	100.27	N.D. -4,674.9	262.53 $\pm$ 385.77	N.D.	N.D.-1,939.56
Farnesol	465.25 $\pm$ 653.25	90.32	N.D. -3,994.24	576.70 $\pm$ 646.14	331.54	N.D.-2,237.58
Isoeugenol	328.65 $\pm$ 648.64	42.69	N.D. -8,639.93	50.11 $\pm$ 101.58	N.D.	N.D.-256.12
Hydroxycitronellal	276.55 $\pm$ 324.54	N.D.	N.D. -4,671.66	511.55 $\pm$ 235.85	N.D.	N.D.-4,105.49
Anise alcohol	182.72 $\pm$ 443.76	N.D.	N.D. -2,519.72	112.59 $\pm$ 421.21	N.D.	N.D.-2,873.28
Isoeugenyl acetate	168.39 $\pm$ 192.64	N.D.	N.D. -2,966.88	80.18 $\pm$ 86.18	N.D.	N.D.-2,010.87
Coumarin	159.91 $\pm$ 240.14	N.D.	N.D. -1,848.25	616.80 $\pm$ 925.15	N.D.	N.D.-3,337.49
Benzyl benzoate	148.95 $\pm$ 342.54	15.68	N.D. -4,933.87	224.79 $\pm$ 1,245.99	N.D.	N.D.-5,623.91
Eugenol	140.44 $\pm$ 259.94	53.28	N.D. -2,474.49	69.00 $\pm$ 54.12	35.76	N.D.-303.27
Benzyl alcohol	100.86 $\pm$ 493.22	N.D.	N.D. -4,094.71	33.35 $\pm$ 55.83	6.66	N.D.-183.35
Benzyl cinnamate	92.40 $\pm$ 89.54	N.D.	N.D. -1,695.65	134.60 $\pm$ 163.38	N.D.	N.D.-1,073.47
Isoeugenyl ethyl ether	79.36 $\pm$ 70.64	11.21	N.D. -655.64	80.18 $\pm$ 42.12	N.D.	N.D.-2,010.87
Methyl 2-octynoate	72.23 $\pm$ 62.54	12.06	N.D. -954.39	14.20 $\pm$ 16.67	N.D.	N.D.-165.68
Methyl isoeugenol	16.58 $\pm$ 13.50	N.D.	N.D. -435.46	4.85 $\pm$ 10.85	N.D.	N.D.-58.49
Cinnamyl alcohol	12.15 $\pm$ 39.96	N.D.	N.D. -308.94	8.38 $\pm$ 5.67	N.D.	N.D.-162.28
Amyl cinnamyl alcohol	2.39 $\pm$ 10.65	N.D.	N.D. -65.84	1.59 $\pm$ 10.95	N.D.	N.D.-19.68

Abbreviations: Lilial, butyl phenyl methyl propional; HICC, 4-(4-hydroxy-4-methylpentyl)-3-cyclohexene-1-carboaldehyde; α -isomethyl ionone, 3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one; N.D., not detected.

QRA evaluation for selected fragrance ingredients

Results from the QRA evaluation for fragrance ingredients are shown in Tables 4 and 5 and reported as mean, median, and maximum concentration (%) in men's and women's perfume (hydroalcoholic products for unshaved skin), respectively. There are two factors involved in risk assessment conducted using the recommended method. These are the AEL, and comparison of AEL to CEL. The AEL is determined by dividing the WoE NESIL by the product type SAF (100). For an acceptable risk assessment, the AEL needs to be greater than or equal to the CEL (AEL $\geq$ CEL).

As summarized in Tables 4 and 5, the AEL/CEL ratios for all sensitizing fragrance ingredients analyzed in this study were greater than 1, indicating the safe use of these potential skin sensitizers. However, assuming a worst-case scenario, the

AEL/CEL ratios of lilial, HICC, citral, isoeugenol, and methyl 2-octynoate measured at maximum concentration for women's perfume were 0.53, 0.67, 0.19, 0.13, and 0.57, respectively. Because these AEL:CEL ratios were below 1, this indicates it is not safe to use these potential skin sensitizers.

Discussion and conclusions

In this study, human health risk assessments were performed with regard to consumer exposure to the materials used in perfumes available in the Korean market. Previous investigators reported safety assessments for fragrance ingredients such as linalool, eugenol, amyl cinnamaldehyde, benzyl alcohol, α -isomethyl ionone, and cinnamyl cinnamate (2015d; Api et al. 2016b, 2015b, 2015c; Bhatia et al. 2007; Lapczynski et al. 2007). This study noted the prevalence and QRA of skin contact allergy related to

Table 4. Dermal sensitization quantitative risk assessment (QRA) of fragrance allergens in 76 women's perfumes (hydroalcoholic product for unshaved skin).

Allergen	WoE NESIL ¹⁾ (µg/cm ²)	SAF ²⁾	AEL (µg/cm ²)	Product exposure ³⁾ (mg/cm ² /day)	Mean concentration of fragrance allergens in the product		Median concentration of fragrance allergens in the product		Max. concentration of fragrance allergens in the product	
					CEL (µg/cm ²)	AEL/CEL	CEL (µg/cm ²)	AEL/CEL	CEL (µg/cm ²)	AEL/CEL
Lilial	4,100	100	41	2.2	8.86	4.63	4.64	8.84	76.88	0.53
Limonene	10,000	100	100	2.2	8.50	11.77	5.49	18.22	57.63	1.74
HICC	4,000	100	40	2.2	6.09	6.57	N.C.	N.C.	59.63	0.67
Linalool	15,000	100	150	2.2	4.98	30.13	3.42	43.88	48.06	3.12
Isomethyl ionone	71,000	100	710	2.2	4.09	173.55	0.22	3255.92	33.23	21.37
Geraniol	11,800	100	118	2.2	3.59	32.90	0.56	211.30	19.79	5.96
Benzyl salicylate	17,700	100	177	2.2	2.48	71.32	N.C.	N.C.	14.24	12.43
Citronellol	29,500	100	295	2.2	2.21	133.38	0.56	529.23	17.37	16.98
Citral	1,400	100	14	2.2	1.79	7.84	0.07	199.80	75.27	0.19
Hexyl cinnamaldehyde	23,600	100	236	2.2	1.48	159.84	0.22	1,069.84	10.28	22.95
Farnesol	2,700	100	27	2.2	1.02	26.38	0.20	135.88	8.79	3.07
Isoeugenol	250	100	2.5	2.2	0.72	3.46	0.09	26.62	19.01	0.13
Hydroxycitronellal	5,000	100	50	2.2	0.61	82.18	N.C.	N.C.	10.28	4.86
Anise alcohol	1,500	100	15	2.2	0.40	37.31	N.C.	N.C.	5.54	2.71
Isoeugenyl acetate	N.C.	100	N.C.	2.2	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
Coumarin	3,500	100	35	2.2	0.35	99.49	N.C.	N.C.	4.07	8.61
Benzyl benzoate	59,000	100	590	2.2	0.33	1,800.48	0.03	17,103.43	10.85	54.36
Eugenol	5,900	100	59	2.2	0.31	190.96	0.12	503.34	5.44	10.84
Benzyl alcohol	5,900	100	59	2.2	0.22	265.90	N.C.	N.C.	9.01	6.55
Benzyl cinnamate	4,700	100	47	2.2	0.20	231.21	N.C.	N.C.	3.73	12.60
Isoeugenyl ethyl ether	N.C.	100	N.C.	2.2	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
Methyl 2-octynoate	120	100	1.2	2.2	0.16	7.55	0.03	45.23	2.10	0.57
Methyl isoeugenol	N.C.	100	N.C.	2.2	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
Cinnamyl alcohol	3,000	100	30	2.2	0.03	1,122.33	N.C.	N.C.	0.68	44.14
Amyl cinnamyl alcohol	3,500	100	35	2.2	0.01	6,656.52	N.C.	N.C.	0.14	241.63

Abbreviations: WoE NESIL, no expected sensitization induction level (NESIL) for fragrance ingredients derived by application of weight of evidence (WoE); SAF, sensitization assessment factors; AEL, acceptable exposure level; CEL, consumer exposure levels; lilial, butyl phenyl methyl propional; HICC, 4-(4-hydroxy-4-methylpentyl)-3-cyclohexene-1-carbaldehyde; α-isomethyl ionone, 3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one; N.C. not calculated. Data from¹⁾ (Api et al. 2008);²⁾ (Feldmann and Maibach 1967);³⁾ (Tozer et al. 2004)

Table 5. Dermal sensitization quantitative risk assessment (QRA) of fragrance allergens in 31 men's perfumes (hydroalcoholic product for unshaved skin).

Allergen	WoE NESIL ¹⁾ ($\mu\text{g}/\text{cm}^2$)	SAF ²⁾	AEL ($\mu\text{g}/\text{cm}^2$)	Product exposure ³⁾ ($\text{mg}/\text{cm}^2/\text{day}$)	Mean concentration of fragrance allergens in the product		Median concentration of fragrance allergens in the product		Max. concentration of fragrance allergens in the product	
					CEL ($\mu\text{g}/\text{cm}^2$)	AEL/CEL	CEL ($\mu\text{g}/\text{cm}^2$)	AEL/CEL	CEL ($\mu\text{g}/\text{cm}^2$)	AEL/CEL
Limonene	10,000	100	100	2.2	11.02	9.08	9.82	10.19	38.18	2.62
Linalool	15,000	100	150	2.2	5.76	26.05	3.62	41.39	18.29	8.20
Isomethyl ionone	7,100	100	710	2.2	4.77	148.80	N.C.	N.C.	24.43	29.10
HICC	4,000	100	40	2.2	4.23	9.45	1.42	28.21	20.49	1.95
Geraniol	11,800	100	118	2.2	2.52	46.77	0.05	2,367.01	18.61	6.34
Lilial	4,100	100	41	2.2	2.48	16.50	0.85	48.15	15.11	2.71
Benzyl salicylate	17,700	100	177	2.2	1.96	90.44	N.C.	N.C.	17.94	9.87
Coumarin	3,500	100	35	2.2	1.36	25.79	N.C.	N.C.	7.34	4.77
Farnesol	2,700	100	27	2.2	1.27	21.28	0.73	37.02	4.92	5.48
Citronellol	29,500	100	295	2.2	1.20	244.98	0.59	496.06	9.36	31.52
Hydroxycitronellal	5,000	100	50	2.2	1.13	44.43	N.C.	N.C.	9.03	5.54
Hexyl cinnamaldehyde	23,600	100	236	2.2	0.58	408.61	N.C.	N.C.	4.27	55.31
Benzyl benzoate	59,000	100	590	2.2	0.49	1,193.03	N.C.	N.C.	12.37	47.69
Citral	1,400	100	14	2.2	0.36	38.63	0.26	53.72	1.77	7.92
Benzyl cinnamate	4,700	100	47	2.2	0.30	158.72	N.C.	N.C.	2.36	19.90
Anise alcohol	1,500	100	15	2.2	0.25	60.56	N.C.	N.C.	6.32	2.37
Isoeugenyl acetate	N.C.	100	0	2.2	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
Eugenol	5,900	100	59	2.2	0.15	388.67	0.08	749.95	0.67	88.43
Isoeugenol	250	100	2.5	2.2	0.11	22.68	N.C.	N.C.	0.56	4.44
Benzyl alcohol	5,900	100	59	2.2	0.07	804.14	0.01	4,026.75	0.40	146.27
Isoeugenyl ethyl ether	N.C.	100	0	2.2	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
Methyl 2-octynoate	120	100	1.2	2.2	0.03	38.41	N.C.	N.C.	0.36	3.29
Cinnamyl alcohol	3,000	100	30	2.2	0.02	1,627.25	N.C.	N.C.	0.36	84.03
Methyl isoeugenol	N.C.	100	0	2.2	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
Amyl cinnamyl alcohol	3,500	100	35	2.2	0.003	10,005.72	N.C.	N.C.	0.04	808.39

Abbreviations: WoE NESIL, no expected sensitization induction level (NESIL) for fragrance ingredients derived by application of weight of evidence (WoE); SAF, sensitization assessment factors; AEL, acceptable exposure level; CEL, consumer exposure levels; lilial, butyl phenyl methyl propional; HICC, 4-(4-hydroxy-4-methylpentyl)-3-cyclohexene-1-carbaldehyde; α -isomethyl ionone, 3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one; N.C. not calculated. Data from ¹⁾ (Api et al. 2008); ²⁾ (Feldmann and Maibach 1967); ³⁾ (Cano and Rich 2001); (Tozer et al. 2004); (Cano 2006).

exposure to sensitizers associated with the utilization of perfumed products.

In addition to the widespread use of skin sensitizers, the probability of consumers coming into contact with fragrance allergens as well as the concentration of the fragrance allergens in the perfumes are important factors. The fragrances with the lowest sensitizing potential, D-limonene, linalool, linal, and geraniol, were identified at relatively high concentrations in perfumes. However, it is worthwhile noting that potential problems may also arise when frequently used compounds with low sensitizing potentials are metabolized by air constituents or on the skin, leading to the formation of more potent allergens. Some investigators demonstrated that hydroperoxides formed after oxidation of limonene were more potent allergens than limonene (Karlberg, Magnusson, and Nilsson 1992; Karlberg et al. 1994; Matura et al. 2002). In addition, derivatives of isoeugenol, employed as alternatives for isoeugenol because of their lower sensitizing potencies, may be again converted into isoeugenol (Rastogi and Johansen 2008). Therefore, this study provided methods and a strategy for the mandatory regulation of perfumes in the Korean market, including derivatives of isoeugenol such as isoeugenyl acetate, isoeugenyl ethyl ether, and methyl isoeugenol.

In total, 25 allergens established as 'safe' according to guidelines for perfumes were quantitatively analyzed. The relatively low concentrations and low detection rate in the products reflect their safety ratings. The Ministry of Food and Drug Safety (MFDS) regulates the use of these allergens in consumer products, including perfumes and cosmetics. Perfumes that exceed maximum concentrations are prohibited for sale on the market. The purpose of this study was to formulate a logical suggestion for the limit of allergens employed in perfumes using a dermal sensitization QRA method. According to our findings, allergens were not present in an amount sufficient to require a further dermal risk assessment. For perfumes, fragrance ingredients used frequently exhibited allergenic potential. For chemicals that demonstrated an estimated higher risk for consumers, the toxicological parameters of the properties of the ingredients were defined by making use of several standards, as toxicity data for the ingredients are limited (Kim et al. 2018b; Larsen and Holmberg 2005).

There is an important issue regarding risk assessment studies of consumer products in Korea, as many of these are registered as existing chemicals (Kim et al. 2018b). Few data are available regarding dermal and inhalation risk assessments of fragrances. In the case of deodorants, inhalation and dermal exposure are considered the main exposure routes to consumers (Bickers et al. 2003; Schnuch et al. 2007). Basketter et al. (1999) reported that quantitative considerations in risk assessment need to include dose-response assessment, exposure assessment, and characterization of uncertainty. In addition, reliable exposure factors are essential in determining the health risks posed by the ingredients in products (Bickers et al. 2003). Englund, Hùglund, and Skjødt (2004) demonstrated that fragrance ingredients (isoeugenol, HICC, atranol, and chloroatranol) were present in 25 perfume products in the Danish market. The amounts of isoeugenol, HICC, atranol, and chloroatranol in perfumes were found to be 56%, 72%, 59%, and 36%, respectively. The concentration of isoeugenol was below the recommended maximum concentration of 0.02%. However, the maximum HICC concentration was determined to be 0.2%, which is 10-fold higher than the maximum tolerable concentration considered safe by the European Union (EU) Scientific Committee. The maximum content of fragrances in the products at concentrations of 15.6–36.94% and linalool was found at the highest level. The amount of benzyl alcohol, cinnamyl alcohol, citral, eugenol, α -amyl cinnamyl alcohol, benzyl salicylate, coumarin, geraniol, linal, linalool, benzyl benzoate, citronellol, hexyl cinnamaldehyde, limonene, and α -isomethyl ionone in deodorants showed maximal levels greater than 10,000 mg/kg. Certain deodorants contained benzyl benzoate at a maximal quantity of 142,344 mg/kg.

In the present study, 25 fragrance ingredients assessed in men's and women's products were quantified at concentrations of 6.45–87.1% and 19.74–88.16%, respectively (Figure 1). Fragrance ingredients that were most frequently found in men's and women's perfumes were D-limonene, linalool, linal, and citronellol, and are all classified as (extremely) weak sensitizers. Among these frequently utilized fragrances, linal and citronellol

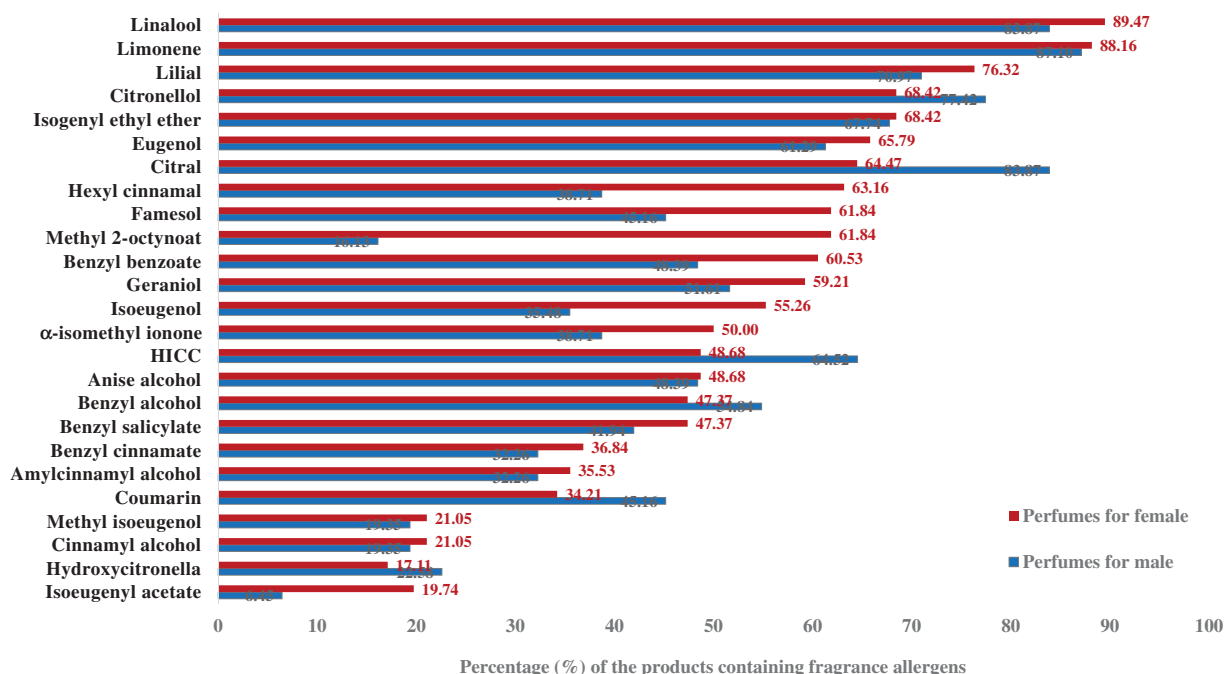


Figure 1. Percentage (%) of the products containing fragrance allergens in women's perfumes (n = 76) and men's perfumes (n = 31). Abbreviations: Lilial (butyl phenyl methyl propional), HICC (4-(4-hydroxy-4-methylpentyl)-3-cyclohexene-1-carbaldehyde), α-isomethyl ionone (3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one).

are classified as less important sensitizers, and D-limonene and linalool as rare sensitizers. In contrast, allergens that are seldom detected in products, such as farnesol, hydroxycitronellal, HICC, and isoeugenol are classified as important sensitizers in consumers. Mondello et al. (2007) also noted that 4 perfumes contained limonene (1,618–3,183 mg/kg), linalool (886–2,559 mg/kg), citronellol (43–880 mg/kg), citral (5–48 mg/kg), geraniol (36–98 mg/kg), cinnamaldehyde (N.D.–127 mg/kg), hydroxycitronellal (N.D.–254 mg/kg), cinnamyl alcohol (N.D.–37 mg/kg), eugenol (N.D.–103 mg/kg), coumarin (N.D.–1,460 mg/kg), α-isomethyl ionone (N.D.–1,030 mg/kg), lilial (N.D.–3,222 mg/kg), HICC (N.D.–1,679 mg/kg), and hexyl cinnamaldehyde (N.D.–90 mg/kg). The acceptable criterion for human safety is a margin of safety (MoS) >100, which indicates no significant risk for human health (Kang, Lee, and Shin 2014; Kim et al. 2013; Scientific Committee on Consumer Safety (SCCS) 2012). Corea et al. (2006) and Basketter et al. (2010) assessed the risk arising from the use of 24 known fragrance sensitizers employed in fabric-washing products. Exposure to the amounts of fragrance sensitizers in washed fabrics resulted

in a MoS exceeding 100 (safety threshold). However, the MoS of methyl heptene carbonate reported in washed fabrics was 55. Andersen et al. (2006) reported the daily exposure calculated for the highest measured content of fragrance substances in massage, body, and essential oils in the Danish market. Based upon the demonstrated NOEL values in numerous studies, the MoS for a 60-kg adult was calculated for the highest concentrations of fragrance substances found in massage, body, and essential oils. The MoS of benzyl alcohol, benzyl benzoate, benzyl cinnamate, cinnamal, citral, citronellol, coumarin, eugenol, geraniol, d-limonene, and linalool in selected massage and body oils were founded to be 135, 3,130, 12,200, 17,700, 350, 650, 182, 8,620, 373, 162, and 40, respectively. The MoS of benzyl alcohol, benzyl benzoate, cinnamal, citral, citronellol, eugenol, geraniol, d-limonene, and linalool in selected essential oils were noted to be 2,174, 74,000, 40,000, 185, 60, 20,600, 206, 368, and 385, respectively. Normally, a cosmetic product with a MoS greater than 100 is considered to be an acceptable safety risk.

In conclusion ingredients of the perfumes evaluated were shown to pose no apparent significant

health risk at the maximum concentration used, except for linal, HICC, citral, isoeugenol, and methyl 2-octynoate. This risk assessment approach is recommended to be used to establish improved guidelines for specific ingredients in consumer products, and for setting limits for newly developed raw ingredients that might pose potential dermal hazards.

Acknowledgment

This research was supported by the Ministry of Trade, Industry & Energy (MOTIE), Korea Institute for Advancement of Technology (KIAT) through the Encouragement Program for The Industries of Economic Cooperation Region

Conflicts of interest

The authors declare that there are no conflicts of interest.

References

- Andersen, D. N., R. D. Holmberg, J. R. Larsen, I. Søborg, and K. H. Cohr. 2006. Survey and health assessment of chemical substances in massage oils. Survey of chemical substances in consumer products, no. 78. Available at: https://www.researchgate.net/profile/KarlHeinz_Cohr/publication/267232731_Survey_and_health_assessment_of_chemical_substances_in_massage_oils/links/5492e7910cf225673b3e1054/Survey-and-health-assessment-of-chemical-substances-in-massage-oils.pdf
- Api, A. M., D. A. Basketter, P. A. Cadby, M. F. Cano, G. Ellis, G. F. Gerberick, P. Griem, P. M. McNamee, C. A. Ryan, and R. Safford. 2008. Dermal sensitization quantitative risk assessment (QRA) for fragrance ingredients. *Regul. Toxicol. Pharmacol.* 52:3–23. doi:10.1016/j.yrtph.2007.10.008.
- Api, A. M., D. Belsito, S. Bhatia, M. Bruze, P. Calow, M. L. Dagli, W. Dekant, A. D. Fryer, L. Kromidas, S. La Cava, J. F. Lalko, A. Lapczynski, D. C. Liebler, Y. Miyachi, V. T. Politano, G. Ritacco, D. Salvito, T. W. Schultz, J. Shen, I. G. Sipes, B. Wall, and D. K. Wilcox. 2016a. RIFM fragrance ingredient safety assessment, isoeugenol, CAS Registry Number 97-54-1. *Food Chem. Toxicol.* 97: S49–S56. doi:10.1016/j.fct.2015.12.021.
- Api, A. M., D. Belsito, S. Bhatia, M. Bruze, P. Calow, M. L. Dagli, W. Dekant, A. D. Fryer, L. Kromidas, S. La Cava, J. F. Lalko, A. Lapczynski, D. C. Liebler, Y. Miyachi, V. T. Politano, G. Ritacco, D. Salvito, T. W. Schultz, J. Shen, I. G. Sipes, B. Wall, and D. K. Wilcox. 2016b. RIFM fragrance ingredient safety assessment, Eugenol, CAS Registry Number 97-53-0. *Food Chem. Toxicol.* 97: S25–S37. doi:10.1016/j.fct.2015.12.013.
- Api, A. M., D. Belsito, S. Bhatia, M. Bruze, P. Calow, M. L. Dagli, W. Dekant, A. D. Fryer, L. Kromidas, S. La Cava, J. F. Lalko, A. Lapczynski, D. C. Liebler, Y. Miyachi, V. T. Politano, G. Ritacco, D. Salvito, J. Shen, I. G. Sipes, B. Wall, and D. K. Wilcox. 2015a. RIFM fragrance ingredient safety assessment, α -butylcinnamaldehyde, CAS Registry Number 7492-44-6. *Food Chem. Toxicol.* 84:S100–S109. doi:10.1016/j.fct.2015.09.006.
- Api, A. M., D. Belsito, S. Bhatia, M. Bruze, P. Calow, M. L. Dagli, W. Dekant, A. D. Fryer, L. Kromidas, S. La Cava, J. F. Lalko, A. Lapczynski, D. C. Liebler, V. T. Politano, G. Ritacco, D. Salvito, T. W. Schultz, J. Shen, I. G. Sipes, B. Wall, and D. K. Wilcox. 2015b. RIFM fragrance ingredient safety assessment, Linalool, CAS registry number 78-70-6. *Food Chem. Toxicol.* 82:S29–S38. doi:10.1016/j.fct.2015.01.005.
- Api, A. M., D. Belsito, S. Bhatia, M. Bruze, P. Calow, M. L. Dagli, W. Dekant, A. D. Fryer, L. Kromidas, S. La Cava, J. F. Lalko, A. Lapczynski, D. C. Liebler, Y. Miyachi, V. T. Politano, G. Ritacco, D. Salvito, J. Shen, I. G. Sipes, B. Wall, and D. K. Wilcox. 2015c. RIFM fragrance ingredient safety assessment, α -amylcinnamaldehyde, CAS registry number 122-40-7. *Food Chem. Toxicol.* 82:S20–S28. doi:10.1016/j.fct.2015.01.008.
- Api, A. M., D. Belsito, S. Bhatia, M. Bruze, P. Calow, M. L. Dagli, W. Dekant, A. D. Fryer, L. Kromidas, S. La Cava, J. F. Lalko, A. Lapczynski, D. C. Liebler, V. T. Politano, G. Ritacco, D. Salvito, T. W. Schultz, J. Shen, I. G. Sipes, B. Wall, and D. K. Wilcox. 2015d. RIFM fragrance ingredient safety assessment, Benzyl alcohol, CAS Registry Number 100-51-6. *Food Chem. Toxicol.* 50:S1–S14.
- Api, A. M., and M. Vey. 2008. Implementation of the dermal sensitization quantitative risk. *Regul. Toxicol. Pharmacol.* 52:53–61. doi:10.1016/j.yrtph.2008.05.011.
- Basketter, D. A., C. J. Clapp, B. J. Safford, I. R. Jowsey, P. McNamee, C. A. Ryan, and F. G. Gerberick. 2008. Preservatives and skin sensitization quantitative risk assessment. *Dermatitis* 19:20–27.
- Basketter, D. A., L. J. Lea, A. Dickens, D. Briggs, I. Pate, R. J. Dearman, and I. Kimber. 1999. A comparison of statistical approaches to the derivation of EC3 values from local lymph assay dose responses. *J. Appl. Toxicol.* 19:261–66.
- Basketter, D. A., A. Pons-Guiraud, A. van Asten, C. Laverdet, J. P. Marty, L. Martin, D. Berthod, S. Siest, F. Giordano-Labadie, D. Tennstedt, M. Baeck, M. Vigan, G. Lainé, C. J. Le Coz, M. C. Jacobs, O. Bayrou, and M. A. Germaux. 2010. Fragrance allergy: Assessing the safety of washed fabrics. *Contact Derm.* 62:349–54. doi:10.1111/j.1600-0536.2010.01728.x.
- Bhatia, S. P., A. Wellington, J. Cocchiara, J. Lalko, C. S. Letizia, and A. M. Api. 2007. Fragrance material review on cinnamyl cinnamate. *Food Chem. Toxicol.* 45: S66–S69. doi:10.1016/j.fct.2007.09.030.
- Bickers, D. A., P. Calow, H. Greim, J. M. Hanifin, A. E. Rogers, J. H. Saurat, I. G. Sipes, R. L. Smith, and H. Tagami. 2005. Toxicologic and dermatologic assessment of cinnamyl alcohol, cinnamaldehyde and cinnamic acid when used as fragrance ingredients. *Food Chem. Toxicol.* 43:799–836. doi:10.1016/j.fct.2004.09.013.

- Bickers, D. R., P. Calow, H. A. Greim, J. M. Hanifin, A. E. Rogers, J. H. Saurat, S. I. Glenn, R. L. Smith, and H. Tagami. 2003. The safety assessment of fragrance materials. *Regul. Toxicol. Pharmacol.* 37:218–73.
- Cano, M. F. 2006. Personal communication on exposure data for hydroalcoholic products for shaved skin. *Chem. Toxicol.* 40:1719–26.
- Cano, M. F., and K. Rich. 2001. Presentation at the 2001 Colipa Scientific Forum.
- Cheng, J., and K. A. Zug. 2014. Fragrance allergic contact dermatitis. *Dermatitis* 25:232–45. doi:10.1097/DER.0000000000000067.
- Choi, S. M., T. H. Roh, D. S. Lim, S. Kacew, H. S. Kim, and B. M. Lee. 2018. Risk assessment of benzalkonium chloride in cosmetic products. *J. Toxicol. Environ. Health B* 21:8–23.
- Corea, N. V., D. A. Basketter, C. Clapp, A. Van-Asten, J. P. Marty, A. Pons-Guiraud, and C. Laverdet. 2006. Fragrance allergy: Assessing the risk from washed fabrics. *Contact Derm.* 55:48–53. doi:10.1111/j.0105-1873.2006.00872.x.
- EC (European Commission). 1999. Opinion Concerning Fragrance Allergy in Consumers. SCCNFP. 8. Available at: http://europa.eu.int/comm/health/ph_risk/committees/sccp/documents/out98_en.pdf.
- EC (European Commission). 2000. Opinion concerning methyleugenol adopted by the SCCNFP during the 14th plenary meeting of 24 October 2000. SCCNFP. 2000 Oct 24. Available at: http://europa.eu.int/comm/health/ph_risk/committees/sccp/sccp_opinions_en.htm.
- EC (European Commission). 2003. Directive 2003/15/EC of the European Parliament and of the Council of 27 february 2003 amending Council Directive 76/768/EEC on the approximation of the laws of the Member States relating to cosmetic products.
- Engelund, B., L. Høglund, and D. Skjødt. 2004. Survey of chemical substances in consumer products no. 43. Mapping of stain removers. *Danish EPA*
- Feldmann, R. J., and H. I. Maibach. 1967. Regional variation in percutaneous penetration of ¹⁴C Cortisol in man. *J. Invest. Dermatol.* 48:181–83.
- Frosch, P. J., B. Pilz, K. E. Andersen, D. Burrows, J. G. Camarasa, A. Dooms-Goossens, G. Ducombs, T. Fuchs, M. Hannuksela, and J. M. Lachapelle. 1995. Patch testing with fragrances: Results of a multicenter study of the European Environmental and Contact Dermatitis Research Group with 48 frequently used constituents of perfumes. *Contact Derm.* 33:333–42.
- Gerberick, G. F., M. K. Robinson, C. A. Ryan, R. J. Dearman, I. Kimber, D. A. Basketter, Z. Wright, and J. G. Marks. 2001. Contact allergenic potency: Correlation of human and local lymph node assay data. *Am. J. Contact Dermatitis* 12:156–61. doi:10.1053/ajcd.2001.23926.
- JECFA (Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives). 2010. *Summary and conclusions of the seventy-second meeting of the Joint FAO/WHO Expert Committee on Food Additives*, 16–25. Rome: FAO/WHO.
- Kang, B., B. M. Lee, and H. S. Shin. 2014. Determination of polycyclic aromatic hydrocarbon (PAH) content and risk assessment from edible oils in Korea. *J. Toxicol. Environ. Health Part A* 77:1359–71. doi:10.1080/15287394.2014.951593.
- Karlberg, A. T., K. Magnusson, and U. Nilsson. 1992. Air oxidation of d-limonene (the citrus solvent) creates potent allergens. *Contact Derm.* 26:332–40.
- Karlberg, A. T., L. P. Shao, U. Nilsson, E. Gafvert, and J. L. Nilsson. 1994. Hydroperoxides in oxidized d-limonene identified as potent contact allergens. *Arch. Dermatol. Res.* 286:97–103.
- Kim, J. H., T. Kim, H. Yoon, A. Jo, D. Lee, P. Kim, and J. Seo. 2018b. Health risk assessment of dermal and inhalation exposure to deodorants in Korea. *Sci. Total Environ.* 625:1369–79. doi:10.1016/j.scitotenv.2017.12.282.
- Kim, J. H., D. Lee, H. Lim, T. Kim, K. Suk, and J. Seo. 2018c. Risk assessment to human health: Consumer exposure to ingredients in air fresheners. *Regul. Toxicol. Pharmacol.* 98:31–40. doi:10.1016/j.yrtph.2018.05.015.
- Kim, K. B., Y. W. Kim, S. K. Lim, T. H. Roh, D. Y. Bang, S. M. Choi, D. S. Lim, Y. J. Kim, S. H. Baek, M. K. Kim, H. S. Seo, M. H. Kim, H. S. Kim, J. Y. Lee, S. Kacew, and B. M. Lee. 2017. Risk assessment of zinc oxide, a cosmetic ingredient used as a UV filter of sunscreens. *J. Toxicol. Environ. Health B* 20:155–82. doi:10.1080/10937404.2017.1290516.
- Kim, M. K., K. B. Kim, K. Yoon, S. Kacew, H. S. Kim, and B. M. Lee. 2018a. IL-1 α and IL-1 β as alternative biomarkers for risk assessment and the prediction of skin sensitization potency. *J. Toxicol. Environ. Health Part A* 18:1–14.
- Kim, Y. W., M. J. Kim, B. Y. Chung, D. Y. Bang, S. K. Lim, S. M. Choi, D. S. Lim, M. C. Cho, K. Yoon, H. S. Kim, K. B. Kim, Y. S. Kim, S. J. Kwack, and B. M. Lee. 2013. Safety evaluation and risk assessment of d-Limonene. *J. Toxicol. Environ. Health B* 16:17–38.
- Lalko, J., and A. M. Api. 2008. Citral: Identifying a threshold for induction of dermal sensitization. *Regul. Toxicol. Pharmacol.* 52:62–73. doi:10.1016/j.yrtph.2008.01.006.
- Lapczynski, A., J. Lalko, V. T. Politano, D. McGinty, S. Bhatia, C. S. Letizia, and A. M. Api. 2007. Fragrance material review on alpha-iso-methylionone. *Food Chem. Toxicol.* 45:S280–289. doi:10.1016/j.fct.2007.09.051.
- Larsen, J. R., and R. D. Holmberg. 2005. Survey of chemical substances in consumer products No. 55. Survey of lip care products with fragrance and flavour. In *Danish EPA*.
- Larsen, W., H. Nakayama, T. Fischer, P. Elsner, P. Frosch, D. Burrows, W. Jordan, S. Shaw, J. Wilkinson, J. Marks, M. Sugawara, M. Nethercott, and J. Nethercott. 2001. Fragrance contact dermatitis: A worldwide multicenter investigation (Part 2). *Contact Derm.* 44:344–46.
- Larsen, W., H. Nakayama, T. Fischer, P. Elsner, P. Frosch, D. Burrows, W. Jordan, S. Shaw, J. Wilkinson, J. Marks, M. Sugawara, M. Nethercott, and J. Nethercott. 2002. Fragrance contact dermatitis - A worldwide multicenter investigation (Part III). *Contact Derm.* 46:141–44.
- Lim, D. S., T. H. Roh, M. K. Kim, Y. C. Kwon, S. M. Choi, S. J. Kwack, K. B. Kim, S. Yoon, H. S. Kim, and B. M. Lee.

- 2018a. Risk assessment of N-nitrosodiethylamine (NDEA) and N-nitrosodiethanolamine (NDELA) in cosmetics. *J. Toxicol. Environ. Health Part A* 81:465–80.
- Lim, D. S., T. H. Roh, M. K. Kim, Y. C. Kwon, S. M. Choi, S. J. Kwack, K. B. Kim, S. Yoon, H. S. Kim, and B. M. Lee. 2018b. Non-cancer, cancer, and dermal sensitization risk assessment of heavy metals in cosmetics. *J. Toxicol. Environ. Health Part A* 81:432–52. doi:10.1080/15287394.2018.1451191.
- Loretz, L. J., A. M. Api, L. M. Barraj, J. Burdick, D. A. Davis, W. Dressler, E. Gilberti, G. Jarret, S. Mann, L. Pan, T. Re, K. Renskers, C. Scrafford, and S. Vater. 2006. Exposure data for personal care products: Hairspray, spray perfume, liquid foundation, shampoo, body wash, and solid antiperspirant. *Food Chem. Toxicol.* 44:2008–18. doi:10.1016/j.fct.2006.06.020.
- Masuck, I., C. Hutzler, and A. Luch. 2011. Estimation of dermal and oral exposure of children to scented toys: Analysis of the migration of fragrance allergens by dynamic headspace GC-MS. *J. Sep. Sci.* 34:2686–296. doi:10.1002/jssc.201100360.
- Mattia, A. 2005. WHO FOOD ADDITIVES SERIES 46: CinnamylAlcohol and Related Substances 1-50. Available at: <http://www.inchem.org/documents/jecfa/jecmono/v46je07.htm>.
- Matura, M., A. Goossens, O. Bordalo, B. Garcia-Bravo, K. Magnusson, K. Wrangsjö, and A. T. Karlberg. 2002. Oxidized citrus oil (R-limonene): A frequent skin sensitizer in Europe. *J. Am. Acad. Dermatol.* 47:709–14.
- Mondello, L., D. Sciarone, A. Casilli, P. Q. Tranchida, P. Dugo, and G. Dugo. 2007. Fast gas chromatography-full scan quadrupole mass spectrometry for the determination of allergens in fragrances. *J. Sep. Sci.* 30:1905–11. doi:10.1002/jssc.200600541.
- Nardelli, A., A. Carbonez, W. Ottoy, J. Drieghe, and A. Goossens. 2008. Frequency of and trends in fragrance allergy over a 15-year period. *Contact Derm.* 58:134–41. doi:10.1111/j.1600-0536.2007.01287.x.
- Nohynek, G. J., E. Antignac, T. Re, and H. Toutain. 2010. Safety assessment of personal care products/cosmetics and their ingredients. *Toxicol. Appl. Pharmacol.* 243:239–59. doi:10.1016/j.taap.2009.12.001.
- NTP (National Toxicology Program). 1989. Support for chemical nomination and selection cinnamaldehyde. Available at: https://ntp.niehs.nih.gov/ntp/htdocs/chem_background/
- OECD (The Organisation for Economic Co-operation and Development). 2001. OECD Screening Information DataSet (SIDS). Citral, CAS No:5392-40-5. 1–113. UNEP Publications. Available at: <http://www.inchem.org/documents/sids/sids/5392-40-5.pdf>
- Pronk, I. M. E. J. 2004. WHO FOOD ADDITIVES SERIES: 52. Aliphatic branched-chain saturated and unsaturated alcohols, aldehydes, acids, and related esters. 1–37. Available at: <http://www.inchem.org/documents/jecfa/jecmono/v52je15.htm>
- Pybus, D. H., and C. S. Shell. 1999. The Chemistry of Fragrances. *The Royal Society of Chemistry*, Cambridge, UK.
- Rastogi, S. C., and J. D. Johansen. 2008. Significant exposures to isoeugenol derivatives in perfumes. *Contact Derm.* 58:278–81. doi:10.1111/j.1600-0536.2007.01283.x.
- Rastogi, S. C., J. D. Johansen, and R. Bossi. 2007. Selected important fragrance sensitizers in perfumes—Current exposures. *Contact Derm.* 56:201–04. doi:10.1111/j.1600-0536.2007.01067.x.
- Rastogi, S. C., T. Menne, and J. D. Johansen. 2003. The composition of fine fragrances is changing. *Contact Derm.* 48:130–32.
- Ryan, C., L. Cruse, R. Skinner, R. Dearman, I. Kimber, and G. Gerberick. 2002. Examination of a vehicle for use with water soluble materials in the murine local lymph node assay. *Food Chem. Toxicol.* 40:1719–25.
- SCCNFP (Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers). 2003. Opinion of the Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers Concerning An update of the initial list of perfumery materials which must not form part of fragrance compounds used in cosmetic products. Available at: http://ec.europa.eu/health/ph_risk/committees/sccp/documents/out252_en.pdf
- SCCS (Scientific Committee on Consumer Safety). 2012. The SCCS's Notes of Guidance for the testing of cosmetic substances and their safety evaluation 8th revision. Available from: https://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_s_006.pdf
- Scheinman, P. L. 1996. Allergic contact dermatitis to fragrance: A review. *Am. J. Contact Dermatitis* 7:65–76.
- Scheinman, P. L. 2002. Prevalence of fragrance allergy. *Dermatology (Basel)* 205:98–102. doi:10.1159/000063156.
- Schnuch, A., W. Uter, J. Geier, H. Lessmann, and P. J. Frosch. 2007. Sensitization to 26 fragrances to be labelled according to current European regulation. Results of the IVDK and review of the literature. *Contact Derm.* 57:1–10. doi:10.1111/j.1600-0536.2007.01088.x.
- Tozer, S. A., L. O'Keeffe, C. E. Cowan-Ellsberry, and K. Rich. 2004. Use of probabilistic analysis in the refinement of exposure data for hydroalcoholic perfume products. *Toxicology* 202:123–24.
- WHO/IPCS. 2012. Guidance for Immunotoxicity Risk Assessment for Chemicals. Harmonization Project Document No. World Health Organization (JECFA/72/SC). Available at: http://www.who.int/foodsafety/chem/summary72_rev.pdf