Ph.D. THESIS

OCULAR AND RESPIRATORY SYMPTOMS ELICITED BY PERFUME AND FRAGRANCE PRODUCTS

Jesper Elberling

The National Allergy Research Centre
Gentofte Hospital

University of Copenhagen, Denmark
Faculty of Health Sciences

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II Elberling J, Linneberg A, Mosbech H, Dirksen A, Frølund L, Madsen F, Nielsen NH and Johansen JD. A link between skin and airways regarding sensitivity to fragrance products? Br J Dermatol 2004 December;151(6):1197-203.

III Elberling J, Linneberg A, Mosbech H, Dirksen A, Menne T, Nielsen NH, Madsen F, Frølund L and Johansen JD. Airborne chemicals cause respiratory symptoms in individuals with contact allergy. Contact Dermatitis 2005 February;52(2):65-72.

IV Elberling J, Johansen JD, Dirksen A and Mosbech H. Exposure of eyes to perfume: a double-blind, placebo-controlled experiment. 2005; manuscript.

V Elberling J, Dirksen A, Johansen JD and Mosbech H. The capsaicin cough reflex in patients with respiratory symptoms elicited by perfume. 2005; manuscript.

Supervisors

Holger Mosbech, MD, DMSc.

Allergy Unit, Rigshospitalet, University of Copenhagen, Denmark

Asger Dirksen, Professor, MD, DMSc.

Department of Respiratory Medicine, Gentofte Hospital, University of Copenhagen, Denmark

Jeanne Duus Johansen, MD, DMSc.

The National Allergy Research Centre, Department of Dermatology

University of Copenhagen, Denmark.

PREFACE

The present Ph.D. thesis was carried out during my employment at the National Allergy Research Centre, Department of Dermatology, Gentofte Hospital, University of Copenhagen, Denmark from September 2001- July 2005.

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The National Allergy Research Centre, June 2005

Jesper Elberling

SUMMARY

The thesis was carried out during my employment at the National Allergy Research Centre, Department of Dermatology, Gentofte Hospital, University of Copenhagen, Denmark from September 2001- July 2005.

Ocular and respiratory symptoms related to environmental perfume exposure are frequently reported by subsets of individuals in general populations and by patients referred to clinics investigating diseases in skin and airways. The underlying pathophysiological mechanisms of such symptoms are unknown, and the relative importance of allergic and or inflammatory diseases in skin or airways in relation to the symptoms has been only scantly investigated.

The PhD work was carried out as two separate projects: an epidemiological study (Part 1) and a clinical experimental study (Part 2).

For **Part 1** a questionnaire on respiratory symptoms related to perfume and fragrance products was developed and posted to 1189 individuals who had recently participated in a population-based study of allergic diseases. The questionnaire-study **1)** described the occurrence, the character and the severity of ocular and respiratory symptoms related to perfume and fragrance products in the population and **2)** investigated the association between reporting such symptoms and skin prick test reactivity (atopy), metachline bronchial hyperreactivity (BHR), allergic rhinitis, asthma, hand eczema and patch test reactivity.

For **Part 2** an exposure device for double-blind eye-challenge with perfume was developed. A case control study investigated **3)** the association between reactivity to perfume-provocation and reporting of ocular and respiratory symptoms and **4)** the association between cough responsiveness to capsaicin and the symptoms related to perfume.

The results from **Part 1** indicated that symptoms related to perfume and fragrance products in the population were most frequently reported from the nose, followed by the eyes, lungs, and mouth or throat. No associations were found between these symptoms and atopy. Positive, independent and significant ($p<0.05$) associations were found between the symptoms and BHR, perfume contact allergy, hand eczema, psychological vulnerability and being a woman. In addition, a positive association was found between respiratory symptoms related to perfume and to airborne chemicals other than perfume. The occurrence of respiratory symptoms related to airborne chemicals other than perfume increased significantly ($p<0.05$) with increasing number of positive patch tests.

In study **Part 2**, subjective irritation was elicited in the eyes by vapours of perfume independent of olfaction, but the relative importance of ocular chemoperception in relation to elicitation of eye and respiratory symptoms from environmental exposure to perfume is yet to be clarified. Lower but not upper respiratory symptoms were associated with increased capsaicin cough responsiveness.

In conclusion, we found no indications that respiratory symptoms from environmental perfume exposure were caused by IgE-mediated allergy, but various other objective findings were associated with the symptoms independent of psychological vulnerability. These objective findings included BHR, increased capsaicin cough responsiveness, perfume contact allergy as well as self reported hand eczema.

RESUMÉ

Afhandlingen er udarbejdet i perioden september 2001 til juli 2005, under min ansættelse som klinisk assistent ved Videncenter for Allergi, Dermatologisk afd. K, Københavns Universitet, KAS Gentofte.

I befolkningen samt blandt patienter henvist til udredning for lidelser i hud og slimhinder er det ikke sjældent at parfumerelaterede symptomer fra øjne og luftveje bliver rapporteret. Den eksisterende viden om patofysiologien bag sådanne symptomer er sparsom og det er ligeledes uklart i hvilken grad symptomerne er relateret til allergiske og eller inflammatoriske sygdomme i hud og luftveje.

Ph.d. studiet blev gennemført som to selvstændige projekter; Et epidemiologisk **delstudie 1** og et klinisk eksperimentelt **delstudie 2.**

Et spørgeskema vedrørende symptomer fra slimhinderne (i øjne og luftveje) relateret til udsættelse for parfume og parfumerede produkter blev udviklet under **delstudie 1**. Spørgeskemaet blev udsendt til 1189 personer, som tidligere havde deltaget i en befolkningsundersøgelse fokuseret på allergiske lidelser. Delstudie 1 beskrev **1)** forekomsten, karakteren og sværhedsgraden af slimhindesymptomer relateret til parfume og parfumerede produkter i befolkningen og undersøgte **2)** sammenhængen mellem sådanne symptomer og priktestning (atopi), metakolin bronkial hyperreaktivitet (BHR), allergisk rhinitis, astma, håndeksem og lappetestning (kontaktallergi).

Et instrument til dobbeltblindede øjeneksponering med parfume blev udviklet under **delstudie 2**. Et case kontrol studie undersøgte sammenhængen **3)** mellem følsomheden for parfume eksponering i øjnene og selvrapporterede symptomer over for parfume og parfumerede produkter og **4)** mellem hostereaktivitet over for capsaicin og selvrapporterede symptomer relateret til parfume og parfumerede produkter.

Resultaterne fra delstudie 1, vedrørende symptomer relateret til parfume og parfumerede produkter i befolkningen, viste at symptomerne hyppigst rapporteres fra næsen efterfulgt af øjne, lunger, mund og hals. Undersøgelsen fandt ingen sammenhænge mellem symptomerne og atopi. Derimod blev der fundet en positiv og signifikant ($p<0.05$) sammenhæng mellem slimhindesymptomerne og BHR, parfume kontaktallergi, håndeksem, kvinder og psykisk sårbarhed. Selvrapporterede slimhindesymptomer relateret til parfume var mere relateret til symptomer fra andre luftbårne kemiske stoffer end til luftbårne proteinstoffer. I en analyse af slimhindesymptomer relateret til luftbårne kemiske stoffer (som ikke var parfumestoffer) fandt vi en signifikant ($p<0.05$) positiv sammenhæng mellem sådanne symptomer og kontaktallergi, som viste sig at afhænge af antallet af positive lappeprøver.

I delstudie 2 blev det påvist, at parfume eksponering i øjnene kunne fremkalde øjensymptomer uafhængigt af lugtesansen. Det kunne dog ikke påvises med sikkerhed om sådan en følsomhed havde relation til symptomer fra øjne og luftveje fra udsættelse for parfumestoffer i dagligdagen. Nedre luftvejssymptomer, men ikke øvre luftvejssymptomer, var positivt og signifikant ($p<0,05$) associeret til en øget capsaicin hostereaktivitet.

Vi fandt således ingen holdepunkter for, at slimhindesymptomer relateret til parfume er forårsaget af IgE-allergi, derimod identificerede vi en række objektive tegn, som uafhængige af køn og psykisk sårbarhed, var relateret til symptomerne. Disse objektive tegn inkluderede BHR, øget capsaicin hostereaktivitet, parfume kontaktallergi såvel som selvrapporteret håndeksem.

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1 BACKGROUND

Ocular and respiratory symptoms from environmental perfume exposure are frequently reported by subsets of individuals in general populations and by patients referred to clinics investigating diseases in skin and airways. However, it is unknown to what extend such symptoms are associated with allergy, hyperreactivity and or inflammatory diseases in skin and airways.

1.1 Fragrance chemicals

A perfume is a blend of oderous ingredients composed of a diluent, usually ethanol, and a mixture of 10 to 300 different fragrance ingredients (1). Most fragrance chemicals are volatile aliphatic or aromatic molecules that can be categorised according to their functional groups including, aldehydes, ketones, acids, esters, ethers, terpenes, alcohols etc. (2). Approximately 3000 different fragrance chemicals are currently used in the perfume industry and are often combined to create characteristic scents (2).

1.2 Exposure

Perfumes and virtually all cosmetics and toiletries contain fragrance chemicals e.g. deodorant, aftershave, skincare products, lipstick, powder, shampoo and soaps etc. In addition, fragrance chemicals are added to various household products e.g. fabric softeners, detergents, cleaning agents, polishes, air fresheners etc. (1); products as cars, candles and toys may be perfume scented, and fragrance chemicals are also added to various branded articles.

Fragrance chemicals may come in contact with the skin, and ocular or respiratory organs (referred to as respiratory organs). This contact may be intentional from direct product application e.g. by using perfumed cosmetics and household products, as well as perfumed toothpaste, mouth-fresheners, eye-drops, medical inhalators for nasal application, or unintentionally by evaporation from consumer products or from other persons' wearing of perfume.

1.3 Adverse effects

Fragrance chemicals may have adverse effects on humans (1;3-8). Such effects may be categorised according to localisation (e.g. to the skin or respiratory organs) or pathophysiology (e.g. type of immunological response). The pathophysiology, terminology, and epidemiology of adverse reactions related to the skin are well established (1;3). Adverse skin reactions are predominated by contact allergy to fragrance chemicals, one of the commonest causes of contact allergy in both the general population (9;10) and in eczema patients (11).

The adverse effects of fragrance chemicals related to the respiratory organs are not well described and the underlying pathophysiology of the symptoms is unclear. In a single case, anaphylaxis has been reported after spraying perfume in the eyes (7), which could indicate mechanisms associated with IgE-mediated allergy. Fragrance chemicals may also cause occupational asthma (5;12) and elicit symptoms in individuals with asthma and rhinitis (4;6). However, lower respiratory symptoms associated with perfume and other fragrance products are also frequent among non-asthmatic and non-allergic individuals (13-15).

1.4 Physiological signs of underlying pathology

Studies in selected groups of patients with lower respiratory symptoms have shown that both inhalation (13) and exposure of only the eyes to vapours of perfume may elicit lower respiratory symptoms, in addition to eye symptoms (16). A decline in lung function after perfume inhalation has been reported in individuals with severe asthma (6) and in cases after occupational exposure to perfume (5;12). It has, however, been disputed whether all the respiratory symptoms elicited by fragrance products in persons with asthma can be attributed to bronchial obstruction (14). Inhalation of capsaicin (the pungent principle in hot chilli pepper) stimulates the afferent C-fibres and Aδ-fibres in the airways (17) and triggers the cough reflex in a dose dependent way in normal individuals (18). In non-asthmatic patients with lower respiratory symptoms elicited by perfume, increased capsaicin cough responsiveness has been observed, suggesting an underlying sensory hyperreactivity of the airways (15).

1.5 Terminology

Respiratory symptoms elicited by fragrance chemicals have been investigated in confined groups of patients, but objective findings were sparse and pathophysiology is not well understood (6;13;14;16;19;20). Although, a sensory hyperreactivity (SHR) has been suggested as an underlying mechanism of lower respiratory symptoms (13-15); no overall accepted terminology exists to classify respiratory symptoms related to perfume. As a consequence, the symptoms are often ascribed to an underlying disease (e.g. asthma) or categorised in broader syndromes such as multiple chemical sensitivities (MCS), environmental illness (EI), or idiopathic environmental intolerance (IEI) (21-29). These syndromes are not confined entities but share some overall characteristics such as 'subjective annoyance to environmental exposures' that make them useful for describing and handling patients with unexplained conditions. However, they may be less suitable for research in underlying pathophysiology, because they most likely cover heterogeneous conditions.

1.6 Epidemiology

The epidemiology of adverse respiratory effects associated with fragrance chemicals has been only scantly investigated in population-based studies, often with limited precision regarding the character of the symptoms and the type of exposure (24;27;30-34) as mentioned above.

A classification of respiratory symptoms related to perfume and fragrance products (according to character and severity) may uncover hidden patterns and enable comparisons between subgroups that may give new indications of the underlying pathophysiology.

2 AIMS OF THE STUDY

The aims of the thesis were to investigate ocular and respiratory symptoms elicited by perfume and fragrance products.

- To classify symptoms related to perfume and fragrance products according to their character and severity in a population-based sample (Paper I)

- To investigate the association between respiratory symptoms related to perfume and previously recognised atopy, BHR, allergic rhinitis, asthma, hand eczema and perfume contact allergy in a population-based sample (Paper I and II)

- To investigate the association between respiratory symptoms related to airborne chemicals other than perfume and contact allergy in a population-based sample (Paper III)

- To investigate the responsiveness to perfume exposures in the eyes of eczema patients with respiratory symptoms related to perfume as compared to healthy controls (Paper IV)

- To investigate the cough responsiveness to capsaicin in eczema patients with respiratory symptoms related to perfume as compared to healthy controls (Paper V).

3 MATERIAL AND METHODS

The PhD work was carried out as two separate projects: an epidemiological study (Part 1) and a clinical experimental study (Part 2).

3.1 Part 1: The epidemiological study

3.1.1 Baseline data

The study population had previously participated in a population-based study of allergic diseases in Copenhagen, Denmark (The Copenhagen Allergy Study) (35;36). A screening questionnaire about respiratory symptoms had been sent to random samples of the general population. Random samples of responders (n=868) and a sample of those who had reported symptoms of respiratory disease on exposure to pollen or furry animals (n= 348) participated in the study. Thus, individuals with IgE-mediated allergy were somewhat overrepresented (37). Clinical examinations were between October 1997 and November 1998. The study included measurement of skin prick test (SPT) reactivity, methacholine bronchial hyperreactivity (BHR), patch testing, history of hand eczema, forced expiratory volume in the first second, serum eosinophilic cationic protein and data on psychological and social variables.

3.1.1.1 Patch testing

The patch tests used were TRUE Test ® (ALK-Abelló, Hørsholm, Denmark) (38-40), as previously described (9). Reactions were classified according to international guidelines (41). A positive reaction (+) was defined as a minimum of homogeneous redness and palpable infiltration in the test area. The markers of perfume contact allergy in the panel consisted of: balsam of Peru (450 $\mu g/cm^2$), colophony (1500 $\mu g/cm^2$), and fragrance mix (450 $\mu g/cm^2$) composed of alpha-amyl cinnamic aldehyde, cinnamic aldehyde, cinnamic alcohol, eugenol, geraniol, hydroxycitronellal, isoeugenol and oak moss absolute

3.1.1.2 Skin prick testing

The skin prick tests (SPT) were performed with a standard panel of 10 inhalation allergens, Soluprick SQ® (ALK-Abelló, Hørsholm, Denmark) as previously described (36). A positive SPT was defined as giving a mean wheal diameter of 3 mm or more to at least one allergen.

3.1.1.3 Hand eczema

All participants completed a questionnaire in 1997/98 including questions about hand eczema. The questionnaire described eczema as follows: 'Eczema is an itching skin disease showing redness, dryness, and eventually vesicles and exudation. Eczema is present in the same skin area for some time'. The question was: Have you had eczema on your hands within the last 12 months? An affirmative answer to this question was used to determine the one-year prevalence of hand eczema.

3.1.1.4 Metacholine bronchial hyperreactivity

Metacholine bronchial hyperreactivity (BHR) was measured with a modification of the method of Yan (42). Briefly, a maximal cumulated dose of 1200 µg metacholine was inhaled beginning with 9.39 µg and ending at a dose of 600 µg, each inhalation representing a doubling of the cumulated dose. Lung function was measured one minute after each dose and the test was stopped if forced expiratory volume in the first second (FEV_1) was 20% below the post-saline value. The provocative dose of methacholine required to cause a 20% fall in FEV_1 (PD_{20}) was calculated by linear interpolation between the last two readings on the log dose-response plot.

3.1.1.5 Forced expiratory volume in the first second

Forced expiratory volume in the first second (FEV_1) was measured with a dry bellow spirometer (Vitalograph®, Vitalograph Ltd, Buckingham, UK) as the higher of two measurements within 100 ml. The spirometer was checked each study day with a three-litre calibration syringe (43). Volumes were reported at body temperature, atmospheric pressure and saturated with water vapour (BTPS).

3.1.1.6 Serum eosinophilic cationic protein

UniCAP® ECP (Pharmacia Diagnostics AB, Sweden) measured serum ECP according to the manufacturer's instructions. The measuring range was 2–200 µg/l in undiluted serum and the cross-reactivity with other granulocyte proteins was less than 0.1%.

3.1.1.7 Test of Psychological Vulnerability

Psychological vulnerability was based on a 22-item questionnaire. The score of psychological vulnerability was calculated as the overall sum of 12 items (possible score, 0-12) that constituted the test of psychological vulnerability described by Kühl and Martini (44). The questions in the test and cut off points on the vulnerability dimension according to age and sex have been described elsewhere (45).

3.1.1.8 Social groups and educational level

Information about social group (white-collar worker, blue-collar worker and others) as well as overall educational level ($\leq$9, 10-11, 12-13, $\geq$14 years) was based on questionnaire data collected at baseline.

3.1.2 The questionnaire on respiratory symptoms

A questionnaire focused on respiratory symptoms related to perfume and fragrance products was developed, and tested in a pilot study.

Symptoms of relevance were investigated by searching articles on Medline dealing with related subjects and by personal semi-structured interviews of 20 individuals who reported moderate-severe respiratory symptoms related to perfume and fragrance products. The participants were recruited among colleagues, their family members, friends, and among patients from the allergy-outpatient clinics of pulmonary medicine and dermatology.

Single questions formulated on the basis of the interviews were evaluated in the working group to achieve a clear and relevant questionnaire. A pilot study tested the preliminary questionnaire, which consisted of 100 questions. A group of 24 individuals with self-reported perfume-related symptoms

were systematically recruited from the department of dermatological and pulmonary medicine for participation. There was a telephone interview two months later using the same questionnaire but without knowledge of the participant's initial answers. Single questions were explained to the participants, if necessary, and their comments were protocoled. The pilot study was approved by the Danish Data Protection Agency.

Questions of irrelevance or ambiguity identified during the pilot study were changed or omitted, reducing the final questionnaire to 67 questions of which 51 had been unchanged. All the unchanged questions were easily understood and answered by the participants in the telephone interview. The test-retest reliability of the questionnaire was judged with kappa statistics to assess concordance between primary and secondary responses on the questionnaire. The mean kappa value for the questionnaire was 0.59 and was regarded as a moderate match

The size of the kappa-value may depend on the quality of the question, and also on the prevalence of the symptoms investigated (46), the time interval between the data collections and the methods by which the data are collected. As some of these parameters were not optimal in favour of obtaining a high kappa-value in the test-retest investigation, it was our interpretation from the interviews that respiratory sensations related to perfume exposure often are uncharacteristic and difficult to express by the affected individuals (especially those sensations related to the mouth).

In the questionnaire, perfume and fragrance products were defined as "any cosmetic, skin-care lotion, cleansing agent, washing soap or air-freshener that is perfumed, e.g., fine fragrances, after-shave, eau de cologne, deodorant, moisturisers, powder, toothpaste, soap, shampoo, shaving foam, detergents, fabric softeners, toilet cleanser etc.".

The questionnaire consisted predominantly of binary questions with answer categories yes and no. The questionnaire was structured and initiated with 1) questions about organ-related symptoms from the eyes, the nose, mouth or throat, and lower airways. 2) Then followed a part with questions regarding other characteristics of the symptoms such as duration, exposure time, severity, and sources of fragrance products that triggered symptoms. 3) Questions about respiratory symptoms

related to airborne agents other than perfume and fragrance products were also included i.e. airborne proteins (pollen, dust, animals, and moulds), airborne chemicals (newspaper or printed magazines, laser printers, drying paint, and motor vehicle exhaust).

3.1.3 Design

The questionnaire about respiratory symptoms related to perfume and fragrance products was developed in the present study and posted to the study population. The results from the questionnaire about respiratory symptoms were considered as outcome variables and compared with data collected during the health examination at baseline 1997/98 (predictor variables).

3.1.3.1 Definitions of predictor variables (baseline data)

Perfume contact allergy was defined as a positive patch test to one or more of the three test substances: fragrance mix (FM), balsam of Peru (PB) and colophony. *Nickel contact allergy* was defined as a positive patch test to nickel sulphate. *Atopy* was defined as a positive SPT to at least one of the allergens in the panel. *Bronchial hyperreactivity* was expressed as $PD_{20,}$ transformed into a dichotomous variable with a cut-off value of 1200 µg. Values of serum *ECP* were transformed into a dichotomous variable with a cut-off value of 20 µg /L. *Age* was dichotomised in two groups: 20 to 45 and 46 to 82 years. The dimension of psychological vulnerability score was dichotomised. *Allergic rhinitis* was defined as whether the participant had had an itchy or stuffy nose or had been sneezing within the last 12 months: 1) when near grass, trees, or flowers, 2) after exposure to furry animals, e.g. horse, dog, cat, rabbit, guinea pig, or hamster or 3) after exposure to dust when cleaning rooms or making beds, or when in bed, concurrent with a positive SPT to an allergen relevant to the symptoms (47). *Allergic asthma*: shortness of breath or trouble breathing within the 12 months after the aforementioned exposure, concurrent with a positive SPT to an allergen relevant to the symptoms. *Non-allergic asthma*: affirmative answer to the question "Within the last 12 months have you experienced wheezing?" and no positive SPT.

3.1.3.2 Definitions of outcome variables (questionnaire data)

Outcome variables were derived from the questionnaire about respiratory symptoms elicited by perfume and fragrance products. In paper I and II the outcome variables were categorised as 1) organ-related symptoms, and 2) more general variables describing frequency and severity. In paper III, the outcome variables were divided in two categories: eye and respiratory symptoms elicited by 3) airborne chemicals, and 4) airborne proteins.

1. Organ-related symptoms comprised four variables, defined as at least one symptom from the eyes, nose, mouth (or throat) or lungs, within the last 12 months elicited by fragrance products (see table 2 for details).

2. The following variables about frequency and severity of symptoms were defined:

'Affecting daily activity' was defined as an affirmative answer to the question 'Is your everyday life troublesome as a result of symptoms from your eyes, nose, mouth, throat or lungs elicited by perfume or fragrance products (e.g. by choice of shopping places, means of transportation or adjustment of working situations)?'.

'Affecting application pattern' of personal use of fragrance and cosmetics was defined from the questions 'Do you ever apply perfume, deodorant or after-shave', and 'If no, is this a consequence of symptoms from eyes, nose, mouth, throat or lungs?'.

'Frequently' was defined as at least one organ-related symptom occurring at least once a week.

'Several elicitors' was defined as eyes or respiratory symptoms elicited in at least three of the following situations: 1) when other persons are wearing perfume, 2) when other persons' clothes are washed in fragrant fabric softener, 3) when entering places with air fresheners and 4) when entering recently cleaned places.

'Extensive target organs' refers to symptoms elicited by fragrance products from all mucous-membrane areas (eyes, nose, mouth/ throat and lungs).

3. Symptoms related to airborne chemical comprised respiratory symptoms within 12 months elicited by: newspaper (or printed magazines), laser printers, drying paint, or car exhaust emission.

4. Symptoms related to airborne proteins comprised respiratory symptoms within 12 months elicited by: furry animals, pollen, dust, or mould.

3.1.4 Data entering

Each questionnaire was entered twice by two different keyboarders in SPSS "Data Entry Builder" 2.0. Any mismatch was identified by comparing files, and the correct information was entered. The average mismatch detected per entered value was 1.6%.

Missing values in the final data shied were those questions not answered or with ambiguous answers disclosed by double entering. The average frequencies of missing values were 4%.

3.2 Part 2: The clinical experimental study

3.2.1 Study population

The study population consisted of 21 eczema patients attending a dermatological outpatient clinic for patch testing, and 21 control subjects.

The patients were recruited consecutively according to the inclusion criterion of having at least one respiratory symptom elicited by perfume. The patients were stratified according to patch-test results, 50% had contact allergy to perfume. A total of 22 patients were included, but one dropped out as she failed to attend to the test procedures. Healthy volunteers were recruited by advertising. Exclusion criteria for the controls were atopic airway disease, eczema, smoking, and respiratory symptoms elicited by perfume. Table 1 shows the basic characteristics of cases and controls.

All participants gave their informed consent. Approval was obtained from the Danish Local Ethical Committee of Copenhagen and the Danish Data Protection Agency.

3.2.2 Design

The study was planned to be much larger, including five study groups with 20 individuals in each group. However, it was not possible to recruit enough patients for this design. In particular, eczema patients without respiratory symptoms related to perfume were not willing to participate.

Accordingly, the design was changed to a simpler case-control study where healthy control persons were selected to match the cases concerning sex and age. All participants completed symptom questionnaires; and underwent lung function test (LFU), skin prick test (SPT), bronchial challenge with capsaicin and double-blind, placebo-controlled perfume exposures in the eyes.

3.2.3 Perfume exposure in the eyes

In a protocol, the following specifications were formulated: the perfume exposure should be performed in a way that enabled: 1) carrying out eye exposures with perfume, at controlled flow rates 2) performing double-blind and placebo-controlled experiments 3) randomising between the eyes, and during an exposure in one eye, exposure of the contra-lateral eye with clean air.

3.2.3.1 Developing the exposure model

We decided to develop a portable exposure device with goggles as exposure chambers. We wanted this device to be inexpensive to run, easy to operate, and not uncomfortable for the participant. The last specification implied that the exposure device had to be perceived as odourless, the goggles and straps should cause only minimal pressure on orbits and head, and it should be possible for the participant to breathe freely through the mouth and nose during the exposures.

The goggles for the exposure device were selected from several tested; most were rejected because they gave off odours, did not fit unless under tight pressure, were uncomfortable to wear, or were designed in ways that made it difficult to fit on the fittings for the vapour transfer tubes. Unfortunately, the goggles became perfume scented after one perfume exposure, which could be perceived even after a week in a fume cupboard, so a new pair of goggles had to be used for each perfume exposure. The significant adsorbing or absorbing quality of silicone became obvious when we drew vapours of perfume, with a flow rate of about 300 ml/min, through a silicone tube, length 1 metre, internal diameter 2 mm, and no scent of perfume was perceived at the other end (at least for 15 min). By applying new silicone tubes in the out-flow part of the exposure device in each study, the pumps were prevented from being polluted with fragrance chemicals and it diminished the risk

of perfume escaping to the surroundings to be smelled. Teflon® tubes were found suitable for transportation of perfume vapours from the test tube with perfume to the goggles (the eye chambers). However, the ethanol had to be evaporated to prevent it dominating the exposure. When vapours of perfume were drawn through the Teflon® tubes in the preliminary testing, it caused severe burning and stinging in the eyes. These effects were due to the diluent in the perfume, which was ethanol 95%. Evaporating ethanol from the perfume theoretically gave a more realistic exposure comparable with exposure to e.g. other persons' wearing of perfume.

3.2.3.2 The exposure device

The eye exposure-chambers were a pair of goggles, made of odourless transparent silicone (Softeril®), with clear vision lenses (Plexisol®). Each eye-chamber was an integrated part of the exposure-device (Photo1) consisting of two identical airflow systems with components appearing in the order illustrated in Figure 1.

To prevent escape of perfume-vapour, the pumps were installed next to the exhaustion filter (Figure1). During exposure the pumps created a slight vacuum that in addition to generating the airflow also provided a close draw-fit between the soft rims on the goggles and the skin. To monitor the flow-rate and register a possible air-leakage (as a drop in the flow rate), a flow meter was placed in the entrance of each system.

A uniform airflow was generated by voltage adjustment on two power supplies (7416, 8-16V, Mascot, RS Components A/S, Copenhagen, Denmark) driving the pumps. The air provided to an eye chamber was drawn through a test tube, and a T-union connection made it possible to shift between an A and B test tube for each eye chamber. The A test tubes (Aright and Aleft) were empty and the B test tubes (Bright and Bleft) contained filter paper strips (Crepe MN750, Buch & Holm A/S, Herlev, Denmark).

3.2.3.3 The perfume

The perfume used for challenging was OPIUM® eau de parfum (Yves Saint Laurent, Paris, France). Fragrance chemicals in this perfume had previously been characterised regarding contents of various contact allergens (48;49). Fifty µL of perfume was placed in the centre of a filter paper strip (10x100mm) with an Eppendorf Multipette® plus. Before being enclosing in a test tube, the scented strip was kept in a fume cupboard for 10 min to evaporate all ethanol, see Figure 2. A similar dimensioned but unscented filter paper strip (the placebo) was enclosed in the other test tube.

The amount of perfume used for testing was chosen as half the quantity that could just be perceived during initial testing procedures. In a pilot study, 5 healthy control persons were exposed to perfume (50 µL pre-evaporated for 10 min) in a double-blind, placebo-controlled experiment. None of the tested persons could smell, taste or in any other way perceive the perfume as an active exposure as compared to a placebo exposure.

3.2.4 Bronchial provocation with capsaicin

The capsaicin cough responsiveness was measured with a modification of the method described by Millqvist et al. (15). A stock solution of capsaicin (UNIKEM, Copenhagen, Denmark) 100 µmol/L in ethanol (99.5%) was prepared. Aqueous solutions of 0.4, 2.0 and 10 µmol/L were made from the same stock solution of capsaicin by dilution with 0.9% saline containing 1% by volume of ethanol. Participants inhaled 1 ml of isotonic saline from a Paryboy® PARI LC PLUS nebulisor (Pari-Werk, Starnberg, Germany), breathing in tidal volume for 6 min, followed by 4 min of room-air breathing at ambient temperature and humidity. The particle median mass diameter was 3.5 µm, with at least 68% of the particles smaller than 5µm. The nebulisers were operated by a Pari Turboboy ® N compressor (Pari-Werk, Starnberg, Germany). Subsequently, participants were provoked in the same way with incremental concentrations of capsaicin 0.4, 2.0 and 10 µM in 1 ml solutions. Lung function was measured before the saline provocation and after the end of inhalation of the last dose

of capsaicin. The number of coughs was registered with a tape recorder. The capsaicin inhalation test was terminated if the number of coughs during and after one provocative dose exceeded fifty. Participants were instructed to withhold bronchodilator medication for 12 hours and antihistamines for 72 hours and not to smoke for at least 4 hours before the study visit. The capsaicin test was not carried out during or within 4 weeks after a respiratory tract infection. The cough responsiveness was expressed as the cumulative number of coughs during inhalation of each of the 3 doses. Participants were instructed to focus on subjective symptoms during the test and were not informed that the number of coughs was the objective outcome measurement of the test.

3.3 Statistical analysis

Statistical analyses were performed with SPSS version 11.0 and 12.0 for Windows. The chi-square test, Fishers exact test as well as the Mann Whitney U test (in Paper V) were used to compare the groups. Associations were expressed as odds ratios (OR) with 95% confidence intervals (95% CI). The association between various outcome variables and predictor variables was further analysed in logistic regression models including relevant confounders. Statistical significance was defined as $p<0.05$.

4 RESULTS

4.1 Epidemiological data (Part 1)

4.1.1 Response and participation pattern

The questionnaire was posted (in October 2002) to 1189 subjects still alive (97.8% of the baseline participants n=1216). A reminder was posted to non-respondents one month later. The overall response rate to the questionnaire about mucosal symptoms elicited by fragrance products was 79.6% of the 1189 participants still alive. Forty-two questionnaires (3.5%) were returned because the address was unknown. Twelve individuals (1%) declined to participate with the following reasons: severe illness (n=2), high age (n=2), out of the country (n=3), no explanation (n=5). A response was not received from 189 individuals (15.9%). The mean age among responders was 45.8 years (range: 20-82). Non-responders were older and more psychological vulnerable than responders (Table 1). The percentage of predicted FEV1 was significantly lower in the deceased (mean 89.5%).

4.1.2 Perfume-related outcome variables (Paper I)

Symptoms were most frequently reported from the nose followed by the eyes, lungs and mouth or throat (Table 2). The frequency of reporting at least one mucous membrane symptom elicited by fragrance products within the last 12 months was 42% in the total population (50% in women and 30% in men). Symptoms at least once a week were reported by 14%, 5% reported that the symptoms influenced their choice of shopping places, means of transportation or resulted in adjustments of working situations, and 4% avoided personal use of perfume and perfumed cosmetics because of their mucosal symptoms. In those who reported symptoms, the symptoms occurred as follows: other persons' wearing of perfume in 56%, air-fresheners in 32%, and other persons' wearing of newly washed clothes in 28%.

4.1.3 Atopy, BHR and perfume related outcome variables (Paper I)

Bronchial hyperreactivity (OR 2.3, 95% CI 1.6-3.5) and female sex (OR 2.3, 95% CI 1.7-3.1) were consistently associated with at least one organ-related symptom, also when adjusting for atopy, ECP, FEV_1, age and smoking. No associations were found between atopy and at least one organ-related symptom (OR 1.2, 95% CI 0.9-1.5). Consistent associations between allergic asthma (OR 1.9, 95% CI 1.2-2.8), non-allergic asthma (OR 3.3, 95% CI 2.1-5.0) and at least one organ-related symptom were found. Allergic rhinitis was associated with symptoms elicited by fragrance products from the nose (OR 1.6, 95% CI 1.2-2.1), mouth or throat (OR 1.5, 95%CI 1.0-2.4), only.

4.1.4 Perfume allergy, hand eczema and perfume-related outcome variables (Paper II)

Perfume contact allergy and hand eczema were significantly associated with the various organ-related symptoms, also when adjusting for nickel contact allergy, hand eczema, BHR, atopy, psychological vulnerability, age and sex. (Table 3a and 3b) and also when adjusting for social groups and educational level.

4.1.5 Contact allergy and non-perfume-related outcome variables (Paper III)

Eye and respiratory symptoms within the last 12 months elicited by airborne chemicals other than perfumes were seen in 37% of the study population. A positive and significant association was found between patch test reactivity and respiratory symptoms elicited by airborne chemicals other than perfume (Figure 3), and the OR increased with the number of positive patch tests with a significant trend, also when excluding individuals with positive patch tests to fragrance chemicals from the analysis (Table 4). Respiratory symptoms related to airborne chemicals other than perfume were more associated with respiratory symptoms related to perfume and fragrance products than were respiratory symptoms related to airborne proteins.

4.2 Experimental data (Part 2)

4.2.1 Eye exposures with perfume (Paper IV)

The exposure-device is illustrated in Photo 1 and Figure 1.The patients more frequently ($p<0.05$) reported various symptoms during an exposure than did the healthy control persons and eye symptoms were significantly associated with an active exposure ($p<0.05$) as compared with placebo. However, symptoms from the nose, throat, lower airways, or CNS were reported independently of an active exposure. As only one eye was exposed to perfume there were three control exposures for comparison (i.e. the contra lateral eye during perfume exposure and both eyes during placebo exposure). Delta values of eye symptoms from active vs. placebo exposure were calculated. Eye symptoms from an active exposure were regarded as a true positive reaction to perfume. Thus, eye symptoms from placebo exposures were regarded as false positive reactions to placebo.

True positive reactions to perfume were seen in 38% (8/21) of the patients and 10% (2/21) of the healthy control persons. The association between a true positive reaction to perfume and being a patient was an OR 5.8 and this association was borderline significant ($p=0.07$, Fishers exact test).

Further, a true positive reaction to perfume was significantly associated with perceiving perfume as an active exposure (Table 5). Conversely, a false positive reaction to placebo (n=4) was not associated with perceiving an exposure as active (Table 5).

A true positive reaction were associated with a history of symptoms related to broader chemical exposures in everyday life other than- and/ or additional to perfume with a significant trend, also when adjustment were made for sex and participation status (24% of the healthy control persons had a history of respiratory symptoms to at least one of these chemical agents within the last 12 months).

4.2.2 Bronchial challenge with capsaicin (PaperV)

All patients in this study had at least one upper respiratory and/or eye symptom elicited by perfume within the last 12 months, and 11 patients (54%) also had lower respiratory symptoms (LRS) (Table 6). Thus 10 patients had only upper respiratory and/or eye symptoms (URS).

In the capsaicin challenge test, the median number of coughs was significantly higher during each of the 3 inhalation series in patients reporting URS compared with the healthy volunteers (Figure 4). However, only a slight and non-significant difference in the cough responsiveness was found between the patients reporting URS and the healthy volunteers. Lower respiratory symptoms were significantly associated with capsaicin cough responsiveness in each of the 3 inhalation series compared with both the group with URS and the healthy volunteers. Reporting severe symptoms to perfume (n=11/21) was not associated with either LRS or increased capsaicin cough responsiveness. The overall frequency of the symptoms provoked by capsaicin inhalation was significantly higher in individuals with LRS compared with the groups with no URS (healthy volunteers) or URS elicited by perfume. Similarly, the overall frequency of the symptoms released by capsaicin was significantly higher in the group with URS than in the group of healthy volunteers.

5 DISCUSSION

5.1 Epidemiological data (Part 1)

Respiratory symptoms related to perfume and fragrance products were common in the study-population. Although most individuals experienced the symptoms as mild and not bothersome, many reported more severe symptoms. The respiratory symptoms were most frequently reported from the nose. The prevalence of symptoms from the nose in individuals with allergic rhinitis was in accordance with a study including 315 patients with rhinitis (4). Unexpectedly, the OR between perfume-related symptoms from the nose and having allergic rhinitis was small and severity of the symptoms was not associated with allergic rhinitis. This suggests that nasal symptoms from perfume exposure may be more attributable to mechanisms related to non-allergic, non-infectious rhinitis (idiopathic rhinitis) than to allergic rhinitis (50). Immunocompetent cells may not be involved in idiopathic rhinitis (51) and neurogenic reflex mechanisms initiated by environmental factors appear to be an alternative explanation for this condition (52).

We investigated the association between respiratory symptoms elicited by perfume and BHR. In the analysis we adjusted for possible confounders: atopy, FEV1, serum ECP and smoking. Consistent associations between the respiratory symptoms and BHR were found. This indicated that such symptoms would be more prevalent in persons with asthma, and when investigating the associations between our epidemiological definitions of asthma and the outcome variable, we found that allergic asthma and especially non-allergic asthma were associated with symptoms elicited by fragrance products. In contrast, smoking and low FEV_1 were not associated with the symptoms.

Respiratory symptoms related to fragrance products were not associated with previously recognised atopy (defined as positive SPT). This suggests pathophysiological mechanisms different from specific IgE-mediated immunological mechanisms.

Increased sensitivity to inhaled capsaicin indicating an alteration of chemosensory perception has been observed in patients with lower respiratory symptoms and SHR has been suggested as underlying the symptoms (15). A decline in lung function after perfume inhalation has been

reported in individuals with severe atopic asthma (6) as well as in patients with occupational asthma to perfume (5;12). These observations support our findings of an association between respiratory symptoms elicited by perfume and BHR as well as by asthma, and because inflammation in the airways may increase the bronchial responsiveness to capsaicin (53), these studies (5;6;12) do not contradict the hypotheses of SHR as an underlying mechanism for perfume-related symptoms (15).

Women, as opposed to men, reported more frequent and more severe mucosal symptoms after exposure to fragrance products. An explanation could be related to a heavier exposure in women to fragrance chemicals from personal cosmetic usage (54). The cough threshold measured by inhaled capsaicin is significantly lower in healthy women compared to healthy men (55). This physiological difference between men and women could indicate the higher frequency of respiratory symptoms among women.

Paper II represents a second step in the data analysis where we investigated the association between respiratory symptoms elicited by perfume and previously recognised perfume contact allergy and hand eczema.

The results suggested that individuals with perfume contact allergy and/or hand eczema were more frequently and more severely bothered by volatile exposure to fragrance products than those without these diagnoses. The respiratory symptoms were mostly reported as elicited within seconds or minutes after airborne exposure to fragrance products. Usually, contact eczema in sensitised individuals develops hours to days after exposure to an allergen and immediate responses are not in agreement with a type IV immunological reaction. However, in a case with occupational asthma to perfume a positive patch test to fragrance chemicals was found, and bronchial obstruction was elicited subsequently after a perfume provocation (12). Airborne exposure to potent allergens such as the preservative methylchloroisothiazolinone/ methylisothiazolinone in sensitised individuals has been reported to result in a systemic reaction with contact eczema and immediate respiratory symptoms (56). Yet, the mechanism remains undetermined. Fragrance ingredients may also cause

non-immunological immediate reactions in the skin (57-59), but data suggest that such symptoms are not related to contact allergy (60).

Hand eczema has multifactorial genesis influenced by both endogenous and exogenous factors (61). History of atopic dermatitis (62), and also genetic factors independent of atopic dermatitis have recently been suggested as predisposing to development of hand eczema (63). Exogenous factors for developing hand eczema constitute exposure to wet work, contact allergens and skin irritants (63). It is tempting to suggest a common endogenous susceptibility to fragrance chemicals in individuals with asthma, perfume contact allergy and hand eczema e.g. atopic dermatitis. However, chronic tissue inflammation is another characteristic of the conditions; asthma and hand eczema, in particular, are more or less chronic inflammatory conditions. During tissue inflammation, sensory nerve activation is altered, which may lower the response threshold to some chemical stimuli (17;64). In this way the results suggest a link between tissue inflammation (local or body regions other than the respiratory organs) and respiratory symptoms related to fragrance chemicals.

Symptoms from the respiratory organs related to fragrance products were reported more frequently among individuals who were psychologically vulnerable. Psychological vulnerability has been associated with upper dyspepsia and irritable bowel syndrome (65;66), and predict prolonged pain after lumber spine surgery and cholecystectomy (67-69). The results from the present study did not disagree with suggestions that psychological vulnerability may predict a poorer health behaviour pattern (44;65-69). However, it is interesting to note that the somatosensory perception of chemicals in respiratory organs i.e. chemestesis (70) is mediated via receptors that are also heat and acid sensitive nociceptors as the capsaicin-receptor (VR1) (71). Individuals with psychological vulnerability and/or inflammatory conditions in skin and respiratory organs may be more sensitive to chemical stimulation of somatosensory neurones, resulting in respiratory symptoms elicited by perfume and fragrance products and in some cases even measurable as an increased bronchial responsiveness to inhalation of capsaicin.

The association we found between perfume contact allergy and respiratory symptoms related to perfume could be influenced by information or attention bias, because individuals with a perfume contact allergy were informed about the patch test result during the health examination at baseline.

In paper III we wanted to explore these obvious biases by investigating the association between respiratory symptoms related to airborne chemicals other than perfume and positive patch tests to contact allergens. In the analysis we adjusted for possible confounders including hand eczema, BHR, psychological vulnerability, sex, and smoking.

The findings of a consistent dose-dependant association between respiratory symptoms related to perfume and fragrance products and patch test reactivity suggest a causal relationship between the respiratory symptoms and cell-mediated allergy to chemicals. However, causality implies a time-sequence, biological credibility, and consistency with other investigations. Although the time-sequence of patch test reactivity might have been indicated (paper III), ideally it would require a prospective study to judge the question about time-sequence. A possible biological mechanism of the association between contact allergy and respiratory symptoms related to airborne chemicals could be an endogenous predisposition to react to chemicals with an inflammatory response. Individuals who are sensitised to more than one allergen 'multiple sensitisation' (41) may belong to a subgroup with increased susceptibility to chemicals (72). Enzymes involved in detoxification of chemicals are subject to genetic polymorphisms (73), which may be of importance for the development of contact allergies (74) and which could also influence the development of respiratory symptoms elicited by airborne chemicals (75). In this relation it is interesting to note that many airborne chemicals with respiratory irritation capacity (76) are also well-known contact allergens (77). No association was found between respiratory symptoms elicited by airborne chemicals and SPT reactivity, which is in agreement with the observation that patch test reactivity appears to be independent of SPT reactivity in the general population (78).

5.2 Validity of the results (Paper I-III)

Although the study was population-based, the study population was not selected as representative of the general population. The selection criterion was participation in the health examination in 1997/98 that included random samples of the general population and a smaller sample of individuals with indications of allergic airway diseases. Thus the frequencies of the symptoms were likely to be overestimated as compared to an unselected population-based sample. However, it was not the intention of the study to estimate the prevalence of respiratory symptoms in the general population.

The associations were independent of the possible confounders included in the analysis. However, Bias could be a problem and deserves further consideration. The predictor variables (atopy, BHR, perfume contact allergy, hand eczema, rhinitis and asthma) were measured four to five years earlier than the respiratory symptoms related to perfume and fragrance products. Theoretically, this may influence the results, but asthma and hand eczema are chronic diseases and the importance of follow-up time is probably of minor relevance for the results. Additionally, recall bias could be a problem: participants with a positive SPT and/or BHR may be more aware of respiratory symptoms, and therefore report more symptoms. However, individuals with atopy did not report more symptoms. In paper II, recall bias could have influenced the results about individuals with a positive patch test to fragrance chemicals. Nevertheless, paper III contradicts the significance of such bias because individuals with positive patch tests were generally more bothered by airborne chemicals.

Hand eczema was not doctor diagnosed. The definition of hand eczema in this study was based on the individual's recognition of a clinical description of eczema in a questionnaire (self-diagnosed eczema). Determination of hand eczema by a questionnaire has been validated in different occupational groups (79;80). The specificity of questionnaire-based, self-diagnosed one-year prevalence of hand eczema compared with subsequent evaluation by a dermatologist was more than 96% and the sensitivity was 53-59% (80).

No difference was found between responders and non-responders concerning BHR, contact allergy, hand eczema and sex, indicating that selection bias between baseline and follow-up was less likely to influence the results.

Thus, there were no indications that the results obtained by the epidemiological study were not valid. However, a judgement of the ability to generalise the results from our study must await future results from epidemiologic investigations on other populations.

5.3 Experimental data (Part 2)

We developed a novel study design: a double-blind, placebo-controlled exposure with perfume vapours in the eyes, where the placebo controlled exposures included both the contra-lateral eye during the perfume exposure as well as both eyes during a 15-min exposure to placebo only. This design made it possible to have very strict criteria for a true positive reaction. A tendency between reporting ocular or respiratory symptoms to environmental perfume exposure and having a true positive reaction to perfume was shown. Further, individuals with a true positive reaction were characterised as having a history of respiratory symptoms related to broader chemical exposures than perfumes alone. A true positive reaction to perfume was significantly associated with identification of perfume as an active exposure. This means that fragrance chemicals in perfumes in at least some individuals have the capacity to induce symptoms in the eyes.

In a previous study of patients with asthma-like symptoms related to strong scents (16) a 30-min perfume exposure elicited eye irritation in a time dependent way. Based on the same study, we had expected the patients with lower respiratory symptoms (n=11) to present cough and dyspnoea when exposed in the eyes with perfume. The patients with lower respiratory symptoms in the present study did not report symptoms from the lower airways in excess during a perfume vs. a placebo exposure. However, they were exposed for only 15 minutes; longer exposures of 20 minutes during preliminary testing gave some participants a perception of smell or taste. This indicated that chemical components may pass the nasolacrimal duct depending on the exposure time and

presumably also influenced by the concentration of the provocative dose as well as anatomical variations.

The ophthalmic branch of the trigeminal nerve innervate cornea, conjunctiva, lacrimal glands and eyelids (70). Since olfactory sensations are not localised to either side (81), it is likely that the true positive reaction to perfume appeared independent of olfaction. Studies have shown good agreement between the thresholds of corneal and nasal irritation regarding single agents (alcohols, ketones and alkylbenzenes) and mixtures of volatiles (82;83). This suggests that individuals with a 'true positive reaction' may have a lower threshold for both ocular and nasal chemestesis, which is in good accordance with the observation that a history of respiratory symptoms to broader chemical exposures was associated with a true positive reaction to perfume.

Lower but not upper respiratory symptoms elicited by perfume were associated with increased capsaicin cough responsiveness (Paper V). Despite normal cough responsiveness to capsaicin, the patients with URS reported significantly more symptoms due to capsaicin challenge than the group of healthy control persons, suggesting that, nevertheless, they were more sensitive to capsaicin.

The patients in the present study were referred to the dermatological outpatient clinic because of eczema and not because of respiratory symptoms elicited by perfume. However, the distribution of symptoms was not significantly different from that of the epidemiologic study (Paper I).

Exposure to inflammatory mediators and chemicals may lead to sensitisation of peripheral nerve endings (64), and neuronal sensitisation of the cough receptor VR1 has been suggested as a possible physiological explanation for increased airway sensitivity to chemicals and strong scents (84). However, damage or loss of normal inhibitory regulation of the cough reflex could also be a physiological explanation of the tendency to continue coughing in patients with LRS.

Fragrance chemicals can be categorised according to the Beilstein system of functional groups (2) of which many show irritant capacity and many are well-known contact allergens (3). Airway exposure to irritants may activate local and central sensory reflexes (17;70;85) and even evoke neurogenic inflammation (86). Sensory neurones play important roles in airway defensive reflexes

such as the physiological response to chemical stimulation in the upper (70) and lower airways (87). Hyperreactivity of airway sensory neurones may underlie cough and asthma-like symptoms related to perfume and airborne chemicals (15). In addition, this sensory hyperreactivity may also include URS related to airborne chemicals in patients with clinical atopic airway diseases (84).

Paper V suggest that sensory hyperreactivity either may be a local phenomenon only measurable in the relevant organ, or that URS may be caused by other mechanisms. Although 95% of the patients reported symptoms from the nose, only 28% had allergic rhinitis. Nasal application of capsaicin in normal individuals triggers protective reflexes of the upper airways such as sneezing, runny nose and lacrimation (88;89) but it is unknown whether the responsiveness to nasal capsaicin provocation would be altered in patients with nasal symptoms elicited by perfume. However, high doses of intranasal capsaicin reduce nasal hyperreactivity in idiopathic rhinitis (90), indicating the importance of capsaicin sensitive neurones of non-allergic nasal symptoms.

When measuring the cumulative number of coughs during 6 minutes of tidal breathing of capsaicin and 4 minutes of rest, the outcome of the test may reflect another aspect of the cough reflex than the exact threshold of elicitation. In normal individuals, tachyphylaxsis occurs within the first minute of bronchial exposure to capsaicin, (18) which could explain the low (expected) capsaicin cough responsiveness seen in our group of healthy volunteers and individuals with URS. The method used in this study determined the tendency to continue coughing rather than a precise threshold for triggering the cough reflex.

We hypothesise that the site of respiratory symptoms elicited by perfume covers hyperreactivity of specific defensive reflexes due to sensitisation of receptors on afferent nerve endings, or loss of normal inhibitory regulation.

5.4 Methodological considerations (Part 2)

In paper IV blinding the exposures to fragrance chemicals had importance, because eye or respiratory irritation might be confounded by smell or taste, as most volatile chemicals stimulate the olfactory system at concentrations well below those at which they elicit irritation (91;92) and some fragrance chemicals are also flavour substances stimulating gustation (2). Subjective, as well as objective responses to chemicals may be influenced by expectations and beliefs about odorant exposures (81;91). If a chemical exposure with a certain smell has previously caused severe sensory irritation, olfaction may contribute with a protective biological function in detecting the potentially harmful chemical (by its odorant properties) at a lower concentration than elicitation of an irritative response (93). This theoretic involvement of olfaction may be established at the expense of specificity regarding the chemicals that initially caused problems. Compounds that are perceived as similar odours may have chemical structures that are totally different (2); therefore, if olfaction plays a role in the elicitation of respiratory symptoms related to fragrance chemicals, broader groups of chemicals without relevance for the initial symptoms might be included.

There may be several reasons for the patients not all having a true positive reaction to perfume.

First, the type of perfume used for exposure may have had limited relevance for the symptoms in the group tested. Perhaps some of the patients are sensitive to only specific fragrance chemicals or to perfumes dominated by functional groups different from those in the perfumed vapour used for exposure. This could be supported by the observation that individuals with a true positive reaction more often had a history of symptoms related to broader chemical exposures additional to perfume (Table 5). Second, the concentration of perfume may not have been high enough or the exposure time not long enough to elicit the symptoms in all patients; this could be supported by the fact that no participant experienced a provocation as severe.

The model of capsaicin cough responsiveness used in Paper V has shown good reproducibility after short-term retesting (15) and reliability when saline and capsaicin solutions are inhaled in randomised, double-blind order (94). Some methodological limitations were attached to the

capsaicin test used in the present study. We had intended to perform the capsaicin challenges according to a previously described procedure (15); however, the nebulisor used in that study was no longer available on the market. The nebulisor used in our study was designed to drive a minimum of 2 ml. solution giving a total output rate of 500 mg/min, and the particle median mass diameter was 3.5 μm, with at least 68% of the particles smaller than 5μm. In the original, described model the output rate was 0.63 ml/min and the median mass diameter of the particles produced was larger (9.2 μm) (95). Although the output rate of the nebulisor used in our study was comparable with the original capsaicin model (95), the capsaicin in our model was more likely to be delivered to lower parts of the airways. We did not perform double-blind capsaicin provocations. However, we camouflaged the outcome of the test by instructing the participants to be focused on subjective symptoms during the test, and we did not disclose that it was the number of coughs and not the subjective sensations that was the most important outcome.

6 CONCLUSIONS AND PERSPECTIVES

With regard to the aims of the thesis formulated in paragraph 2, the following conclusions can be drawn:

- Exposure to perfume and fragrance products may elicit various symptoms in the respiratory organs, mostly localised in the upper airways.
- Bronchial hyperreactivity, asthma, hand eczema and perfume contact allergy were all associated with the respiratory symptoms related to perfume. Allergic rhinitis was associated only with respiratory symptoms from the upper airways, and IgE-mediated allergy to proteins was not associated with the respiratory symptoms related to perfume
- Respiratory symptoms related to airborne chemicals other than perfume were associated with contact allergy, and the association increased with the number of positive patch tests
- Vapours of perfume may cause irritation in the eyes independent of olfaction, although the relative importance of the ocular chemoperception in relation to elicitation of eye and respiratory symptoms from environmental exposures to perfume is yet to be clarified.
- Lower but not upper respiratory symptoms were associated with increased capsaicin cough responsiveness.

In conclusion, we found no indications that respiratory symptoms from environmental perfume exposure were caused by IgE-mediated allergy, but various other objective findings were associated with the symptoms independent of psychological vulnerability. These objective findings included BHR, increased capsaicin cough responsiveness, perfume contact allergy as well as self-reported hand eczema.

6.1 Perspectives

There may exist several physiological pathways by which fragrance chemicals elicit symptoms in respiratory organs. The present thesis suggests that such pathways might be positively influenced by individual factors, including the physiological threshold of protective reflexes triggered by chemicals (e.g. the bronchial obstruction reflex triggered by metacholine and the cough reflex triggered by capsaicin), inflammatory conditions in skin and airways (asthma and hand eczema), immunological type IV allergic reactions or endogenous predisposition to react to chemicals with an inflammatory response (contact allergy), sex and psychological factors.

Further, the thesis supports the observation stated by other researchers that respiratory symptoms related to perfume can, to some extent, be quantified by bronchial provocations with capsaicin.

The perfume exposures in this study were performed as double-blind and placebo-controlled experiments in concentrations of perfume close to the detection threshold. Future studies might benefit from comparing dose-response effects describing the relationship between the detection threshold and the threshold of objective signs of irritation (e.g. lacrimation) between cases and controls. Since a true positive reaction to perfume indicated the active exposure with great precision for the participant, subjective eye irritation may influence the blindness of an exposure, where other symptoms are measured.

More insight into the pathophysioloical mechanisms that cover the various respiratory symptoms is a precondition for development of more precise terminology, and also for developing better hypotheses and generating new test methods to further describe the epidemiology of respiratory symptoms related to environmental perfume exposure.

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8 TABLES

Table 1: Characteristics of responders and non-responders (Part 1)

	Responders			Non-responders		
	Total (n=946) (%)	Men (n=416) (%)	Women (n=530) (%)	Total (n=270) (%)	Men (n=137) (%)	Women (n=133) (%)
Patch test reactivity to perfume-related haptens †	4.5	2.2	6.3	3.8	2.2	5.4
Patch test reactivity to nickel sulphate	10.9	2.7	17.3	12.5	2.2	23.4
Hand eczema	15.5	9.6	20.2	12.6	11.7	13.5
Bronchial hyperreactivity	12.9	11.2	14.2	15.7	16.0	15.4
Atopy	40.1	46.4	35.1 *	37.5	48.9	25.8 *
Age (< 46 years)	54.8 *	51.2 *	57.5 *	73.3 *	70.8 *	75.9 *
Psychological vulnerability	9.7 *	9.4	9.8 *	14.8 *	13.1	16.5 *

Including individuals who emigrated or died in the follow-up period.
† Fragrance mix, colophony, balsam of Peru.
* The difference between responders and non- responders was statistically significant ($p<0.05$)

Table 2: The prevalence of various symptoms occurring within the last 12 months (Part 1)

Do perfume or fragrance products induce any of the following symptoms?	Total (*n*=946) (%)	Atopy (*n*=378) (%)	Allergic rhinitis (*n*=258) (%)	BHR (*n*=118) (%)	Allergic asthma (*n*=96) (%)	Non-allergic asthma (*n*=109) (%)
Nose						
Sneezing	29	34	39	45	44	49
Runny nose	17	20	21	26	27	31
Itching	14	17	19	21	27	22
Pricking sensation	12	12	14	16	16	25
Irritation (stinging and dryness)	9	8	10	13	17	16
Stuffed-up nose	8	10	10	18	11	14
Eyes						
Itching eyes	15	18	20	27	30	29
Eye-irritation (stinging and dryness)	12	12	13	19	16	27
Tearful eyes	11	10	12	18	16	25
Swelling or flushing of surroundings	8	9	11	15	12	17
Lower airways						
Troublesome breathing	11	11	13	32	25	29
Coughing	11	10	11	26	20	24
Wheezing	7	8	10	24	20	22
Phlegm when coughing	6	4	4	18	9	16
Chest tightness	5	4	4	12	9	15
Mouth or throat						
Discomfort	6	5	6	8	8	17
Irritation (stinging and dryness)	5	6	7	7	10	11
Pricking sensation	4	6	7	6	10	7
Itching	3	4	5	5	8	7

Table 3a: Occurrence of symptoms elicited by fragrance products within the last 12 months in various subgroups (Part I)

		Fragrance products that elicit symptoms from the :							
		Eyes (22%) #		Nose (35%) #		Mouth or throat (12%) #		Lungs (18%) #	
		Prevalence*	OR **	Prevalence*	OR **	Prevalence*	OR **	Prevalence*	OR **
Perfume-related haptens †	Positive ##	43%	**2.4 (1.2-4.8)**	58%	**2.0 (1.0-4.0)**	31%	**3.7 (1.8-7.7)**	33%	**2.4 (1.2-5.0)**
	Negative	21%		34%		11%		17%	
Nickel sulphate	Positive ##	31%	1.1 (0.7-1.9)	45%	1.2 (0.7-1.8)	13%	0.8 (0.4-1.7)	20%	0.9 (0.5-1.5)
	Negative	21%		34%		11%		18%	
Hand eczema ††	Yes	41%	**2.6 (1.7-3.9)**	52%	**1.8 (1.2-2.6)**	21%	**2.0 (1.2-3.4)**	29%	**1.6 (1.0-2.6)**
	No	18%		32%		10%		16%	
BHR	Positive	35%	**1.9 (1.2-3.0)**	47%	**1.6 (1.0-2.4)**	19%	**1.8 (1.1-3.2)**	40%	**4.0 (2.5-6.2)**
	Negative	19%		34%		10%		14%	
Atopy	Yes	23%	1.1 (0.8-1.6)	38%	1.3 (1.0-1.7)	13%	1.2 (0.7-1.8)	18%	1.0 (0.6-1.4)
	No	21%		33%		11%		18%	
Psychological Vulnerability	Yes	36%	**1.9 (1.1-3.2)**	47%	**1.7 (1.0-2.7)**	21%	**2.3 (1.3-4.2)**	31%	**2.0 (1.2-3.5)**
	No	20%		34%		10%		16%	

the prevalence of the above symptoms in the total population
* the prevalence of the above symptoms in the groups in the row
** odds ratio (95% confidence interval) adjusted for the other values in the table (plus sex and age).
positive patch test to the haptens tested for
† fragrance mix, colophony, balsam of Peru.
†† hand eczema within the last 12 months (in 1998)

Table 3b: Occurrence of symptoms indicating severity in various subgroups.(Part1)

		Symptoms elicited by fragrance products:									
		Affecting daily activity (5%) #		Frequently (15%) #		By several elicitors (8%) #		In extensive target organs (6%) #		Affecting application pattern (4%) #	
		Prevalence*	OR **	Prevalence*	OR **	Prevalence*	OR **	Prevalence*	OR **	Prevalence*	OR **
Perfume related haptens †	Positive ##	18%	**3.7**	29%	**2.5**	16%	1.9	21%	**5.1**	10%	3.1
	Negative	5%	**(1.4-9.7)**	14%	**(1.1-5.3)**	7%	(0.7-4.9)	5%	**(2.1-12.1)**	3%	(0.9-9.9)
Nickel sulphate	Positive ##	12%	1.9	17%	0.9	13%	1.4	6%	0.6	5%	1.1
	Negative	5%	(0.8-4.1)	14%	(0.5-1.7)	7%	(0.7-2.9)	6%	(0.2-1.5)	4%	(0.4-3.1)
Hand eczema ††	Yes	14%	**3.4**	27%	**2.1**	16%	**2.5**	13%	**2.5**	7%	**2.6**
	No	4%	**(1.7-6.7)**	12%	**(1.3-3.3)**	6%	**(1.4-4.6)**	5%	**(1.3-4.8)**	3%	**(1.1-6.0)**
BHR	Positive	12%	**2.3**	24%	**1.8**	14%	1.7	12%	**2.1**	7%	1.8
	Negative	4%	**(1.1-4.7)**	13%	**(1.1-3.1)**	7%	(0.9-3.2)	5%	**(1.1-4.2)**	3%	(0.8-4.4)
Atopy	Yes	6%	1.0	14%	0.8	8%	0.9	6%	0.9	4%	1.0
	No	5%	(0.5-2.0)	15%	(0.6-1.3)	8%	(0.5-1.6)	6%	(0.5-1.7)	4%	(0.5-2.2)
Psychological Vulnerability	Yes	13%	2.1	31%	**2.5**	17%	**2.5**	14%	**2.6**	7%	1.2
	No	4%	(0.9-4.8)	13%	**(1.5-4.3)**	7%	**(1.3-4.9)**	5%	**(1.3-5.4)**	3%	(0.4-3.8)

the prevalence of the above symptoms in the total population
* the prevalence of the above symptoms in the groups in the row
**odds ratio (95% confidence interval) adjusted for the other values in the table (plus gender and age).
positive patch test to the haptens tested for
† fragrance mix, colophony, balsam of peru.
†† hand eczema within the last 12months

Table 4: Logistic regression model with eye and airway symptoms elicited by airborne chemicals or airborne proteins within the last 12 months as dependent variables, and patch test reactivity and skin prick test reactivity as explanatory variables

	Eye and airway symptoms elicited by			
	Airborne chemicals ¶		Airborne proteins¶¶	
	% (n/total)	OR (95%CI)	**% (n/total)**	OR (95%CI)
Patch test reactivity				
No positive patch test †	33.3 (227/682)	**1 *P* < 0.001##**	51.8 (350/676)	1 *P* = 0.3 ##
One positive patch test †	47.8 (65/136)	**1.6 (1.1-2.4)**	53.9 (73/137)	1.0 (0.6-1.5)
Two or more positive patch tests ††	58.3 (28/48)	**2.2 (1.2-4.0)**	71.4 (35/49)	1.7 (0.8-3.5)
Skin prick test reactivity				
No positive SPT	34.9 (184/527)	1 *P* = 0.3##	33.1 (171/517)	**1 *P* < 0.001##**
One positive SPT *	41.0 (50/122)	1.2 (0.8-1.9)	69.8 (88/126)	**5.0 (3.2-7.7)**
Two or more positive SPT**	39.6 (86/217)	1.2 (0.8-1.7)	90.1 (199/219)	**21.0 (12.6-35.1)**

¶ eye and airway symptoms elicited by exposure to at least one of the following: newsprint, drying paint, car exhaust emission, or laser printers within the last 12 months
¶¶ eye and airway symptoms elicited by exposure to at least one of the following: furry animals, pollen, dust, or mould within the last 12 months
† reactivity to only one of the 24 patches in the panel
†† reactivity to two or more of the 24 patches in the panel
* reactivity to only one of the 10 skin prick tests in the panel
** reactivity to two or more of the 10 skin prick tests in the panel
# odds ratio (95% confidence interval) adjusted for the other values in the table and bronchial hyperreactivity, hand eczema, psychological vulnerability; plus age, sex, and smoking habits
p value for test for trend

Table 5: The association between eye symptoms elicited by an exposure and perceiving the exposure as active.

Eye symptoms:	Perceived perfume as active (n=14) (%)	No indications of an active exposure (n=21) (%)	Perceived placebo as active (n=7) (%)
True positive reaction (n=10)	**9 (90.0)***	1 (10.0)	0 (-)
False positive reaction (n=4) **	1 (25.0)	3 (75.0)	0 (-)
No reaction from the eyes (n= 28)	4 (14.3)	17 (60.7)	7 (25.0)

* A true positive reaction to perfume was associated with the perception of perfume as active ($p<0.05$, Fishers exacts test)

** An excess of eye symptoms during placebo exposure or from the contra-lateral eye as compared with the perfume exposed eye.

Table 6: Basic characteristics for cases and controls

	Patients with eczema (n=21)	**Healthy volunteers (n=21)**
Mean age, years (range)*	50.5 (38-68)	48.1 (22-70)
Women/men *	15/6 (75.6)	15/6 (75.6)
Contact allergy (% of total) #	10 (47.6)	-
FEV1 % predicted (range)	94.3 (69-122) †	103.2 (83-127) †
At least one positive skin prick test (% of total)	8 (38)	2 (9.5)
Allergic asthma (% of total) #	4 (19.1)	-
Non-allergic asthma (% of total) #	1 (5)	-
Allergic rhinitis (% of total) #	6 (28.7)	-
Present smokers (% of total) #	6 (28.6)	-
Past smokers (% of total)	11(53.7)	8 (42.1)
Mean pack years (range)	13.8 (0-60) †	1 (0-5.2) †

* Matching criteria for the healthy volunteers
\# Exclusion criteria for the healthy volunteers
† The difference between the patients and healthy volunteers was statistically significant ($p<0.05$)

9 PHOTO OF FIGURES

Photo 1: The exposure-device. Numbers correspond to descriptions in legend to Figure 1

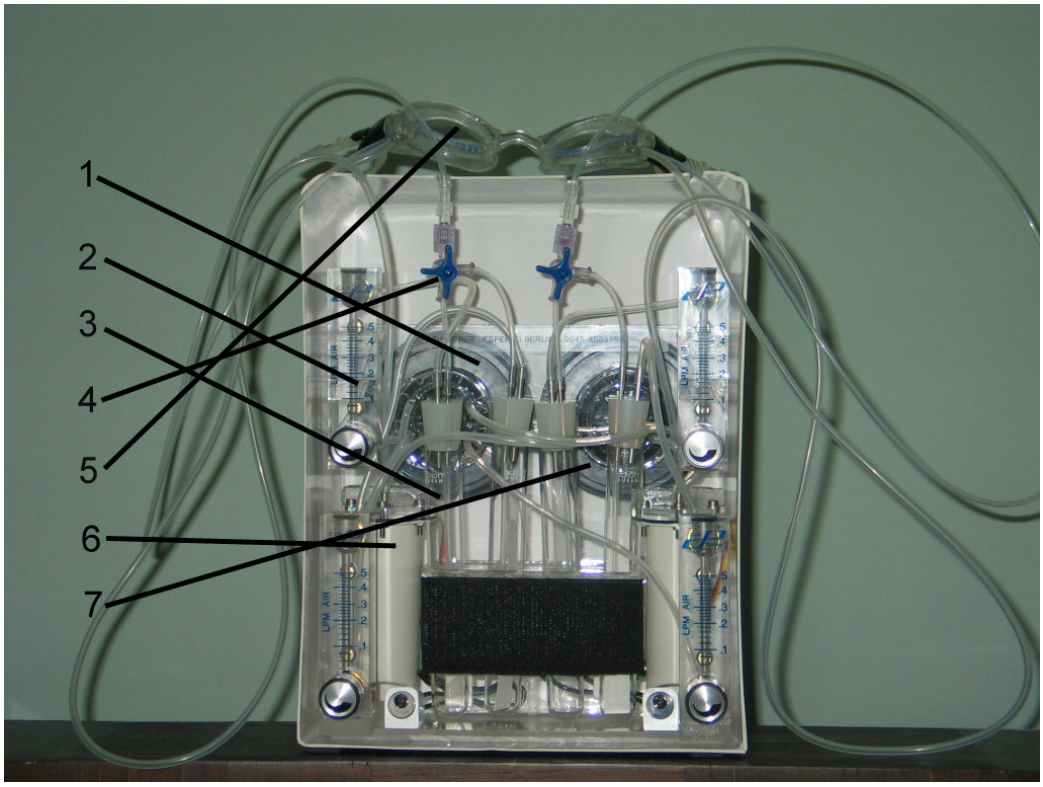
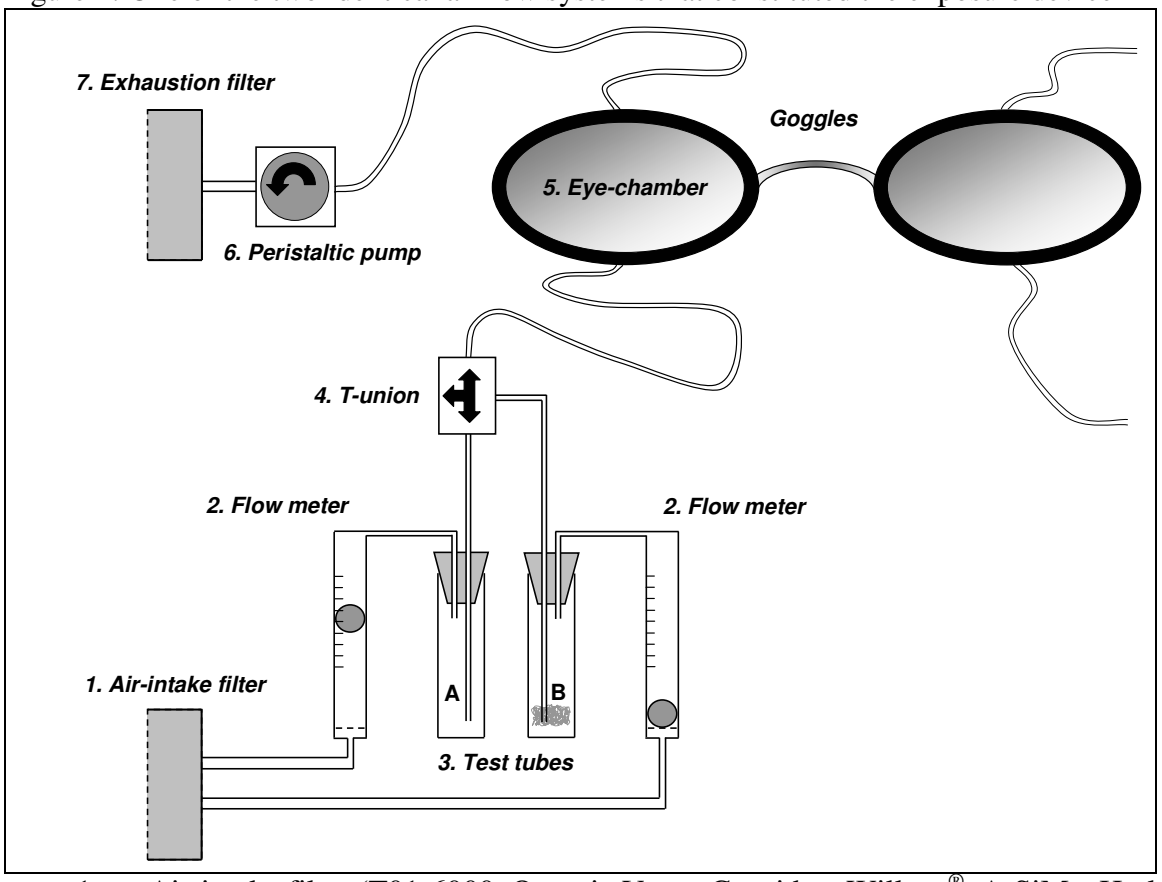


Figure 1: One of the two identical airflow systems that constituted the exposure device

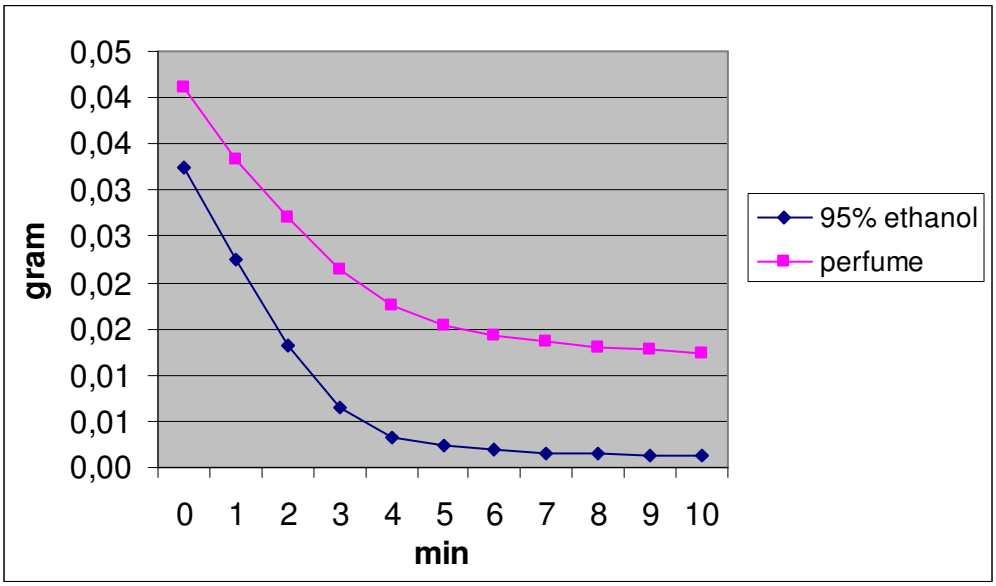


1. Air-intake filter (T01-6000, Organic Vapor Cartridge, Willson®, ArSiMa, Herlev, Denmark)
2. Flow meter (U-32460-40, Cole-Parmer, Vernon Hills, USA)
3. Test tube A and B (Duran 2613, glass, height 150 mm i.d. 20 mm, Buch & Holm A/S, Herlev, Denmark)
4. T-union (5206634, Combidan Adaptor female-female Luer-Lock, B. Braun Medical A/S, Copenhagen, Denmark)
5. Eye chamber (Goggles, Kaiman Aqua Sphere, AquaSport, Copenhagen, Denmark)
6. Peristaltic pump (MT-10, Interacoustics A/S, Assens, Denmark)
7. Exhaustion filter (T01-6000, Organic Vapor Cartridge, Willson®,ArSiMa, Herlev, Denmark).

The components in each system were connected by flow tubes

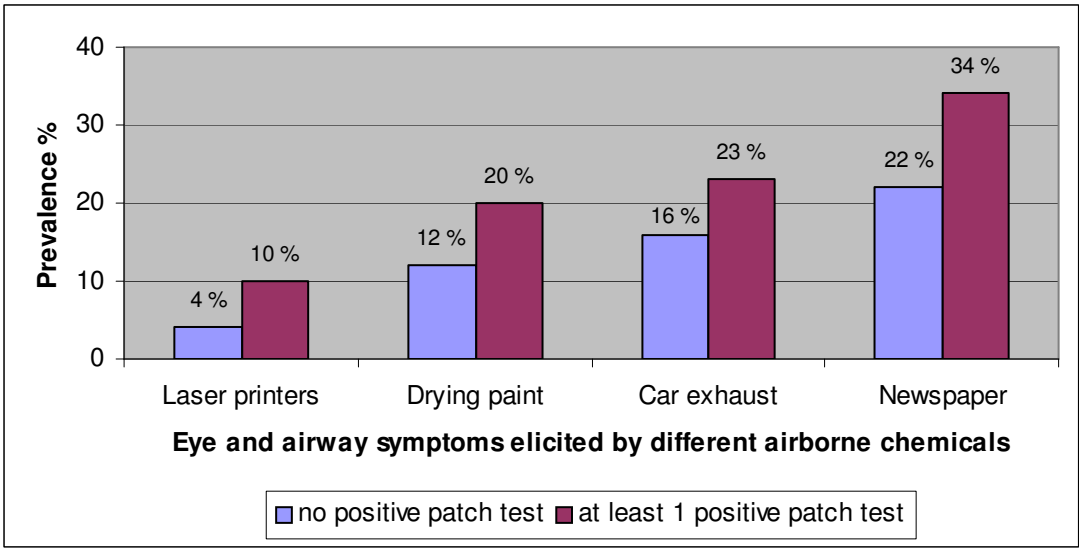
- The test tubes and the goggles were connected with Teflon® tubes (Buch & Holm A/S, Herlev, Denmark) with a total length of 1 m, and a inner diameter of 2mm
- All other components were connected with Silicone tubes (Buch & Holm A/S, Herlev, Denmark) with a total length of 1.5 m, and a inner diameter of 2mm

Figure 2:
Evaporation of 50 µL ethanol and perfume from filter paper strips



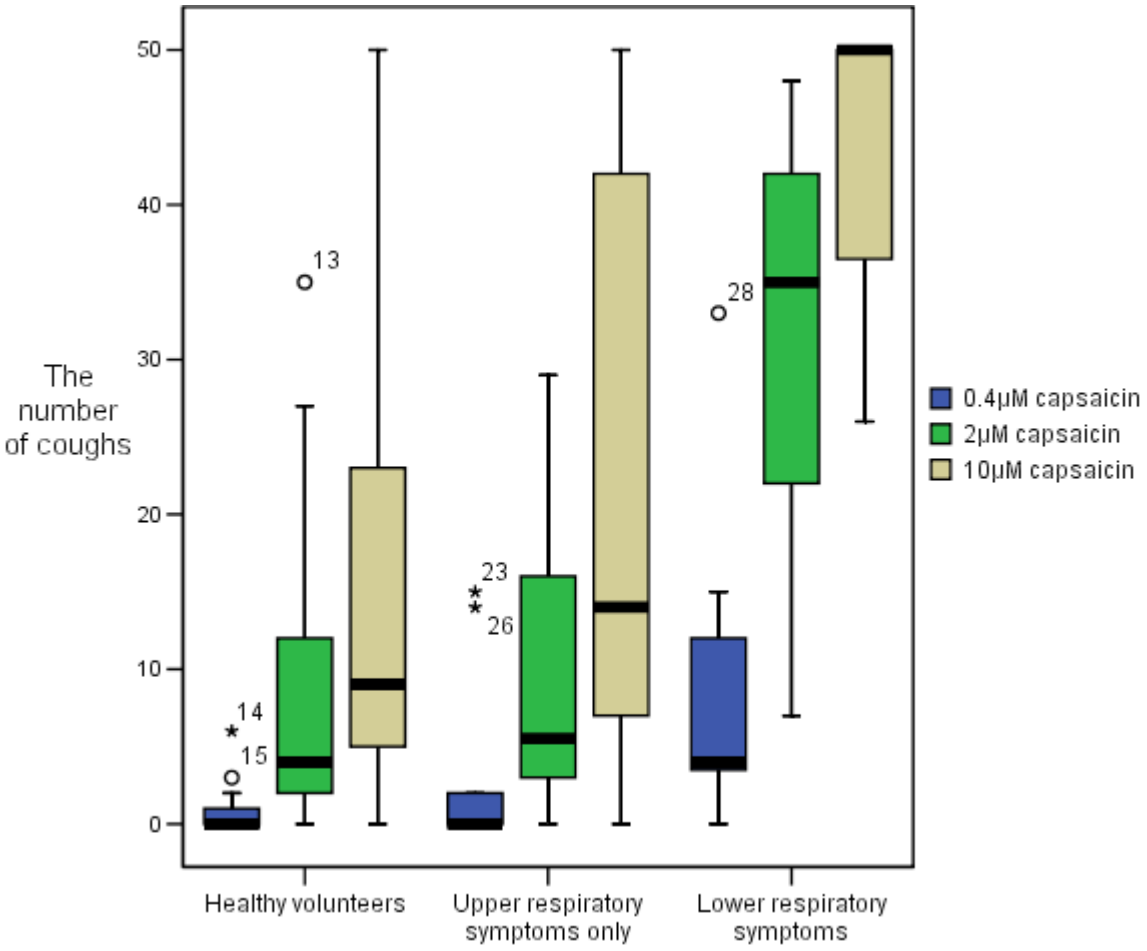
The time dependent fall in weight when evaporating 50 µL perfume and 50 µL 95% ethanol for 10 minutes. The units of measurement are average delta values based on 10 measurements.

Figure 3:
The occurrences of eye and airway symptoms triggered by various agents within the last 12 months



The difference between the groups was statistically significant $P < 0.05$

Figure 4:
Results from capsaicin bronchial challenge test. Number of coughs at the three concentrations of inhaled capsaicin, (median, quartiles and range) for healthy volunteers, patients with only upper respiratory symptoms elicited by perfume, and patients with lower respiratory symptoms elicited by perfume



PAPERS I-V