

ENVIRONMENTAL DETERMINANTS

Fragrance materials in asthma: a pilot study using a surrogate aerosol product

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Abstract

Objective: Many household products contain fragrances. Little is known about exposure to fragrances on human health, particularly within the airways. This study aimed to evaluate how common household fragrance products (i.e. air fresheners, cleaning products) affect people with asthma, who frequently report sensitivity to these products. Many of these products have volatile organic compounds or semi-volatile organic compounds. This study evaluated nine fragrance materials in an aerosol formulation to assess effects on airway physiology, airway inflammation and symptom perception in normal controls and those with asthma. **Methods:** The effects of fragrances were evaluated in people without asthma, people with mild asthma and people with moderate asthma in a four-way crossover placebo-controlled study. Subjects were exposed twice to a fragranced aerosol and twice to a placebo aerosol (15 and 30 min each). Subjects completed a questionnaire for 29 symptoms during and up to 3 h after each exposure scenario. Spirometry was performed prior to and 3 h post-exposure; sputum induction was conducted 3 h post-exposure. **Results:** Nasal symptoms showed the greatest frequency of response in all three subject groups, and moderate asthmatics reported the greatest symptom severity and symptom types. No significant differences were noted in physiology or cellular inflammation. **Conclusion:** A trend for increased symptoms was noted in moderate asthmatics, suggesting that asthma severity may play a factor in fragrance sensitivity.

Keywords

Airway inflammation, fragrance sensitivity, mild asthma, moderate asthma, symptom perception

History

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Introduction

Many household consumer products, including cosmetics, toiletries, air fresheners and laundry products contain fragrance materials. There is limited evidence to suggest that exposure to certain fragrance materials may induce allergic and/or asthma-like symptoms in sensitive individuals. Although considerable information exists on dermal effects from exposure to fragrance materials [1–4], relatively little information exists on inhalation exposure to these substances [5,6].

A 2009 survey requested respondents to comment on health effects (e.g. headaches, respiratory difficulties) from exposure to scented products [7]. Results indicated that 30.5% of respondents found scented products irritating, and 19% reported adverse health effects, with higher prevalence in those with asthma or allergies.

Asthmatics may be more sensitive to fragrance exposure than non-asthmatics, although the degree of increased

sensitivity and the relationship to asthma severity is unknown. A few studies have looked at fragrance exposure in asthmatics and subjects with sensory over-perception, as well as airway hyper-responsiveness [8–13]. One study considered inhalation effects of perfume strips in asthmatics. Worsening respiratory symptoms (chest tightness, wheeze) with exposure to the product were noted, along with decrements in forced expiratory volume in 1 s (FEV₁) post-challenge [13]. It appeared that the number of exacerbations also increased with asthma severity, as well as with positive atopic status determined by allergen skin tests.

Asthma is characterized by paroxysmal or persistent respiratory symptoms in conjunction with demonstrable physiologic findings of improvement in large airway caliber noted on spirometry with bronchodilator and/or airway hyper-responsiveness [14,15]. In addition to self-reporting of perceived symptoms, the effects of fragrance inhalation can be evaluated through measures of airway physiology and inflammation. Measures of airway inflammation, such as cell counts in induced sputum, provide reliable and reproducible analysis of lower airway inflammatory profiles [16]. Different types of lower airway inflammation can be characterized through standardized sputum cell counts and analysis of the supernatant [16–18].

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While individuals with asthma may be more sensitive to fragrance exposure than non-asthmatics, the relationship between asthma severity and the degree to which fragrance products affect these individuals is unknown. This study examined the effects of fragrance exposure from a surrogate aerosol product containing a mixture of nine fragrance materials among non-, mild and moderate asthmatics using measures of symptom perception (subjective) and changes in airway physiology/inflammation.

Methods

Study subjects

Subjects were recruited from a database of potential participants held by Toxcon Health Sciences Research Centre, Inc. (Toxcon; Edmonton, Alberta, Canada) and patients attending the University of Alberta Hospital's (UAH) Asthma Program. This study was approved by the University of Alberta Health Research Ethics Board (No: 05-003-R101).

Inclusion criteria

Males or females, ages 18–70 years, in good mental and physical health, able to provide informed consent, non-smokers within the last 6 months (with a total history <10 pack years), and with the ability to produce sputum for analysis.

Exclusion criteria

Individuals with pre-existing respiratory conditions (other than physician-diagnosed mild and moderate asthma); underlying cardiac, liver and/or renal conditions; diabetes; immunosuppression; a history of drug or alcohol abuse; or a history of severe and/or life-threatening allergy. Pregnant or lactating women, those with a body mass index >35, and individuals unable to complete the questionnaires were also excluded.

Screening

Individuals who met initial inclusion criteria were referred to the UAH Asthma Program for further review. This involved a detailed clinical review to ensure current asthma status, and that all medical conditions were properly identified and managed prior to study exposures. Subjects were then assigned to the appropriate stratified study groups. Atopic status was assessed through skin prick testing (12 common environmental allergens) along with a Dermatology assessment which included dermal patch testing to the nine fragrance materials used in the study.

As part of the clinical screen, subjects underwent pulmonary function tests (PFT) and, where necessary, a methacholine challenge to confirm current asthma. PFTs were performed according to the American Thoracic Society Guidelines [19,20]. Subjects were characterized as non-, mild and moderate asthmatics according to the Canadian Asthma Consensus 2003 Guidelines [21] and Global Initiative for Asthma guidelines [15] in accordance with current guidelines at study initiation (2006).

All subjects underwent an Otolaryngology assessment to identify any abnormalities that would preclude them from

participation in the study. Olfactory responsiveness was captured through the Smell Identification Test™ (Sensonics, Inc., Haddon Heights, New Jersey, USA; University of Pennsylvania Smell Identification Test, UPSIT).

Study design

A four-way crossover design was used (stratified by asthma status). Each subject served as their own control for each exposure to the test substance (TS) and the control substance (CS). Each individual was exposed on four occasions for either 15 or 30 min to the TS (TS-15 and TS-30) and CS (CS-15 and CS-30). The order of fragrance challenges (15 min versus 30 min; CS versus TS) was done through a randomization process through Toxcon (Edmonton, Alberta, Canada). The subjects were not told which substance was given ahead of time, but the odor may have been perceptible during the exposure, and as such the study was not blinded. There was a 2-week minimum washout period between exposures, and a minimum 4-week hold period if a subject developed any upper/lower respiratory tract (or other) infection, asthma exacerbation or allergen exposures with consequent reactions (respiratory, cutaneous or other).

Exposure protocol

Exposure days began with a brief clinical review, followed by baseline spirometry. Data recorded comprised FEV₁; percent reference of FEV₁ based on age, gender, height and race (FEV₁ %REF); forced vital capacity (FVC) and percent reference value of FVC (FVC %REF) [22].

Prior to exposure, subjects completed a Symptom questionnaire indicating the presence/severity of 29 symptoms using a visual analog scale (VAS), ranging from “None” to “Incapacitating” (Table 1). This questionnaire was also completed during the exposure period every 5 min and at 60, 120 and 180 min post-exposure.

After completing the clinical review, subjects were exposed to either the TS or CS for 15 or 30 min in an environmentally controlled exposure chamber (9.1 m³) with an air exchange rate of 0.6/h (±0.1), previously established to simulate typical residential ventilation [23]. This incorporated a standardized product instilled through a spray canister (two sprays per exposure) with measurement of background levels of the substances captured using absorbent tubes with carbon capture (Dr CJC; Toxcon, Edmonton, Alberta, Canada).

A Fragrance Perception questionnaire was completed using a VAS from “Extremely Unpleasant” to “Extremely Pleasant”, rating the strength of the fragrance in the chamber, and the subject's comfort level.

Three hours post-exposure, spirometry was repeated and sputum induction was performed according to established protocols [16]. Cell counts were conducted on blinded specimens by a certified cytotechnologist.

TS and CS were provided by the sponsor. The TS was composed of 0.2% butylated hydroxyanisole (BHA; antioxidant/stabilizer), 0.5% Witconol (food grade emulsifier; polyglycerol ester of oleic acid), 29% hydrocarbon propellant (~20% propane and 80% isobutene), 69.76% water and 0.06% each of the following fragrance materials: benzyl acetate, eugenol, α -hexyl cinnamic aldehyde, 1,3,4,6,7,8-hexahydro-

Table 1. Symptom categories and individual symptoms rated by subjects using a VAS.

Chest	Eyes	Nose	Throat	Skin	Non-specific
Cough	Burning	Runny	Drip at back of throat	Dry	Headache
Wheezy sound	Itching	Stuffy	Sore	Irritated	Dizziness
Tightness	Dry	Itchy	Scratchy	Rash	Nausea
Shortness of breath	Runny/watery	Sneezing	Dry		Unusual tiredness
			Tickling		Fatigue beyond attributable to exercise
			Tight/choking feeling		Abdominal pain
					Dry mouth
					Tension/nervousness

4,6,6,7,8,8-hexamethyl cyclopenta- γ -2-benzopyran, hydroxy-citronellal, β -ionone, *d*-limonene, linalool and methyl dihydrojasmonate. The CS aerosol preparation was identical to the TS except the fragrance materials were replaced with deionized distilled water. Double distilled water made up the percentage in the placebo of what was the fragrance formulation in the test aerosol [23].

Study timelines

A defined end date was specified after which recruitment was stopped. The non- and mild asthmatics were recruited and safety data obtained prior to recruitment of moderate asthmatics. Due to the specified end date, only eight moderate asthmatics were recruited.

Analysis of symptoms

Changes in symptom scores following CS and TS exposures were computed by subtracting baseline scores from post 15 and 30-min exposures (performed on an individual basis). Positive scores for any symptom indicated an increase in severity scores from baseline. For each subject, the number of symptoms where an increase from baseline occurred was computed within symptom categories (Lower and Upper Respiratory, Eyes/Skin, and Non-Specific) for the exposures. For example, the Lower Respiratory category comprised four clinical symptoms (cough, wheeze, chest tightness and dyspnea). If a subject in the TS exposure, after 15 min, had a positive score change in all symptoms, their score would be four for this category. Differences in the number of positive symptom responses were noted between CS and TS and they were compared using a Wilcoxon two-sample test for statistical significance in each group. A *p* value <0.05 was considered significant.

Analysis of airway physiology/inflammation

The FEV₁ values in the data set for the TS and CS were recorded at baseline and 3 h post-exposure. Percent change from baseline was calculated for each individual. Repeated measures analysis of variance was then performed with the percent change in FEV₁ value as an outcome variable and the type of substance exposure, asthma stratification status, gender and age as factors. Analysis similar to that used for FEV₁ was applied to induced sputum characteristics on raw scores instead of percent changes.

Descriptive statistics and results for three characteristics, describing fragrance perception for CS-15, CS-30, TS-15 and TS-30, were examined with profile plots under repeated

Table 2. Subject demographics.

Characteristics	Non-asthmatics (<i>n</i> = 12)	Mild asthmatics (<i>n</i> = 12)	Moderate asthmatics (<i>n</i> = 8)
Males (female)	4 (8)	4 (8)	3 (5)
Average age ^a	46 (11.87)	43 (9.89)	42 (11.84)
Atopic ^b	6	12	7
Fragrance sensitivity (self-report) ^c	3	4	4
Dermal patch sensitivity (urticarial scoring system) ^d	1	4	0
Olfactory test ^e			
Normosmic	10	10	4
Mild microsmic	2	1	3
Moderate microsmic	0	1	1

^aAge (standard deviation); ^bDetermined by the skin prick test performed at UAH; ^cSelf-reported by subjects; ^dOnly mild ($\pm$ or 1+) reactions; ^eDetermined by UPSIT criteria.

Table 3. Number of subjects with an increase in symptom severity after exposure to nine fragrance materials (TS).

Exposure duration (min)	Symptom group	Number of subjects with a greater increase in symptom severity for TS as compared to CS		
		Non-asthmatics	Mild asthmatics	Moderate asthmatics
15	Chest	2	3	3
	Eye	3	2	4
	Nose	5	5	5
	Throat	3	4	6
	Skin	1	0	1
	Non-specific	3	2	4
30	Chest	2	1	3
	Eye	4	2	1
	Nose	2	5	2
	Throat	4	3	2
	Skin	0	3	0
	Non-specific	2	1	1

measure model analysis to show a trend over time (four time points for 15-min exposures and seven time points for 30-min exposures).

Results

Subject demographics

Fifty-five potential selected subjects underwent further clinical screening at UAH Asthma Program. Thirty-nine subjects completed all screening requirements and thirty-two subjects

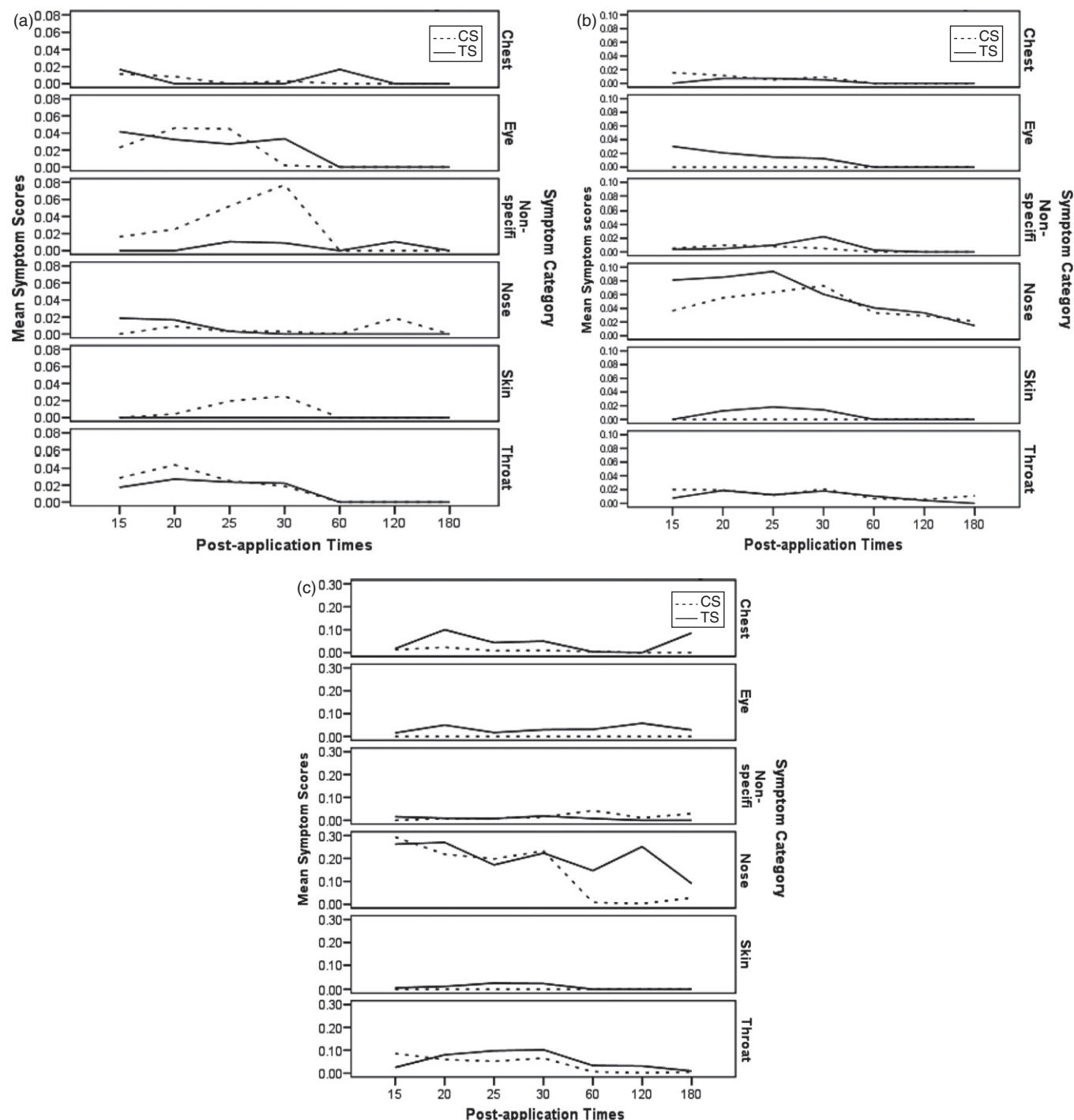


Figure 1. Mean change in symptom severity scores from baseline for (a) non-asthmatics (b) mild asthmatics and (c) moderate asthmatics. Dashed line represents CS and solid line represents TS.

completed all four exposures: 12 non-, 12 mild and 8 moderate asthmatics.

The mean age was 46 years for the non-asthmatic group, 43 years for the mild asthmatic group and 42 years for the moderate asthmatic group. Eleven of the 32 subjects (34%) were male and 21 (66%) were female. Twenty five (78%) were atopic, as assessed by allergy skin tests. No subjects had remarkable ENT/nasal endoscopy findings.

Of the 12 non-asthmatics, 10 were normosmic and 2 were mild microsmics. Among the mild asthmatics, 10 were normosmics, one mild microsmic and one moderate microsmic. Finally, for the moderate asthmatics, four were

normosmic, three were mild microsmics and one moderate microsmic (Table 2).

Symptom perception

The “nose” category showed the greatest frequency of response to the TS in all subject groups, particularly after the 15-min exposure. Table 3 summarizes the number of subjects who perceived more severe symptoms after exposure to TS compared to CS. In general, the severity of symptoms decreased with increasing time of exposure (15 versus 30 min). For the non-asthmatic group (Figure 1a), statistically

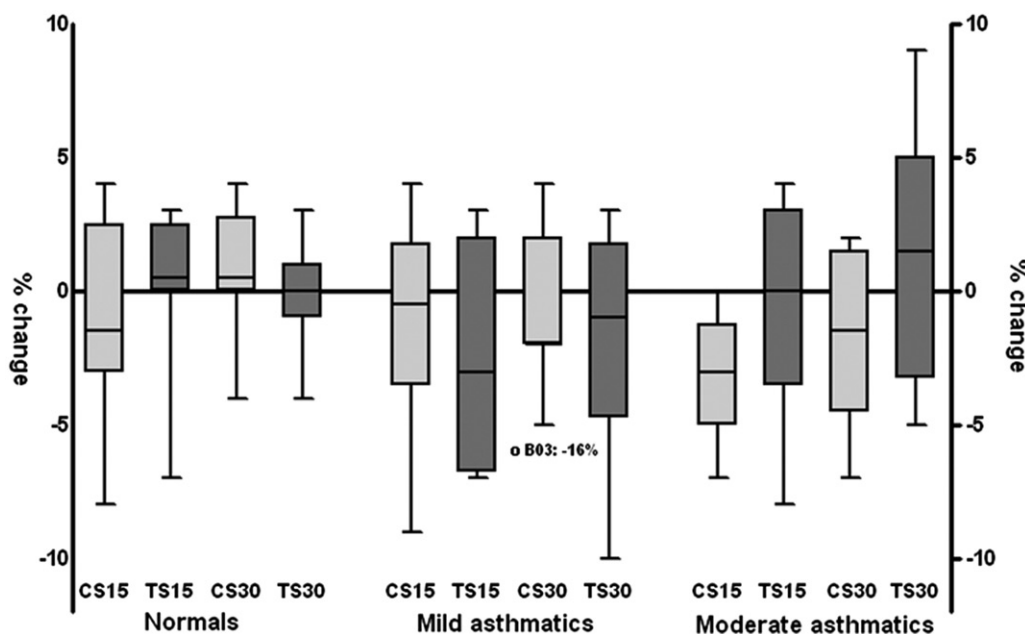


Figure 2. Mean change in FEV₁ from baseline (%REF) following exposure to TS-15, TS-30, CS-15 and CS-30.

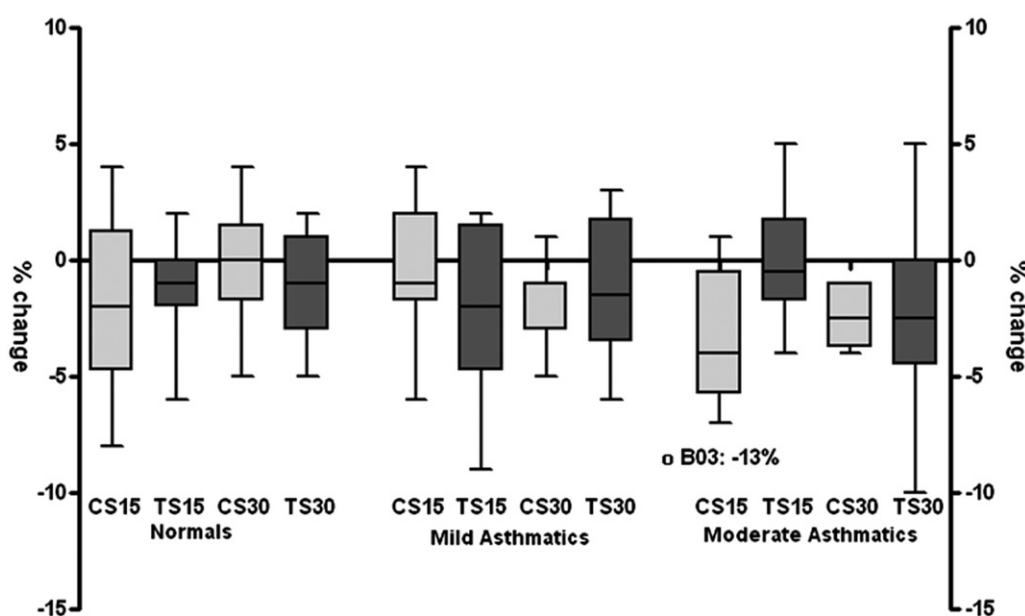


Figure 3. Mean change in FVC from baseline (%REF) following exposure to TS-15, TS-30, CS-15 and CS-30.

significant differences were found after TS-15 for the nose symptoms 3 h post-exposure ($p = 0.02$). In mild asthmatics (Figure 1b), only chest symptoms 3 h post TS-30 exposure showed a statistically significant increase ($p = 0.01$). Moderate asthmatics (Figure 1c) reported the greatest severity in symptoms (particularly after TS-15) as well as the most symptom types. For this group, only the nose and skin symptom categories showed statistically significant differences. For nose symptoms after TS-15, there was a statistically significant increase in symptom reporting after 5 min ($p = 0.03$) and at 25 and 30 min post TS-30 ($p = 0.03$). For skin symptoms after TS-30, a marginal difference ($p = 0.047$) was observed at 2 and 3 h post-application.

Airway physiology

CS exposures at 15 and 30 min did not have any apparent effect on airway physiology (Figures 2 and 3). Lung function, as assessed by spirometry, showed no significant difference after CS and TS-15 and TS-30 for both absolute and percent reference changes in FEV₁ and FVC (Figures 2 and 3) across all subject groups.

Lower airway inflammation

CS exposures at 15 and 30 min did not have any apparent effect on airway inflammation, as assessed by sputum cell counts. No change was noted in percent sputum eosinophils

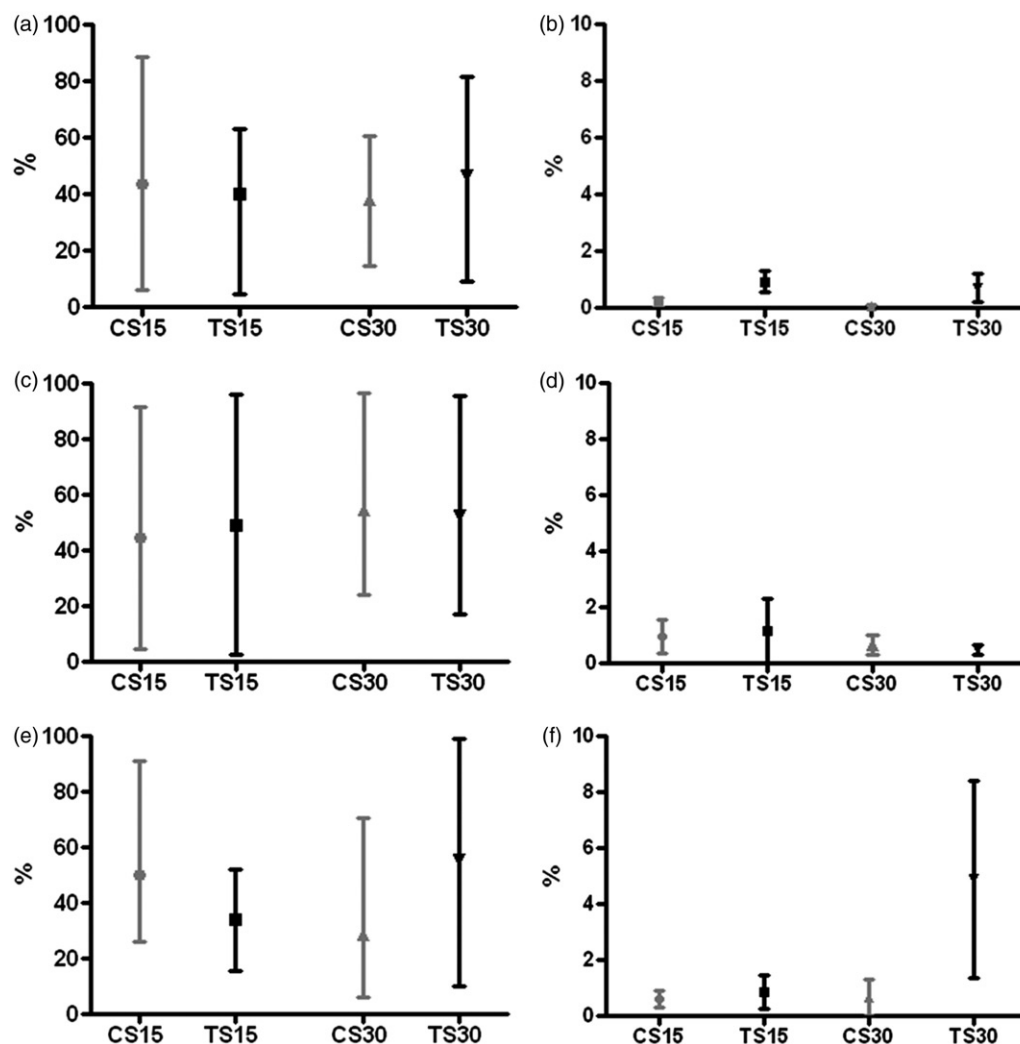


Figure 4. Lower airway inflammation measured by induced sputum for cell counts following fragrance exposures. (a) percent sputum neutrophils: non-asthmatics, (b) percent sputum eosinophils: non-asthmatics, (c) percent sputum neutrophils: mild asthmatics, (d) percent sputum eosinophils: mild asthmatics, (e) percent sputum neutrophils: moderate asthmatics and (f) percent sputum eosinophils: moderate asthmatics. Values presented for sputum cell counts represent induction 3 h post-exposure to TS-15, TS-30, CS-15 and CS-30.

or neutrophils post-exposure to TS or CS, although a non-significant trend toward increased neutrophils was noted in the moderate asthmatic group (Figure 4a–f).

Discussion

Approximately 3000 chemical substances are used in the fragrance industry [1]. Our study did not reveal, in the individuals assessed both with and without asthma, significant adverse effects following exposure to fragrances. Although fragrances are commonly encountered in our environment, only a small proportion of individuals develop fragrance sensitivity [2,7], which within different reports is not a consistent use of the term, and most often relates to “self-report”. This study showed that non-, mild and moderate asthmatics did not develop marked adverse effects after exposure to fragrances. No clinical effects of fragrance were noted on airway physiology, inflammatory markers or overall symptom scores, although nasal symptoms showed greater frequency. Reviewing individual symptom data, more were reported by moderate asthmatics than non- or mild asthmatics, particularly for TS-15 as opposed to TS-30 (Table 3).

This suggests that moderate asthmatics may be more responsive to fragrance substances. Most of the symptoms reported were related to the throat suggesting a possible irritant effect in some individuals within the upper airway (periglottic structures).

The data from this study aligns with a 2010 study conducted by Schnuch et al. [24]. In contrast to the current study, Schnuch et al.’s study [24] was conducted with subjects selected based on a positive patch test to either isoeugenol or hydroxyisohexyl-3-carboxaldehyde. Each subject was exposed to the vapor of a single substance, at high concentration ($1000 \mu\text{g}/\text{m}^3$ for 60 min), in an environmental exposure chamber (25 m^3) with an air exchange rate of 30/h. The goal of that study was to establish whether inhalation exposure, in dermally sensitized individuals, would cause allergic effects resulting in measureable changes in pulmonary function and/or visible dermal reactivity.

Concentrations of each fragrance material in the pressurized aerosol formulation used in the present study were consistent with relevant concentrations in commercially available aerosol air freshener products (lower than those in the Schnuch et al.’s study). In addition, subjects in the

Schnuch et al.'s study [24] were sensitized to the materials of interest. In contrast, in our study, the non-, mild and moderate asthmatic individuals were not sensitized. In both studies, inhalation exposure did not cause measurable respiratory decline or an increase in airway inflammation as noted by both FEV₁ and differential cell counts.

Study limitations include the small sample size per group. In this study, subjects underwent a number of measures at multiple time points resulting in many data points for each individual. Consequently, if there were time-dependent effects, it is possible that they would be less obvious as the data were collapsed to reduce the number of variables included in analysis. It is unlikely that a major effect would have been missed, but it is possible that some minor differences between groups were lost by this mechanism.

As well, we did not include severe asthma patients; our study focused on safety and the development of clinical data in mild and moderate asthmatics. We are planning further studies that incorporate both upper and lower airway evaluation of moderate and severe asthmatics.

This study was conducted in a manner that corresponded with realistic concentrations of fragrance materials available in aerosol products found routinely on store shelves. We conclude that the concentration of materials in the tested formulation did not exacerbate pre-existing asthma or induce asthma-like symptoms in non-asthmatic individuals. Using this study will be useful toward designing a future larger sampling of individuals with asthma to better understand the effects of fragrance materials in this population.

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All authors contributed to the design of the study (with the exceptions of D.M. and J.B.). D.V., H.V., J.B. and D.M. participated in the study conduct. All authors were involved in the preparation of this article.

This study was conducted at the University of Alberta Hospital, Canada.

Declaration of interest

The authors report no conflicts of interest. The authors are responsible for the content and writing of this article.

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