

ORIGINAL PAPER

Influence of short-term exposure to airborne Der p 1 and volatile organic compounds on skin barrier function and dermal blood flow in patients with atopic eczema and healthy individuals

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Clinical and Experimental Allergy

Summary

Background Epidemiological studies indicate environmental pollutants to be involved in the increase in the prevalence of allergic diseases. In human exposure studies, volatile organic compounds (VOCs) have been shown to cause exacerbations of allergic asthma whereas, no data concerning atopic eczema (AE) are available.

Objective We investigated the effect of airborne VOCs on the skin of patients with AE and controls in the presence or absence of house dust mite allergen, Der p 1.

Methods In a double-blind crossover study, 12 adults with AE and 12 matched healthy volunteers were exposed on their forearms to Der p 1 and subsequently to a mixture of 22 VOCs (M22, 5 mg/m³) in a total body exposure chamber for 4 h. Transepidermal water loss (TEWL) and skin blood flow were measured in all subjects before, during and after exposure. Additionally, an atopy patch test (APT) with Der p 1 was applied to the skin after exposure.

Results A significant increase in transepidermal water loss was observed 48 h after exposure to VOCs as compared with exposure with filtered air in all individuals (mean difference: +34%; 95% Confidence Interval: 7–69%). Prior Der p 1 exposure resulted in a significant rise of dermal blood flow after 48 h in patients with AE but not in controls. Six out of seven patients showed enhanced atopy patch test (APT) reactions to HDM allergen after previous exposure to VOCs.

Conclusion Our results show that exposure to VOCs – at concentrations commonly found in indoor environments – can damage the epidermal barrier and enhance the adverse effect of Der p 1 on sensitized subjects with AE. These findings may contribute to a better understanding of the mechanisms underlying the increase in prevalence and exacerbation of AE.

Keywords atopic eczema, epidermal barrier, exposure chamber, Der p 1, M22, TEWL, VOC

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Introduction

Allergic diseases have increased in prevalence worldwide, while the reasons for this development still remain to be elucidated [1]. The induction of atopy is believed to be dependent on the interaction of individual genetic disposition and the exposure to allergens as well as to other environmental factors. For the clinical manifestation of allergic inflammation, indoor and outdoor pollutants have been discussed as trigger factors having an adjuvant effect [2–4].

In this regard, the indoor environment is of particular interest as the majority of people in industrialized countries spend over 80% of their time indoors and about two-thirds of the day inside their homes [5]. Pollutants frequently detected in the indoor air are organic vapour-phase compounds that are termed volatile organic compounds (VOCs) [6]. The sum of these substances is referred to as total VOCs (TVOCs), which can serve as a screening tool for risk assessment regarding sensory irritation [7] but is of limited value for the prediction of further health effects owing to its heterogeneous nature [8].

As human exposure to VOCs is mainly by inhalation, health effects have been studied with the main focus on airway impairment in adults [9] as well as in children [10–12]. Also, maternal VOC exposure was shown to influence the immune status of the newborn child as an adjuvant for a T helper type 2 (TH2)-polarization [13]. Concerning VOC effects on human skin, only very limited data are available although the dermal route is important for resorption and significantly contributing to the whole body burden [14].

However, in real life, individuals are not exposed to VOCs alone but rather to a mixture of various pollutants/irritants/allergens of the indoor air.

Sensitization to house dust mite (HDM) as one of the most important allergens of the indoor air has been identified as a major risk factor for persistent or relapsing wheezing in a longitudinal, population-based, unselected birth cohort study of childhood asthma followed to adulthood [15]. In patients with allergic rhinoconjunctivitis, HDM was found to be directly triggering clinical symptoms after short-term challenge in exposure chamber experiments [16].

In atopic eczema (AE) direct application of HDM allergen on the skin as an atopy patch test (APT) was shown to be capable to elicit eczematous skin lesions [17].

The effect and possible synergistic/additive actions of pollutants and allergens of the indoor air in the pathogenesis of atopic diseases have not yet been investigated in detail.

In the setting of this pilot study, it was therefore our aim to investigate the impact of VOC-exposure alone as well as in combination with allergen on human skin. Exposure experiments were carried out within a double-blind crossover trial with HDM and M22, a mixture of 22 VOCs frequently found in indoor air and used in numerous exposure studies [18].

Methods

Study subjects

Twelve adults with AE (six males, six females; age 36 ± 11 years) recruited from patients attending our hospital and 12 matched healthy volunteers without a history of atopic diseases as controls (six males, six females, age 36 ± 11 years) were included in the study. All individuals were examined thoroughly before inclusion. Informed consent was obtained from each subject before the study, which was approved by the local ethics committee. Inclusion criteria for patients were a doctor-diagnosed atopic eczema according to the definition of Hanifin and Rajka [19]. The patients included in the study had a mean SCORAD of 51 ± 26 . The forearms of the patients did not display acute skin lesions. Furthermore, the patients had to display specific immunoglobulin E antibodies, a positive skin prick test and a positive APT. Patients using topical or

systemic corticosteroid medication or systemic antihistamine treatment within 3 weeks prior to randomization were excluded.

Whole body exposure: exposure experiments with M22

Each subject was exposed twice with bare forearms and upper back in a 40 m^3 exposure chamber for 4 h ($23 \pm 0.5^\circ \text{C}$, $50 \pm 1\% \text{RH}$): once with VOCs and another time with purified air as control. The VOC mixture was composed of 22 compounds (M22, the 'Mølhave mixture': m/p-xylene and n-butylacetate, each 1.613 mg/m^3 , n-hexane, n-nonane, n-decane, 1-decene, ethylbenzene, α -pinene, n-hexanal, n-butanol, ethoxyethylacetate, 1,2-dichloroethane, each 0.161 mg/m^3 , n-undecane, 1-octene, cyclohexane, 1,3,5-trimethylbenzene, n-propylbenzene, n-pentanal, isopropanol, 2-butanone, 3-methyl-2-butanone, 4-methyl-2-pentanone, each 0.016 mg/m^3) [18]. VOCs were added to the chamber via air supply (3 air changes per hour) by a specially adapted micro-dosing system (Microdrop, Norderstedt, Germany) to generate the final TVOC-concentration of 5 mg/m^3 in the chamber air. Samples from the chamber air were taken every 30 min and analysed subsequently for the single components of the VOC mixture using gas chromatography-mass spectroscopy.

Partial body exposure: aerogenic provocation test of skin to house dust mite allergen

The aerogenic provocation test of skin (APTOS) [20] was performed before the whole body exposure on the volar forearms in an exposure chamber developed for partial body exposure. The device was necessary in order to avoid pulmonary allergen provocation.

In brief, the device consisted of two glass boxes, each with a volume of 0.25 m^3 with acrylic glass front walls allowing the exposure of the forearms of two persons at the same time. Two nebulizers (LCPlus, PARI, Starnberg, Germany) connected to a synthetic air supply were located within each exposure box. The nebulizers contained Der p 1 in buffer (0.15% casein in phosphate-buffered saline (PBS)) or buffer only as control. By operating the nebulizers a distribution of the aerosol on the volunteers forearms was achieved.

Eight milliliters HDM extract (Allergon, Ängelholm, Sweden) containing 20 100 ng Der p 1/mL was nebulized inside the exposure box for 25 min. In order to estimate allergen deposition on human skin, HDM allergens were collected on filters (Isopore membrane filters, Millipore, Bedford, MA, USA) at eight test sites on and beside the forearms as well as by wiping samples (Kimwipes Lite, Kimberly-Clark, Roswell, GA, USA) of the floor. Allergen measurement was carried out by using a two-site monoclonal antibody ELISA (Indoor Biotechnologies,

Charlottesville, VA, USA) and did not differ significantly between the different test sites (mean 11.2 ng Der p 1/cm²).

Occlusive allergen provocation test: atopy patch test

Directly after the whole body exposure to M22 or filtered air in seven out of 12 patients with AE, an APT to HDM in petrolatum and petrolatum alone as control was applied on the exposed skin of the upper back. After 48 h the APT was removed and graded immediately as well as 24 h later according to international guidelines [17]. The investigator evaluating the APT was blinded throughout the study. In five out of the 12 patients with AE, no APT after the exposure experiments was performed as they did not give their consent to this part of the study.

Non-invasive measurements of the skin

Measurement of the transepidermal water loss. Transepidermal water loss (TEWL) was measured according to international guidelines [21] by a Tewameter (TM210, Courage & Khazaka, Cologne, Germany) at the volar forearms at the following time points: immediately before (t0) and after (t1) exposure of the forearms to HDM/buffer as well as after 2 h (t2) and 4 h (t3) of exposure to VOCs or filtered air, and 24 h (t4), 48 h (t5) and 72 h (t6) after termination of exposure. The measurement was performed by a blinded investigator. The measurement of TEWL is a reliable method for the investigation of the skin barrier function *in vivo* being particularly suitable for the assessment of mild skin reactions [22].

Measurement of dermal blood flow. Laser Doppler imaging (LDI) (Moor Instruments, Axminster, England) was applied to monitor dermal blood flow in all subjects participating in the study. Measurements were performed at the volar forearms before (t0) as well as 24 h (t4), 48 h (t5) and 72 h (t6) after termination of the exposure experiments by a blinded investigator. The blood flow value obtained before the experiment at the timepoint t0 was standardized as 100%.

Statistical analysis

TEWL and LDI data showed a log normal distribution: therefore all analysis was carried out after log transformation of the data. The basic analysis variable was the change (difference of the log-transformed values) in the individual's reaction when comparing the exposure condition with the time correspondent control condition. Analysis of variance (ANOVA) was chosen (SPSS for Windows). For the three time points after exposure, we tested whether exposure with VOC and/or HDM or having atopic eczema influenced the change in the individual's reaction.

A result was considered significant if the error probability *P* was less than 5%. Geometric means and their appropriate 95% confidence intervals (95% CI) were used to characterize the location, spread and mean relative changes.

Results

Transepidermal water loss measurements

TEWL values were low when measured in healthy volunteers (Fig. 1a) exposed to (1) filtered air/buffer (mean TEWL from all subjects at the time points t2–t6: 6 g/m²/h) or (2) filtered air/HDM (5 g/m²/h) followed by the exposure to (3) VOCs/buffer (8 g/m²/h) and (4) VOCs/HDM (8 g/m²/h). In patients with AE, the TEWL (Fig. 1b) values were generally higher compared with healthy volunteers: after exposure to (1) filtered air/buffer (13 g/m²/h) and (2) filtered air/HDM (13 g/m²/h) values were lower than after exposure to (3) VOCs/buffer (15 g/m²/h) and (4) VOCs/HDM (15 g/m²/h).

The following mean intraindividual standard deviations were obtained for patients with AE: (1) filtered air/buffer: 2.4 g/m²/h; (2) filtered air/HDM: 2.4 g/m²/h; (3) VOCs/buffer: 3.3 g/m²/h; and (4) VOCs/HDM: 3.8 g/m²/h. The respective values for healthy volunteers were as follows: (1) 1.3 g/m²/h; (2) 1.4 g/m²/h; (3) 3.2 g/m²/h; and (4) 2.8 g/m²/h.

As there were no significant differences between patients and controls, both groups were taken together for further data analysis: a significant rise (*P* < 0.05) of the TEWL could be detected 48 h (t5) after exposure with VOCs without allergen exposure compared with filtered air for all study subjects taken together (mean change: +34%; CI: 7–69%) (Fig. 2a). This result remained significant when using the non-parametric sign-rank test instead of the paired *t*-test. Concerning the TEWL values for both groups separately, the mean change in TEWL 48 h after exposure was very similar with +31% (CI: –10% to 93%) in patients with AE and +37% (CI: –1% to 91%) in healthy volunteers.

Dermal blood flow measurement

In patients with AE (Fig. 1c), there were similar dermal blood flow values (mean out of t4–t6) after exposure to (1) filtered air/buffer 91% and (2) filtered air/HDM 92% whereas higher values were recorded after exposure to (3) VOCs/buffer 112% and even more marked after (4) VOCs/HDM 129%. Furthermore, a significant rise 48 h after the combined exposure to HDM and VOCs compared with exposure with filtered air with HDM (mean change: +41%; CI: 9–83%) (Fig. 2b) or without HDM allergen (mean change: 39%; CI: 7–63%) could be detected. Exposure to VOCs or HDM alone induced no significant rise in dermal blood flow.

In healthy volunteers, the relative dermal blood flow values (Fig. 1d) did not show significant changes after the

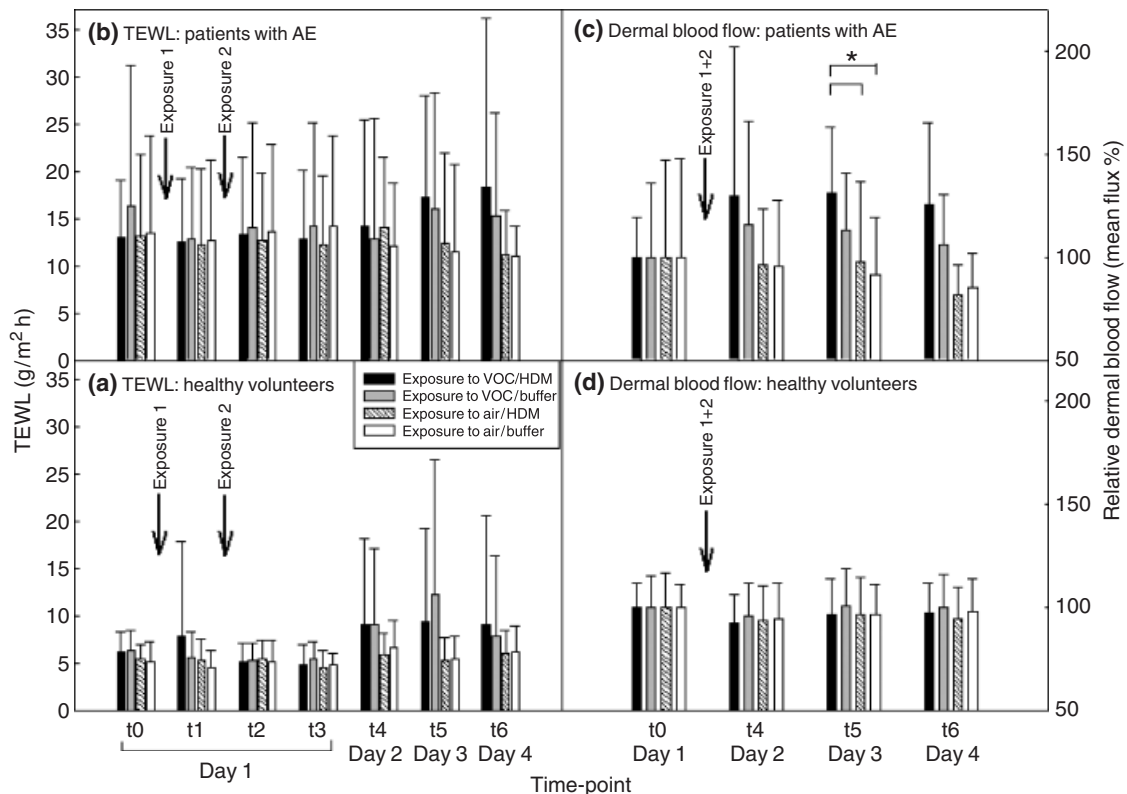


Fig. 1. Measurement of transepidermal water loss (TEWL) ($\text{g}/\text{m}^2/\text{h}$) at the lower volar forearm in (a) healthy volunteers ($n = 12$) and (b) patients with atopic eczema (AE) ($n = 12$) before (t0) and after exposure with house dust mite (HDM), respectively, buffer (t1) as well as after exposure with volatile organic compounds respectively filtered air for 2 h (t2) and 4 h (t3). Further measurements were obtained 24 h (t4), 48 h (t5) and 72 h (t6) after the experiment. Measurement of dermal blood flow (%) at the lower volar forearm in (c) patients with AE ($n = 12$) and (d) healthy volunteers ($n = 12$) before (t0) and 24 h (t4), 48 h (t5) and 72 h (t6) after exposure with HDM respectively buffer as well as VOCs respectively filtered air.

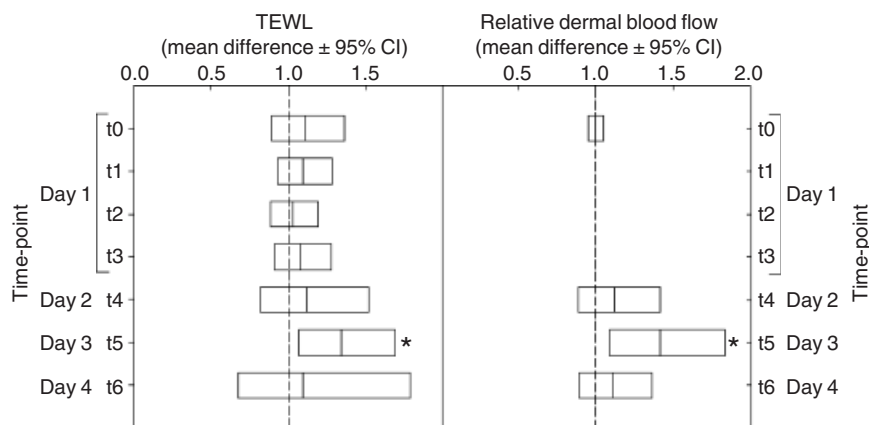


Fig. 2. (a) Mean transepidermal water loss (TEWL) changes in all study subjects ($n = 24$; 12 healthy volunteers and 12 patients with atopic eczema (AE)) at different time points: immediately before (t0), after 2 h (t2) and 4 h (t3) during volatile organic compounds (VOCs) exposure and 24 h (t4), 48 h (t5) and 72 h (t6) after the exposure as compared to the exposure with clean air alone. The boxplot shows the mean and the 95% confidence interval. *significant rise under VOCs exposure compared with clean air. (b) Mean changes in dermal blood flow in patients with AE ($n = 12$) at different time points: before (t0) and 24 h (t4), 48 h (t5) and 72 h (t6) after the exposure with HDM and VOCs as compared with the exposure with clean air alone. The boxplot shows the mean and the 95% confidence interval. *indicates a significant rise under VOCs exposure compared with clean air.

different exposure protocols (mean out of t4–t6): (1) filtered air/buffer 96%; (2) filtered air/HDM 95%; (3) VOCs/buffer 99%; and (4) VOCs/HDM 95%).

The following mean intraindividual standard deviations were obtained for patients with AE: (1) filtered air/buffer: 11.8%; (2) filtered air/HDM: 10.8%; (3) VOCs/buffer:

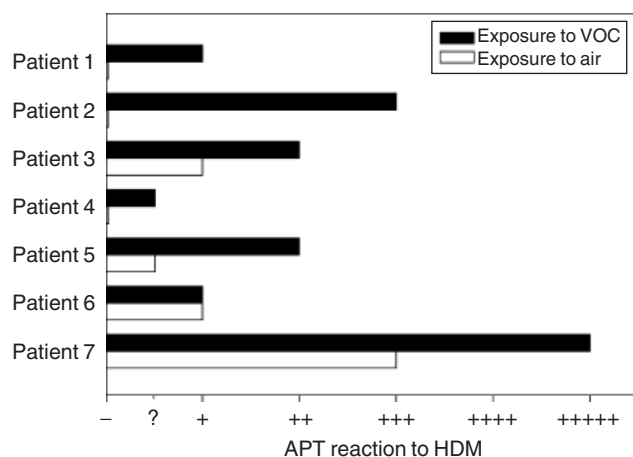


Fig. 3. Results of the atopy patch test (APT) with house dust mite (after 48 h) in seven patients with atopic eczema after exposure to volatile organic compounds, respectively, filtered air.

14.5%; and (4) VOCs/HDM: 20.8%. The respective values for healthy volunteers were as follows: (1) 6.6%; (2) 7.3%; (3) 5.3%; and (4) 5.4%.

Atopy patch test

In the APT, six out of the seven patients tested exhibited an increased skin reaction to HDM after exposure to VOC as compared with exposure with filtered air alone. Only one patient showed no difference after both exposure protocols (Fig. 3, $P = 0.031$).

Discussion

This study shows for the first time that a mixture of 22 VOCs commonly found in indoor air can have an adverse effect on the epidermal barrier function in humans. This effect is independent of the condition of the skin and is seen both in patients with atopic eczema as well as in healthy volunteers.

Furthermore, VOCs enhance the Der p 1-induced rise in dermal blood flow in patients with AE and HDM allergy, but not in healthy volunteers.

As expression of the clinical relevance of these findings the APT to HDM is significantly enhanced after exposure to VOCs in patients with AE.

Volatile organic compound exposure

Investigations concerning the TVOC levels in Austrian households ($n = 180$) showed concentrations ranging from 11 to 6045 $\mu\text{g}/\text{m}^3$ (median 155 $\mu\text{g}/\text{m}^3$), with 82% below 300 $\mu\text{g}/\text{m}^3$ and 5% above 1000 $\mu\text{g}/\text{m}^3$ [23]. The concentration as well as the distribution of important components of VOCs were found to be similar in households in Germany [24]. In both studies more than 25 single

compounds were characterized to allow further assessment of the results as TVOC values alone are not sufficient for estimating health effects because of its heterogeneous nature. At present, guidelines have been set for individual compounds rather than for TVOC concentrations taking into consideration the toxic and irritable properties of the substances.

Our study clearly shows that short-term exposure to a mixture of VOCs (M22) for only 4 h has an adverse effect on human skin, leading to a significant increase in TEWL. The concentration of the single compounds ranged from 1.613 to 0.016 mg/m^3 . The total concentration of 5 mg/m^3 used in our experiments was rather high compared with the results of the surveys mentioned above but can be observed in new buildings and also in established dwellings, especially after recent renovation [25].

Previous human exposure studies of our group showed that formaldehyde and NO_2 at domestic concentrations cause an impairment in the skin barrier function in patients with AE. In healthy volunteers, only NO_2 but not formaldehyde led to an increase of TEWL [26]. In the current study, both patients with AE and healthy individuals reacted in the same way with an impairment of the skin barrier towards the exposure with VOCs, supporting the theory that the observed effect is an overall phenomenon of the exposed VOCs in the concentration used on human skin. Investigating the effect of environmental VOC exposure, other researchers focused mainly on irritation of mucous membranes, whereas the effects on human skin were not studied in detail [27].

In our study, significant changes in TEWL occurred with a delay of 48 h after 4 h of exposure. This may be because of the time required for VOCs to penetrate and exert adverse effects on the epidermal barrier.

Dermal penetration of VOCs had already been shown in an animal model [28]: male Fisher 344 rats were exposed to aqueous solutions of 14 VOCs, including important compounds of M22 such as *m*-xylene, ethylbenzene and 1,2-dichloroethane, up to 24 h, and blood samples were analysed at different time points. The absorption of the VOCs was directly correlated to the exposure concentration and decreased with decreased water solubility of the substances. The blood levels of two VOCs increased during the whole 24 h period (e.g. 1,2-dichloroethane), while the 12 others reached peak concentrations after 4 h (e.g. *m*-xylene, ethylbenzene). These results underline the high capacity of VOCs to penetrate through the epidermal barrier and show the relevance of 4 h of exposure as performed in our study.

The dermal uptake of VOCs occurs via diffusion into the skin. Once VOCs have penetrated the cornified layer, they can damage the viable epidermis and dermis and cause erythema, oedema, leucocyte infiltration, necrosis and increased oxidative species and low-molecular-weight DNA levels in rat skin [29, 30].

Exposure studies with *m*-xylene on viable dermal fibroblasts from male Fischer 344 rats with a sealed exposure system showed a time- and dose-dependent decrease in endogenous catalase that occurred with decreasing cell viability. Thus, *m*-xylene, one of the compounds of M22, induces cytotoxicity and promotes a decrease in cellular antioxidants [31]. A study of rat skin after dermal exposure to *m*-xylene for up to 6 h revealed histopathological changes (epidermal–dermal separation and granulocyte infiltration) as well as increased IL-1 α and iNOS protein expression after 1 h of exposure. These proteins may serve as early indicators of skin irritation [32].

For human exposure, dermal penetration of VOCs was found to be an important route of uptake [33]. To prevent hazardous action on human health in Germany, MAK and TRK values have been defined as exposure limits for individual compounds, based on their toxicity or irritable properties [34]. Our results show that exposure to 22 VOCs in concentrations substantially below MAK and TRK values is capable of affecting human health by disturbing the epidermal barrier. Therefore, it is advisable to minimize human exposure to these VOCs and reduce indoor concentrations to create a 'non-irritating' environment, as also proposed by other authors [35, 36]. In this context, it is important to note that general guidelines for TVOC values are insufficient as pointed out earlier and that a compound-by-compound approach, taking into consideration the characteristics of each individual substance, has become more common [37].

Combined exposure to volatile organic compounds and Der p 1

The newly developed device (APTOS) allowing a combined aerogenous exposure to VOCs and Der p 1 proved as a sufficient provocation method of the skin.

In the real exposure situation e.g. in children's beds, the mean allergen levels were reported to be 49 $\mu\text{g Der p 1/m}^2$ [38]. In US beds, HDM allergen concentrations (Der p 1 and Der f 1) above 10 $\mu\text{g/g}$ dust could be found in 24% whereas in 46%, it ranged between 2 and 10 $\mu\text{g/g}$ dust. In 84% HDM allergen concentrations were found to be between 0.025 and 2 $\mu\text{g/g}$ dust [39]. To elicit skin reactions in the APT, substantially higher concentrations are necessary (125 $\mu\text{g Der p 1/m}^2$) [38]. The allergen application in our study was with approximately 112 $\mu\text{g Der p 1/m}^2$, similar to the concentration used in the APT. In contrast to the APT in our experimental setting, allergen application alone did not cause skin reactions in the patients who reacted to HDM in the APT before inclusion in the study. This is most likely because of the application mode which was short (25 min) and aerogenous in the study, while the allergen is applied occlusively and continuously for 48 h in the APT.

The increased skin blood flow after combined aerogenous exposure to VOCs and Der p 1 might be because of a VOC-induced impairment of the predisturbed epidermal barrier in patients with AE facilitating the penetration of the HDM allergen into the skin. Additionally, Der p 1 exerts a proteolytic activity that can enhance access to the antigen-presenting dendritic cells by cleaving the tight junction proteins, thus facilitating its own penetration as could be shown *in vitro* [40, 41].

In our study, both the TEWL and skin blood flow were significantly increased 48 h after exposure, but not at the other timepoints investigated. This effect is biologically plausible as epidermal changes need time to develop, similar to the eczematous reaction in atopic eczema following skin provocation with allergens or irritants [42]. Other than in immediate-type allergy, aerogenous allergen exposure in real life usually results in disease exacerbation after days. This time frame is also reflected by the skin reaction following diagnostic allergen exposure (APT). The explanation regarding why no effect was seen after 72 h might be the relatively mild and short VOC- and allergen-exposure in our study, which did not even lead to the development of eczema but only to transient barrier disturbance and erythema.

As our exposure study was designed to imitate the real-life conditions as closely as possible, the subjects were allowed to go back to their normal lives in between the medical investigations. Data analysis in this pilot study was based on the mean values of all subjects to minimize the effect of individual differences influencing study outcome but naturally this cannot be fully excluded because of the experimental design.

Atopy patch test after short-term exposure to volatile organic compounds

After exposure to VOCs, the applied APT with HDM was significantly enhanced compared with the control experiments with filtered air alone in all patients but one (in which it was unchanged) ($n = 7$). This result shows that VOC exposure not only leads to an impairment of the epidermal barrier in humans and increases the effect of aerogenous HDM exposure to the skin of patients with AE but also enhances the skin reaction in a standardized allergological provocation test.

The APT is the diagnostic provocation test of the skin in AE that is routinely used to evaluate the clinical relevance of aeroallergens for triggering the disease and causing eczematous skin lesions in individual patients [17]. It shows that the VOC-induced disturbance of the epidermal barrier may increase the susceptibility of patients with AE to develop eczematous skin lesions. The enhanced penetration of aeroallergens through an impaired epidermal barrier may lead to the subsequent induction of an eczematous skin reaction and it was hypothesized that

indoor pollutants and low-grade irritants may increase the vulnerability of skin to allergen exposure because of a disturbed barrier function [43]. Our findings can be considered as a missing link in this context connecting skin barrier function, indoor air pollution and allergen exposure, giving additional experimental proof of this concept.

Concerning the increasing prevalence of AE in the last decades, pollutants are discussed to be involved in this process [44]. The results of this pilot study stress the role of interaction between different indoor air contaminants influencing the epidermal barrier in healthy and diseased skin, and might lead to a better understanding of the pathophysiological mechanisms underlying AE. Future human exposure studies with single VOCs at lower concentrations and greater numbers of subjects are being planned to further investigate the clinical relevance of this concept.

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