Inhalation Toxicology

http://informahealthcare.com/iht
ISSN: 0895-8378 (print), 1091-7691 (electronic)

Inhal Toxicol, 2014; 26(5): 310–318

DOI: 10.3109/08958378.2014.888110

informa healthcare

RESEARCH ARTICLE

Assessment of the risk of respiratory sensitization from fragrance allergens released by air fresheners

Wouter ter Burg[1], Krista Bouma[2], Durk J. Schakel[2], Susan W. P. Wijnhoven[1], Jacqueline van Engelen[1], Henk van Loveren[1,3], and Janine Ezendam[1]

[1]National Institute for Public Health and the Environment, Bilthoven, The Netherlands, [2]Netherlands Food and Consumer Product Safety Authority (NVWA), Utrecht, The Netherlands, and [3]Department of Toxicogenomics, Maastricht University, Maastricht, The Netherlands

Abstract

Consumers using air fresheners are exposed to the emitted ingredients, including fragrances, via the respiratory tract. Several fragrances are known skin sensitizers, but it is unknown whether inhalation exposure to these chemicals can induce respiratory sensitization. Effects on the immune system were assessed by testing a selection of five fragrance allergens in the respiratory local lymph node assay (LLNA). The probability and extent of exposure were assessed by measuring concentrations of the 24 known fragrance allergens in 109 air fresheners. It was shown that the most frequently used fragrances in air fresheners were D-limonene and linalool. In the respiratory LLNA, these fragrances were negative. Of the other tested chemicals, only isoeugenol induced a statistically significant increase in cell proliferation. Consumer exposure was assessed in more detail for D-limonene, linalool, and isoeugenol by using exposure modeling tools. It was shown that the most frequently used fragrances in air fresheners, D-limonene, and linalool gave rise to a higher consumer exposure compared with isoeugenol. To evaluate whether the consumer exposure to these fragrances is low or high, these levels were compared with measured air concentrations of diisocyanates, known human respiratory sensitizers. This comparison showed that consumer exposure from air fresheners to D-limonene, linalool, and isoeugenol is considerably lower than occupational exposure to diisocyanates. By combing this knowledge on sensitizing potency with the much lower exposure compared to diisocyanates it seems highly unlikely that isoeugenol can induce respiratory sensitization in consumers using air fresheners.

Keywords

Air fresheners, fragrance allergens, inhalation exposure, respiratory LLNA, respiratory sensitization

History

Received 25 November 2013
Revised 13 January 2014
Accepted 23 January 2014
Published online 12 March 2014

Introduction

Already in ancient times, people were attracted to products with a pleasant smell. Nowadays, the selection of scented consumer products extends from perfumes to personal care products, cleaning products, air fresheners, home perfumes, and toys. Fragrances are important ingredients in these products, but some of these substances are known to cause allergic contact dermatitis by skin exposure (Uter et al., 2010). In the European Union, 24 fragrance chemicals and two botanical extracts (oak moss and tree moss) have been identified as human skin sensitizers (Schnuch et al., 2007). It is estimated that 1% of the general population suffers from contact allergy to fragrances, making these chemicals the second most frequent cause of contact allergy after metals (Schnuch et al., 2002).

Besides skin exposure, consumers are exposed via the airways to ingredients emitted from scented consumer products, such as air fresheners, cleaning sprays, and toys (Masuck et al., 2011). In the globally harmonized system (GHS) for classification and labeling, skin and respiratory sensitizers are classified in different hazard classes; respectively, H317 and H334. For respiratory sensitizers, it has been shown that the skin can be an important route of exposure for sensitization. Evidence for this is derived predominantly from animal studies (van Triel et al., 2011; Vandebriel et al., 2000; Vanoirbeek et al., 2003). There is limited evidence that in humans this is true as well, although for human exposure it is not always possible to trace back the exact routes of exposure (Redlich, 2010; Heederik et al., 2012).

It is still a matter of debate, whether inhalation of skin sensitizers can induce sensitization of the airways. There is some evidence from animal studies that this is the case (Arts et al., 1998; Garssen et al., 1991; van Triel et al., 2010). In most of these studies, strong skin sensitizers were used, but effects of inhalation exposure to weak or moderate sensitizers, such as fragrance allergens, have not been studied. Other studies, however, fail to demonstrate that inhalation of skin sensitizers induced sensitization or respiratory symptoms (Farraj et al., 2004; Henjakovic et al., 2008; Vanoirbeek et al., 2006). Human evidence for the effects of inhalation exposure to skin sensitizers is scarce. Two case studies showed

Address for correspondence: Janine Ezendam, National Institute for Public Health and the Environment, P.O. Box 1, 3720 BA Bilthoven, The Netherlands. Tel: +31 30 2743447. Fax: +31 30 274 4446. E-mail: Janine.Ezendam@rivm.nl

occupational inhalation exposure to fragrances might induce respiratory allergy. First, a saleswoman working in a perfumery suffered from respiratory distress at work and the allergic symptoms could be reproduced in the hospital after inhalation challenges with different perfumes (Baur et al., 1999). In a second case study, it was shown that rhinitis and asthma in a hair dresser could be reproduced after inhalation challenge with the fragrance eugenol (Quirce et al., 2008).

Assessment of respiratory sensitization hazard is hampered by the lack of validated or widely accepted animal models that can identify these substances (Basketter & Kimber, 2011; Kimber et al., 2007, 2011). Several animal models have been described, all using different routes of exposure and read-outs (Arts & Kuper, 2007). The respiratory local lymph node assay (LLNA) was developed as a short-term exposure model able to identify potential respiratory sensitizers. In the respiratory LLNA, mice are exposed on three consecutive days by inhalation to the test substance. Cell proliferation and cytokine responses in the draining mandibular lymph nodes are used as read-outs (Arts et al., 2008; De Jong et al., 2009; van Triel et al., 2011). We have used this model to investigate whether fragrance allergens can have an effect on the immune system after inhalatory exposure. This model has not been extensively validated and only a limited number of strong respiratory and skin sensitizers were tested up to now. Moderate to weak sensitizers, such as fragrance allergens, have not been tested. Therefore, this model cannot be used for hazard identification, but is used as a tool to assess whether inhalation of fragrances can stimulate the immune system. The risk for consumers exposed to fragrance allergens is dependent not only on the respiratory sensitization potential of this substance but also on the level of exposure. Therefore, the concentrations of 24 fragrance allergens in air fresheners, available in Dutch stores, were measured. The product concentrations were used to calculate consumer exposure for a selection of fragrance allergens for trigger sprays, spray cans, evaporators, and scented blocks. The results of the respiratory LLNA together with the exposure assessment were used to evaluate whether consumers are at risk for respiratory sensitization when they use air fresheners.

Methods

Chemical selection for the respiratory LLNA

In the respiratory LLNA, five fragrance allergens were tested (Table 1). Fragrances were selected either based on their skin sensitizing potency in the LLNA (EC3 values) (Gerberick et al., 2005; SCCS, 2012) or on the frequency of use as ingredient in scented consumer products (Table 2). The fragrances benzyl salicylate (99% purity), isoeugenol (98% purity), and linalool (purity >97%) were purchased from Sigma (St. Louis, MO). Citral (purity >95%) and D-limonene (purity >99%) were purchased from Fluka (Buchs, Switzerland).

Table 1. Skin-sensitizing potency of the fragrances tested in the respiratory LLNA.

Fragrance	LLNA EC3 value skin[a] (%)
Isoeugenol	1.5
Benzyl salicylate	1.5
Citral	1.5–5.6%
d-Limonene	10–69%
Linalool	30–46%

[a]EC3 values represent the concentration that induces a 3-fold increase of cell proliferation in the LLNA and is expressed in % (derived from Gerberick et al., 2005; SCCS, 2012).

Mice

Male BALB/c mice were housed in polycarbonate cages under conventional conditions in light-, humidity-, and temperature-controlled rooms. The mice were fed a standard pellet diet (RM3 [E] SQC, Special Diet Service, Witham, UK) and unfluoridated tap water *ad libitum*. All other husbandry conditions were maintained according to all applicable provisions of the Experiments on Animals Decree and Experiments on Animals Act. All animal experiments had permission from the Commission of Animal Welfare of the Dutch National Institute for Public Health and the Environment.

Experimental design

The respiratory LLNA was performed as described previously (Arts et al., 2008). In short, groups of male BALB/c mice (six animals per group) were exposed nose-only to one of the fragrance chemicals on three consecutive days for 45, 90, 180, or 360 min/d. Control mice were exposed nose-only to the vehicle for 360 min/d.

All fragrances were nebulized in acetone to produce an aerosol of liquid droplets in a target concentration of 75 mg/m^3. Nebulization of benzyl salicylate resulted predominantly in aerosols, whereas nebulization of isoeugenol, citral, D-limonene, and linalool resulted exclusively in vapour

Table 2. Presence and weight fractions of fragrances allergens in air fresheners.

	Presence[a] (%)	Weight fraction[b] (%)
Linalool	87	0.827 (15.03)
D-Limonene	69	0.342 (6.34)
Geraniol	50	0.043 (0.272)
Lilial	50	0.053 (0.732)
Citronellol	46	0.129 (3.06)
Benzyl alcohol	44	0.104 (2.94)
Hexyl cinnamal	42	0.078 (0.927)
Coumarine	32	0.099 (0.994)
γ-Isomethylionone	30	0.210 (2.837)
Eugenol	28	0.072 (0.876)
Benzyl salicylate	28	0.022 (0.130)
Citral	26	0.158 (1.156)
Benzyl benzoate	20	0.052 (0.231)
Amyl cinnamal	17	0.084 (0.914)
Lyral	14	0.038 (0.249)
Hydroxycitronellal	14	0.010 (0.045)
Cinnamyl alcohol	11	0.115 (0.855)
Cinnamal	6	0.080 (0.492)
Isoeugenol	6	0.027 (0.150)
Benzyl cinnamate	2	0.027 (0.053)

[a]The presence of a fragrance is expressed as the percentage of air fresheners ($n = 109$) that contain this fragrance.
[b]For each fragrance, the concentration is expressed as the weight fraction. This table provides the mean and maximum (in brackets) values (in %) based on measurements in 109 air fresheners.

(not shown). The aerosols were sampled on 47 mm Teflon filters at a flow rate of 1 l/min for 5 min. The collected mass was determined gravimetrically immediately after sampling to minimize evaporations of the collected droplets and used for concentration calculations. The vapor in this mixture downstream of the filters was also sampled on activated charcoal. In addition, the test atmosphere was sampled at a flow rate of approximately 1 l/min for 5 min on activated charcoal and these were used for wet chemical determinations and used to calculate the average actual concentrations during the exposures. The actual air concentrations measured were close to the target concentration of 75 mg/m^3. The fluctuations of all test atmospheres on the 3 d of exposure were less than 10% as indicted by continuous mass concentration measurements using a total carbon analyser (TEA).

Mice were necropsied 3 d after the last exposure and mandibular lymph nodes (LN) were excised, pooled for each animal, and suspended in 5 ml RPMI 1640 (Gibson, Life Technologies, Breda, The Netherlands) with 5% heat inactivated fetal calf serum (FCS) (Integro, Zaandam, The Netherlands), 100 U/ml penicillin, and 100 µg/ml streptomycin (standard medium). Cell proliferation was measured *ex vivo* using [^{3}H]-thymidine incorporation and is expressed per animal. Stimulation indices (SI) were calculated by dividing the [^{3}H]-thymidine incorporation of the exposed mice with the mean [^{3}H]-thymidine incorporation of the vehicle group.

Calculation of potency in the respiratory LLNA

In the respiratory LLNA, potency is derived from the dose–response curves by plotting cell proliferation against the duration of exposure. PROAST software (RIVM, Bilthoven, The Netherlands; http://www.rivm.nl/en/Documents_and_publications/Scientific/Models/PROAST) was used to perform a non-linear regression analysis (Slob, 2002) in order to determine the duration of exposure (min) at which a 3-fold increase in proliferation was induced. The ED3 value, which is the estimated dose at which this 3-fold induction is induced is then calculated using the mean actual exposure concentration (75 mg/m^3), the duration of exposure (min) at which a 3-fold increase in proliferation was obtained, the mean body weight, and a standard ventilation rate of 1.5 l/kg mice. Absorption via the lungs was assumed to be 100% (described in detail in Arts et al., 2008).

Statistical analysis

In the respiratory LLNA, proliferation results were statistically analyzed using a one-way analysis of variance (ANOVA). Significant differences of the control group were determined with the Bonferroni *post hoc* test, using a significance level of $p \leq 0.05$.

Measurement of the levels of fragrance allergens in scented consumer products

A total of 109 air fresheners were sampled from Dutch stores in January 2009. The products were divided in four categories: spray cans (aerosols) ($n = 37$), trigger sprays (pump or trigger mechanism) ($n = 13$), liquid evaporators ($n = 38$), and scented blocks ($n = 18$). Three remaining products could not be placed in one of these categories. The concentrations of the 24 fragrances (Table 2) were measured in these products. The botanical extracts oak moss and tree moss, which contain fragrance allergens as well, were not included in this analysis.

The concentrations were determined by the gas chromatography. In short, a proportion of content of the air freshener was transferred to a headspace vial. Then a volume of 2 ml of the product was mixed with 10 ml acetone. This mixture was injected into a gas chromatograph (CPWax 52 CB en CPSil 5 CB column). Detection of the fragrances was done in the total ion mode. Specific target ions were used to quantify each component.

Exposure assessment for spray applications

To perform the exposure assessment of fragrance allergens in air fresheners, the exposure modeling tools ConsExpo 4.1 (RIVM, Bilthoven, The Netherlands) and the RIVM Emission tool (both freely available from www.consexpo.nl) were used to assess the exposure for the four product categories. To compare the exposure to the selected fragrances released from these products (see results for substance selection and substance specific input parameters), a generic exposure scenario for spray applications was described that was adapted for product specific properties only to ensure that differences are the result of product-substance characteristics and not based on scenario settings (Table 3).

Exposure scenarios for sprays and passive room perfumes have been described previously (Park et al., 2006), which

Table 3. Generic input parameters for exposure assessment of air fresheners per product category.

	Spray cans	Trigger sprays		Evaporators	Scented blocks
Input parameters	Instantaneous release	Spray model	Instantaneous release	Evaporation	Emission model
Amount used (g)	1.5	1.5	1.5	375	150
Room volume (m^3)	10	10	10	58	58
Room ventilation (1/h)	2	2	2	0.5	0.5
Use frequency	5/d	5/d	5/d	1/4 week	1/4 week
Exposure duration (min)	10	10	10	672	672
Airborne fraction	–	0.02	–		
Inhalation cut-off (µm)	–	15	–		
Spray duration (s)	–	1	–		
Median particle size distribution (CV) (µm)	–	3.9 (0.65)	–		
Release area (cm^2)				30	30

wk, weeks; CV, coefficient of variation.

partly are adopted. Spray applications (spray cans and trigger sprays) have a high initial peak release to the air. For these applications, peak (event) exposure is assessed, since this is the most relevant exposure metric. For spray cans and trigger sprays, two different exposure models were considered in ConsExpo 4.1. The ''instantaneous release model'' assumes that all substances are released at once, which is a worst-case assumption, considering the very fast evaporation of volatiles from aerosols during the spray process due to a very large surface area of the aerosols. The ''exposure to spray model'' describes the exposure to non-volatiles, wherein the model assumes that volatiles are evaporated immediately, leaving only aerosols. The decision on which of the two models to use is based on the volatility of the substance of interest.

As a reasonable worst-case scenario, the use of a spray can or trigger spray in the bathroom (relatively small room, volume 10 m^3) was considered. The amount used is estimated by multiplying the spray duration (1 s) with the mass generation rate, i.e. 1.5 g. Experimental results of a study on spray cans and trigger sprays demonstrated that the mass generation rate for air refresher spray cans ranged from 1 to 2 g/s (Delmaar & Bremmer, 2009). A mass generation rate of 1.5 g/s was taken as a default value. The same scenario (use in a bathroom) is considered for trigger sprays where only the product characteristics and use will be adjusted. The particle size distribution and airborne fractions are different for trigger sprays compared with spray cans. Trigger sprays will generally release larger aerosols and thus lower fractions are available for inhalation. However, as no recent measurement data are available for trigger spray air fresheners, the same particle distribution was assumed as for spray cans, i.e. a median of 3.9 μm and a coefficient of variation (CV) of 0.65 (Delmaar & Bremmer, 2009).

Exposure assessment for evaporators and scented blocks

As both evaporators and scented blocks have slow long lasting releases, constant (daily) exposure is the relevant exposure to take into account. The scenario for passive room perfumes can be used for both evaporators and scented blocks (adapted from Park et al., 2006) (Table 3). Evaporators are generally used as room perfumes, which can be used throughout the house (including the bedroom) or in cars. According to the survey, the amount of product in a passive room perfume, in the form of a gel or liquid, ranges from 6 to 375 ml. The product is released over several weeks, ranging from 4 to 8 weeks. The product amount released in a day can therefore range from 0.1 to 5 g/d, assuming a specific weight of approximately 1 g/cm^3. The use of a room perfume in the living room was considered. The environmental settings of the living room are 58 m^3 and with a ventilation rate of 0.5 h^{-1}. Product use assumes that all products are dispersed, i.e. 375 ml (approximately 375 g). The worst-case exposure duration is set at 4 weeks, which is equal to duration of 672 h. The release area is estimated at 30 cm^2. The evaporation model further requires data on the mass transfer rate (measure for release from matrix) and the molecular weight of the matrix. Since information is lacking, by default, the Thibodeaux method is used to determine the mass transfer rate and 3000 g/mol was taken forward as a molecular weight matrix, the latter being worst case assumptions for the input parameter as it would approximate the evaporation of a pure substance (for details on molecular weight matrix, see Bremmer et al., 2006).

Scented blocks are generally used as room perfumes, which can be used throughout the house (including the bedroom) or as toilet perfumes. They are very similar to liquid or gel evaporators and may be used interchangeably for the same purpose. For this reason, exposure parameters as release area and environmental settings of evaporators were used for scented blocks. All scented blocks in the measurements had a product size of 150 g. Emission of a substance from solid materials is dependent on the diffusion of the substance through the material and the mass transfer rate from the material to the air (often described by a partition coefficient). Such parameter values are not commonly available and thus the data and methods described previously by Delmaar (2011) have been used to determine the input parameters and to assess the exposure from scented blocks. The diffusion coefficients for substances in a similar range of molecular weights that are used in similar matrices were considered; they ranged from 1×10^{-14} to 1×10^{-10} m^2/s. As a worst case, the upper value was used. The partition coefficient was calculated using either Raoult's law or an equation based on empirical data (described in Delmaar, 2011). The results proved to be rather insensitive to changes in the partition coefficient, and therefore the results from the equation were used (see Table 3 with substance-specific data). The model only allowed inserting deterministic data, and therefore the median weight fraction was used in the calculations. In Table 3, an overview of the input parameters is given for the product-specific scenarios.

Results

Effects of fragrance allergens in the respiratory LLNA

Inhalation exposure to the five tested fragrance chemicals did neither induce any macroscopically visible toxic effects nor affected body weight gain (not shown). The effects of the individual fragrances on cell proliferation in the mandibular lymph nodes are shown in Figure 1. The only fragrance that significantly increased cell number and proliferation in the mandibular lymph nodes was isoeugenol. After 45 min/d exposure, the cell proliferation was already significantly increased more than four-fold compared with the control group. At the time points, 90 min/d and 180 min/d SI values do not further increase and appear to reach a plateau of 3.5-fold. The variation within the experimental groups is relatively high, and these changes were not statistically significant. Prolonged exposure (for 360 min/d) did induce a further increase of cell proliferation to an SI value of 7.2. This increase was statistically significant compared with the vehicle control group. Benzyl salicylate and citral both increased cell proliferation, but these changes were not statistically significant.

Dose–response information of isoeugenol was used to calculate the ED3 value, a measure for the potency of chemicals in the respiratory LLNA. The ED3 value for

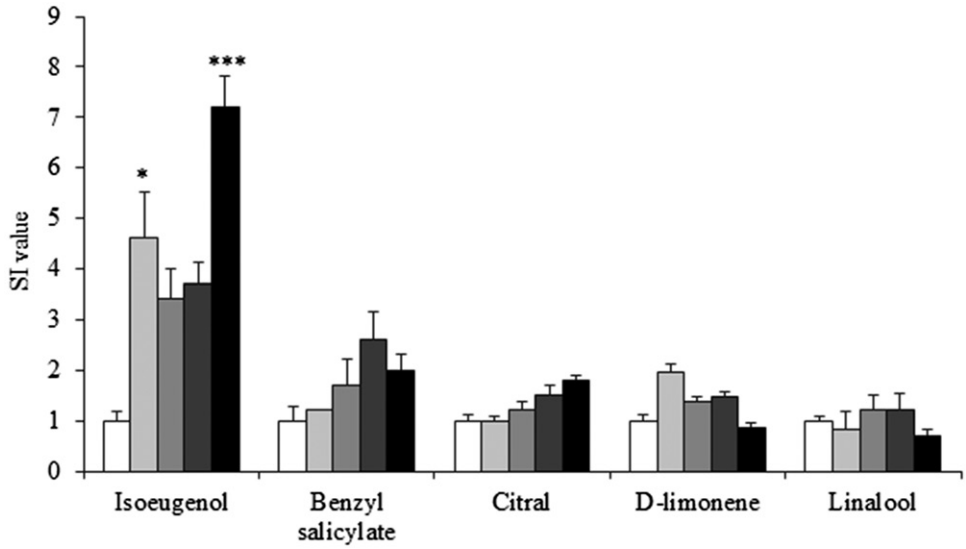

Figure 1. Stimulation index (SI) in the mandibular lymph nodes after inhalation exposure to fragrance allergens. Mice were exposed to the fragrances (75 mg/m^3) via nose-only exposure on days 0, 1, and 2. On day 5, cell proliferation in the mandibular lymph nodes was assessed. Proliferation is expressed as SI value, being the fold-increase compared with vehicle-treated mice (white bars). Mice were exposed for 45 min/d (lightest grey bars), 90 min/d (light grey bars), 180 min/d (dark grey bars), and 360 min/d (black bars). Statistically significant differences were assessed with a one-way ANOVA with a Bonferonni's post hoc test. Asterisks depict significant differences from the control group: *$p < 0.05$ and ***$p < 0.001$.

Table 4. Potency (ED3 values) of sensitizers in the LLNA and respiratory LLNA[a].

Potency	LLNA (EC3 in %)	Respiratory LLNA (ED3 in μg)
Respiratory sensitizers		
Hexamethylene diisocyanate	NA	18
Toluene diisocyanate	0.109	28
Isophorone diisocyanate	NA	44
Phtalic anhydride	0.357	63
Trimellitic anhydride	0.218	156
Skin sensitizers		
Oxazolone	0.013	19
Dinitrochlorobenzene	0.044	173
Isoeugenol	1.5	415

NA, no potency data available. Adapted from Arts et al. (2008). The EC3 value represents the potency determined in the LLNA and is the concentration (in w/v %) that induces a 3-fold induction of proliferation in the auricular lymph nodes. The ED3 value represents the potency determined in the respiratory LLNA and is the dose (in μg) that induces a 3-fold induction of proliferation in the mandibular lymph nodes (for details see Methods section).

isoeugenol was 415 μg. Table 4 shows the potencies of other respiratory and skin sensitizers tested in the respiratory LLNA as well as in the dermal LLNA. In general, isoeugenol is less potent compared with the other skin and respiratory sensitizers tested, especially compared with the diisocyanates tested and oxazolone. This illustrates that for isoeugenol a higher dose is required to induce a 3-fold increase in cell proliferation.

Presence and concentrations of fragrance allergens in air fresheners

Our analysis of 109 air fresheners obtained from the Dutch market shows that 20 of the 24 fragrance allergens were detected in these products. The fragrances eugenol, anisyl alcohol, farnesol, methyl 2-octynoate, and amyl cinnamyl alcohol were not detected. The percentages of products that contain a fragrance allergen together with the mean and maximum concentration levels per product category are summarized in Table 2. More details of the concentrations in the different product categories can be found in the Supplementary data table. The most frequently used fragrances were linalool, D-limonene, and geraniol. Furthermore, the mean and maximum product levels of D-limonene and linalool were higher than the other fragrances.

Calculated exposure to isoeugenol, linalool, and D-limonene

The fragrances linalool, D-limonene, and isoeugenol were selected for the exposure assessment. Linalool and D-limonene were selected because they are the most frequently used ingredients in air fresheners, and therefore consumer exposure is likely. Isoeugenol was selected because it was the only fragrance that induced significant increased cell proliferation in the respiratory LLNA. The physicochemical properties of the selected substances are given in Table 5. It was decided that isoeugenol would ''act'' as an aerosol particle due to its relatively low vapour pressure. Therefore, the ''exposure to spray model'' was used for the calculations concerning spray applications containing isoeugenol. Linalool and D-limonene are volatile and are thus more likely available in vapour form, which is best described by the ''instantaneous release model'' (in case of spray applications). The evaporation model was used for the evaporator and the emission tool was used for the scented block applications for all substances. The weight fractions of the selected fragrances for the different products are given in Table 5.

The calculated air concentrations for the different product categories are shown in Table 6. These concentrations are

Table 5. Chemical properties and partition coefficients of selected fragrances allergens.

		D-Limonene	Linalool	Isoeugenol
Chemical properties				
Mol weight (g/mol)		136.23	154.2	164.2
Log Kow		4.23	2.97	3.04
Vapour pressure (Pa)		190	21	0.7
Diffusion coefficient (m^2/s)		1×10^{-10}	1×10^{-10}	1×10^{-10}
Partition coefficient (air-material)		88	796	23 886
Weight fraction – spray can	Median	0.01	0.007	–
	CV	2.14	2.1	–
Weight fraction – trigger spray	Median	0.064	0.395	0.002
	CV	1.66	0.725	–
Weight fraction – evaporator	Median	0.044	0.097	0.013
	CV	2.09	1.899	1.579
Weight fraction – scented block	Median	0.012	0.022	0.0039
	CV	1.041	1.732	0.116

CV, coefficient of variation.

Table 6. Summary of the average calculated air concentrations (mg/m^3) for the different substances and uses.

	D-limonene	Linalool	Isoeugenol
Peak exposure			
Spray cans			
– Average	0.0128	0.00893	–
– SD	0.00168	0.00106	
– 90th percentile	0.0658	0.0455	
Trigger spray			
– Average	0.0816	0.504	4.64E-7
– SD	0.00784	0.0132	–
– 90th percentile	0.402	1.11	–
Day exposure			
Evaporator			
– Average	0.0542	0.0141	6.37E-5
– SD	0.00624	0.00111	5.16E-6
– 90th percentile	0.342	0.0656	0.000275
Scented block			
– Average	0.00033	0.00061	8.4 E-5
– SD			
– 90th percentile			

Note that the peak exposure is in fact the mean event concentration.

worst-case estimations. Exposures should be best compared within a product category, i.e. sprays (aerosol and trigger), evaporators, scented blocks, as the same assumptions are made within these categories. Comparing the exposure between categories provides insight on differences in orders of magnitude; however, the reader should be aware of the different underlying assumptions and the unknown level of conservatism. Trigger sprays were shown to cause the highest peak concentrations for D-limonene and linalool. This is explained by the fact that the weight fractions of the substances are higher in trigger sprays than in spray cans. For isoeugenol, peak exposures were only calculated for trigger sprays, since isoeugenol was not detected in spray cans. The peak exposure of isoeugenol from trigger sprays is orders of magnitude lower compared with the other two fragrances in trigger sprays, which is explained by the lower weight fractions and a lower airborne fraction due to spray model settings that include gravitational removal of airborne particles. The daily exposure to D-limonene and linalool is the highest in case of evaporators. There were no differences in calculated air concentrations for isoeugenol between evaporators and scented blocks. Again, the exposure to isoeugenol is much lower compared with D-limonene and linalool.

Comparison of fragrance exposure with occupational exposure levels to diisocyanates

In order to estimate whether the exposure levels calculated for isoeugenol, D-limonene and linalool are high in terms of risk on respiratory sensitization, a comparison was made with occupational exposure levels to diisocyanates that are a common cause of occupation asthma (Bernstein, 1996). For this purpose, data from a study measuring actual air concentrations of 23 diisocyanates in the automotive spray painting industry (Pronk et al., 2006, 2007) were used. The air concentrations of a number of diisocyanates in were reported for the individual worker and the different tasks they perform and were expressed in $\mu g/m^3$ NCO (the isocyanate group). Exposure was in the range of 0.002–2643 $\mu g/m^3$ NCO, with median values of 6.67–8.55 (Pronk et al., 2006). We assumed that these reported air concentrations represent peak exposures and could therefore be compared with the calculated air concentrations for the spray applications. The average peak exposures to D-limonene and linalool from trigger sprays were 0.0816 and 0.504 mg/m^3, respectively (Table 6). These calculated values are orders of magnitude lower than the median values reported for the diisocyanates.

To be able to compare the exposure with diisocyanates to consumer exposure to fragrances, information on average daily exposure estimates is necessary. These estimates were based on personal task-based inhalation measurements. It was not possible to derive diisocyanate exposure directly from this study, since diisocyanate exposure was expressed in $mg/m^3 \times h$ (concentration–time product) per month. Daily exposure was dependent on the task performed and ranged from 0.004 to 66.4 $mg/m^3 \times h$, with a median value of 3.68 $mg/m^3 \times h$ per month. This median value was considered to be the cumulative dose per month. The daily concentration was calculated by dividing this value by 82 working-hours per month. A worst-case scenario was calculated as well, using the maximum level of daily exposure. Based on these data, the daily median exposure was estimated to be 0.045 mg/m^3 and the maximum daily exposure was 0.81 mg/m^3.

For air fresheners, it was shown that evaporators give the highest daily exposure to D-limonene and linalool. The calculated air concentrations for the three fragrances liberated from evaporators were, therefore, compared with occupational exposure levels to diisocyanates. For D-limonene and linalool, the calculated air concentrations were 0.0542 and 0.0141 mg/m^3, respectively (Table 6). These exposures are lower than the maximum daily exposure, but were close to or similar to the median concentration estimated for diisocyanates. Exposure to isoeugenol is orders of magnitude lower than the median and maximum daily exposure levels to diisocyanates (Table 6).

Discussion

This study shows that consumers that use air fresheners are likely to be exposed to fragrance allergens that are liberated from these products, but that the extent of exposure can be quite different for each fragrance allergen. Four out of the 24 fragrances analyzed for were not present in the air fresheners that were included in this study. Most frequently used were linalool and D-limonene, whereas cinnamal, isoeugenol, and benzyl cinnamate were only used in a small percentage of the air fresheners. The exposure assessment, in which linalool, D-limonene, and isoeugenol were compared, showed that linalool and D-limonene give rise to higher calculated exposures compared with isoeugenol for all product categories. In a comparison between the different types of air fresheners, it was shown that the use of trigger sprays will result in a higher peak exposure than spray cans, which is explained by the higher weight fractions used in trigger sprays. When the room perfumes were compared, it was shown that evaporators will release higher levels of D-limonene and linalool than scented blocks. The conclusions should, however, be viewed with care as the intrinsic conservatism differs between the applied models. Although we assume worst-case exposure, our calculated results are lower than or in line with values reported by Bureau Européen des Consommateurs (BEUC), who measured actual indoor air concentrations of fragrances after spraying air fresheners in an empty closed room (BEUC, 2005).

The exposure assessment shows that consumer exposure to fragrances is likely, but that the magnitude of exposure differs considerably for the individual fragrances. Isoeugenol is not often used as an ingredient in air fresheners, and if present, exposure is relatively low. This does not mean that there is no risk for consumers; strong potent sensitizers can induce sensitization at low levels of exposure. Isoeugenol was the only fragrance that induced a statistically significant response in the respiratory LLNA, whereas D-limonene and linalool did not induce a response in this assay. It is important to carefully interpret the results of the respiratory LLNA, since this method has not been validated with a large number of chemicals and the chemicals tested were predominantly strong potent skin and respiratory sensitizers. But the respiratory LLNA does provide a warning that a chemical potentially can sensitize via the respiratory tract and as such it might be a hazard to consumers. The absence of a toxicological reference value for respiratory sensitization makes it difficult to determine whether the relatively low exposure to isoeugenol is a cause of concern. To be able to get some insight in the relation among exposure, hazard and possible risks, a comparison was made with occupational diisocyanate exposure levels (Pronk et al., 2007) and the sensitizing potencies of these respiratory sensitizers.

For respiratory sensitizers, it has been shown that no-effect-levels exist, but uncertainties remain whether peak versus average exposure levels are equally important contributors to the risk of sensitization (Vandenplas, 2011). As such, it is not known whether no-effect levels should be based on peak or daily exposure levels. It was decided to compare both the peak and daily exposures. The peak exposures to diisocyanates were orders of magnitude higher than the peak exposures to D-limonene, linalool, and isoeugenol from the spray applications. The daily exposures to linalool and D-limonene calculated for evaporators were also lower compared with the maximum daily exposure for diisocyanates. Exposure to linalool from evaporators was in the same range as the median daily concentration estimated for diisocyanates. Both for peak and daily exposure, consumer exposure to isoeugenol are much lower compared with diisocyanate exposure levels. In this comparison, it is very important that the exposure patterns for consumers in terms of frequency and duration will be quite different from occupational exposure and it is likely that occupational exposures will be more frequent and prolonged. These aspects should be considered as well and impact the total exposure.

Despite many uncertainties and assumptions, the comparison with the diisocyanates shows that overall consumer exposure to D-limonene, linalool, and isoeugenol is lower compared with diisocyanates. In the hazard characterization of sensitizers, the potency of substances is important to consider as well. For example, both linalool and D-limonene are the most frequently used fragrances in cosmetics, but they rarely cause allergic contact dermatitis (Schnuch et al., 2007). This has been explained by the fact that although skin exposure to these fragrances is common, they are weak skin sensitizers. Notably, linalool and D-limonene are known to auto-oxidize when exposed to air for a sufficiently long period, resulting in metabolites that were identified as strong sensitizers (Christensson et al., 2008; Karlberg et al., 1994; Skold et al., 2004). To what extent this actually occurs in cosmetics or air fresheners is unknown, making it difficult to estimate the relevance of auto-oxidation for consumer exposure and risk assessment (SCCS, 2012). Auto-oxidation was, therefore, not taken into account in our approach. When comparing sensitizing potencies, isoeugenol is a stronger potent skin sensitizer than D-limonene and linalool and a lower dose is needed to induce sensitization at least in the skin. Compared with diisocyanates, isoeugenol is far less potent than the diisocyanates both in the LLNA (SCCS, 2012; van Och et al., 2000) and in the respiratory LLNA (Arts et al., 2008). By combining this knowledge on sensitizing potency with the much lower exposure compared to diisocyanates it seems highly unlikely that isoeugenol can induce respiratory sensitization in consumers using air fresheners.

To our knowledge, this is the first study that has measured concentrations of fragrances allergens in air fresheners and estimated consumer exposure to a selection of fragrances. The conclusions for these three fragrances were based on

several assumptions and uncertainties. Therefore, they cannot be extrapolated to other fragrance allergens or to other exposure conditions. For example, in occupational settings, inhalation of fragrances might not be safe and there is limited evidence for this from case studies (Baur et al., 1999; Quirce et al., 2008). Clearly, there is a need for predictive methods that enable risk characterization for these types of consumer products.

Acknowledgements

We thank Anja Redjosentono-Maat of the NVWA for the assessment of the levels of the fragrance allergens in the air fresheners. For technical assistance during the animal experiments, we acknowledge Jolanda Vermeulen, Liset de la Fonteyne, Arja de Klerk, Eric Gremmer, and Bert Verlaan of the RIVM. We thank Miriam Gerlofs, Paul Fokkens, and John Boere of the RIVM for their input in the experimental design for the inhalation experiments and for performing the inhalation exposures. For biotechnical support in the animal experiments, we thank the biotechnicians of the Institute for Translational Vaccinology. We are grateful that Prof. Dick Heederik of the Institute for Risk Assessment Sciences of the Utrecht University and Dr. Anjoeka Pronk of TNO-Innovation for Life for critically reading our manuscript and provided us additional information on their studies on diisocyanate exposure.

Declaration of interest

The authors report no declarations of interest. This work was supported by the Netherlands Food and Consumer Product Safety Authority (NVWA).

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Supplementary material available online

Supplementary table.