

Validation of an environmental exposure unit for controlled human inhalation studies with grass pollen in patients with seasonal allergic rhinitis

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Summary

Background There is an increasing need for allergen inhalation systems to perform basic clinical research and test anti-allergic drugs under well-controlled conditions. This requires stability of environmental conditions like temperature and humidity, as well as allergen concentration and reproducible induction of allergic symptoms.

Objective The aim of this study was to validate an environmental exposure unit for controlled human pollen inhalation studies in participants with seasonal allergic rhinitis.

Methods Temperature, relative humidity, and air flow rate were kept constant with an air conditioning system. Pollen atmosphere was generated using a specially designed feeding system and monitored online by laser counter and offline using rotating rod samplers. Efficacy (total nasal symptom score, nasal air flow rate, nasal secretion) and safety (lung function) parameters were evaluated at different pollen concentrations and repeated allergen challenges.

Results Temperature, humidity, and air flow rate in the environmental exposure unit remained constant within a range of <2%. The spatial distribution and the temporal stability of the pollen concentration varied only slightly over 4 h ($\pm 10\%$ and <4%, respectively). Dose-dependent induction of allergic rhinitis symptoms, reduction in nasal air flow rate, and increase in nasal secretion were observed over time. These effects were reproducible from day to day. Lung function remained clinically normal at all concentrations and from day to day.

Conclusions Thus, pollen exposure in the environmental exposure unit is an effective, reproducible, safe, and suitable method for single-centre clinical studies on the efficacy of anti-allergic treatment or basic clinical research.

Keywords allergen challenge, environmental exposure unit, grass pollen, seasonal allergic rhinitis

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Introduction

Allergic rhinitis affects about 20% of the United States and European population and has the greatest prevalence of any respiratory disease [1]. Therefore, there is an increasing need for new anti-allergic drugs and basic clinical research. Several methods are available to expose subjects to allergens, namely natural exposure in field studies, nasal or inhalative application of allergens, or the environmental exposure unit. The latter is a pollen exposure system designed to evaluate the efficacy of anti-allergic medications. It was developed to reproduce conditions of an outdoor allergy environment without the variability of parameters found in field exposure. These include fluctuation and unpredictability of various parameters such as pollen concentration, pollen antigenicity, humidity, and temperature. Other difficulties are seasonal dependency or, as is the case for topical allergen application,

non-physiologic conditions. Environmental exposure units have already been successfully used and established in drug testing in Vienna, Austria [2, 3], Toronto, Canada [4–7], and Atlanta, USA [8]. They have been used to determine onset of action, efficacy, and safety of antihistamines and topical steroids for the treatment of seasonal allergic rhinitis (SAR) [6, 8–10]. In the Austrian unit, up to 12 and in the other two units about 150 subjects can be tested at once. The pollen concentrations used in these investigations ranged from about 600 to 5000 grains/m³. They were monitored every 20–30 min using a Rotorod system (Rotorod, Multidata, Minneapolis, MN, USA), which was evaluated manually with a light microscope. Day et al. [4] measured pollen emission online with a laser counter in addition to the Rotorod. All groups used questionnaires that participants filled out every 15–60 min to evaluate allergic symptoms. In addition, Horak et al. [9] and Day et al. [10] used rhinomanometry and peak nasal inspiratory flow, respectively, as objective measurements of allergic symptoms. Horak et al. [11] also determined the amount of nasal secretion. Comparison of the different environmental exposure units is difficult, because only limited information has been published and a systematic evaluation

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has not been carried out so far. Furthermore, asthmatics were excluded from previous studies and no data are available that determine the influence of pollen exposure on lung function of the participants. This is the first study that systematically validates the induction and reproducibility of different efficacy parameters and, as a safety parameter, the lung function of the participants in a newly built environmental exposure unit.

Materials and methods

Technical specifications of the environmental exposure unit

The environmental exposure unit is located on the first floor of the clinical inhalation facility of the Fraunhofer Institute of Toxicology and Experimental Medicine. This room measures 47 m² and has a height of 2.80 m. The wall and the ceiling material are stainless steel. The floor is covered with ceramic tile. A lavatory is built into the environmental exposure unit so that volunteers can stay in the room for long-term periods. The air flow and air conditioning of the environmental exposure unit are provided by an air conditioning system, located on the technical floor above the unit. The environmental exposure unit is ventilated with filtered air (HEPA H14 filter, Trox, Neukirchen, Germany) by a push-pull ventilation system with air exchange rates adjustable between $\Lambda = 3/\text{h}$ and $20/\text{h}$. Air is mixed by turbulence generated (1) via a total of 14 swirl inlets for the fresh incoming air and (2) via thermal convection by heating the floor of the room. Temperatures are adjustable in the range of -10°C and $+30^\circ\text{C}$. The relative humidity can be set to values from 20% and, depending on the temperature range, up to 95%. The air parameters are continuously measured by calibrated sensors. For the present studies, 15-min mean values of the continuously recorded signals were calculated and regarded as raw data. The following target values and the tolerable range were chosen for the pollen exposure experiments in the environmental exposure unit: temperature 20°C ($19\text{--}23^\circ\text{C}$), relative humidity 40% (35–45%), volumetric air flow rate $1500\text{ m}^3/\text{h}$ ($1400\text{--}1600\text{ m}^3/\text{h}$).

Volunteers entered the challenge room via an air lock. The air pressure of the exposure room was maintained at a slightly lower level than the adjoining room to prevent pollen loss from the unit. The volunteers wore protective clothing in order to minimize the influx of additional particles (generated by human activity) into the chamber and to prevent pollen from being carried out of the room. During the studies, up to 18 volunteers sat on chairs placed in designated areas of the chamber. They were encouraged to move around while they were in the room. For periodically performed rhinomanometry and spirometry measurements, they sat at a table located in front of the window pointing towards the control room (Fig. 1). Communication between volunteers and control personnel outside the environmental exposure unit was possible via wireless telecommunication with headsets.

Generation of the pollen atmosphere

Commercially obtained grass pollen (orchard grass, *Dactylis glomerata*, average diameter $30\text{ }\mu\text{m}$) were used for the challenge studies (Biopol Laboratory Inc., Spokane, WA,



Fig. 1. Environmental exposure unit: patients with seasonal allergic rhinitis are depicted in the environmental exposure unit during rhinomanometry and spirometry measurements.

USA). They were stored at 4°C until use. The relative potency of the allergenic pollen extract was correlated to the potency of an FDA-provided reference extract by the manufacturer using an ELISA competition assay.

The generation system for the pollen atmosphere was installed on the floor above the environmental exposure unit and consisted of a feeding system and a pneumatic dispersion nozzle (developments of the Fraunhofer Institute). The pollen aerosol, which left the generator, was split into four equal streams, which were individually introduced into the environmental exposure unit via four tubes just beneath an air outlet in the ceiling. To distribute the pollen, a ceiling fan, placed in the centre between the four pollen introduction ports, drew in air from the bottom of the room and dispersed it radially under the ceiling. The pollen generator operated in a stable and reproducible manner with a feedback system, which controlled the feed rate of the pollen feeder. The control signal was based on the data from the online pollen counter (see below).

The pollen concentration was measured online by a laser particle counter (LASAIR Model 550, PMT GmbH, Heimsheim, Germany) at a fixed point in the centre of the challenge room. The particles sampled at a flow rate of $28\text{ L}/\text{min}$ were classified into six size classes: 1–5, 5–10, 10–20, 20–30, 30–50, 50–100 μm . The pollen were attributed to a size range between 20 and $50\text{ }\mu\text{m}$. The LASAIR counter measured 1 min average values of the particle size distribution in the chamber. Fifteen minutes average values were calculated and stored as raw data for further statistical evaluation. Preliminary experiments showed that the influx of non-pollen particles (in the particle size range between 20 and $50\text{ }\mu\text{m}$) originating from the volunteers inside the chamber was about $400\text{--}500\text{ particles}/\text{m}^3$ for 15 subjects. The pollen feeder was adjusted to the target pollen concentration before the volunteers entered the environmental exposure unit. Thus, the values measured by the LASAIR counter during the studies consisted of the target concentration plus 500 (particle influx by volunteers). In addition to the online measurements, the pollen concentration was monitored offline in the centre of the room by two rotating rod samplers (Rotorod) for visual pollen identification and counting under the light microscope. The

Rotorod sampler is an instrument widely used for pollen detection in the atmosphere and an accepted standard [12]. The rod sampler was operated according to the manufacturer's instructions. Immediately after sampling, pollen was counted independently by two technicians. The Rotorod samples were taken over a 4-h daily exposure period. The room was thoroughly cleaned with a wet mop after each pollen exposure.

To investigate the spatial distribution of pollen in the environmental exposure unit, extensive evaluation experiments with a range of size integrating forward scattering particle counter (Fraunhofer Institute development), which counted all particles larger than 15 µm, were performed at heights of 1.2, 1.5, and 1.65 m. The difference between the lower and the upper measuring height was not more than 15%. The 1.5 m height was selected for repeated measurements, because it represented a good average for volunteers exposed to pollen under various conditions (sitting, standing, walking). Pollen concentrations were measured for 5 min at each point of a 41-node grid.

Efficacy and safety measurements

Nasal symptoms (rhinorrhoea, nasal congestion, sneezing, and nasal itching) were self-assessed before and every 20 min during the allergen challenge in the environmental exposure unit according to a four-point severity scale. The subjects recorded their scores on diary sheets using the following scale: 0 = none; 1 = mild; 2 = moderate; 3 = severe. The total nasal symptom score (TNSS) was calculated by adding the four nasal symptoms.

The nasal flow rate was measured before challenge and at 30, 90, 150, and 180 min during each exposure using rhinomanometry (Jaeger Master Scope Rhino, Jaeger GmbH, Wuerzburg, Germany). An active anterior measuring technique at a pressure difference of 150 Pa between anterior and posterior nasal pressure using a face mask was used. Flow rates at 150 Pa were obtained for the right and left nostril (cm³/s). The sum of the flow rates of both nostrils was calculated from the two measurements.

Nasal secretion was collected using paper tissues. Used and unused tissues were saved in a plastic bag by each volunteer and collected every 60 min. The amount of nasal secretion was determined by weighing these paper tissues. The volunteers were provided with a new package of tissues after each tissue collection.

Spirometry was performed according to standard procedures (American Thoracic Society) before and every 60 min during allergen challenge (Jaeger Master Scope, Jaeger GmbH). During the measurements, volunteers sat in an upright position. The highest of three readings was recorded.

Participants measured their peak expiratory flow rate using a Mini-Wright peak flow meter (Clement Clarke, Essex, England). They determined the flow rate before exposure, after exposure, every 2 h until bedtime, and the next morning. Values were recorded on diary sheets.

Study design

Two different validation studies were performed in adult volunteers with SAR. The studies were performed out of tree

and grass pollen season and all subjects were symptom-free at the time of the study. Subjects with a history of SAR due to grass pollen for at least 2 years, a positive skin prick test to orchard grass pollen, and normal lung function (VC ≥ 80%, forced expiratory volume in 1 s (FEV₁) predicted ≥ 80%, FEV₁/VC ≥ 70%) were included in the study. Exclusion criteria were the following: structural nasal defect, large nasal polyps, infection of the upper airways 2 weeks before study begin, sinusitis, asthma, with the exception of mild intermittent asthma (normal lung function as defined above, symptoms ≤ 2 times a week, night-time symptoms ≤ 2 times a month; intake of only short-acting β₂-agonists as needed), and any medication that would affect the outcome of the study. The studies were approved by the ethics committee of the Medical School of Hannover, and all patients provided written informed consent.

The first study was a double-blind, cross-over study in 23 adult volunteers with SAR (18 males and five females, age 20–44 years, mean age 28.2 years; seven asthmatics). It was carried out to assess the efficacy parameters (degree of nasal symptoms, changes in nasal flow rate, and the amount of nasal secretion) and safety parameter (lung function) during inhalation of four different concentrations of grass pollen or clean air. The exposures with allergen at a concentration of 1000, 2000, 4000, and 8000 grains/m³ or clean air were each performed for 4 h on five different occasions at least 7 days apart. The 23 subjects were divided into two groups of 12 and 11 subjects. The different pollen concentrations were administered in a randomized, double-blind manner and only the technical personnel, who were responsible for the generation of the pollen atmosphere, knew the actual pollen concentration. The TNSS, rhinomanometry, nasal secretion, and spirometry were determined as mentioned for efficacy and safety measurements.

The second study was performed in 24 adult volunteers with SAR (18 males and six females, age 19–44 years, mean age 26.3 years; nine asthmatics) to assess the reproducibility of the efficacy and safety parameters in a realistic study situation. This study was part of a double-blind, parallel-group, placebo-controlled clinical trial for a new anti-allergic treatment. The subjects for the current study belonged to the placebo group only. They were divided into three groups of eight volunteers. The participants stayed in the environmental exposure unit on five different occasions (at a screening visit and on days 1, 3, 7, and 14 of randomization) for 4 h each. The pollen concentration was 8000 grains/m³. The determination of TNSS and measurements of nasal flow rate, nasal secretion, and lung function were performed in the same way as described for efficacy and safety measurements. For lung function measurements, 48 volunteers, 24 from the treatment group and 24 from the placebo group, were included for evaluation, because there was no significant difference in lung function between these groups.

Statistical analysis

Groups were compared using the Wilks' Lambda test [13]. Comparisons of pairs were performed with Student's *t*-test. Values of *P* < 0.05 were considered to be significant for both tests. Statistics using both tests were calculated using SAS Proprietary Software Release 6.12 (Cary, NC, USA).

Results

Technical details

Temperature, relative humidity, and volumetric air flow rate were evaluated using data from 28 exposure days for 4 h per day (raw data 15 min mean values). All parameters were kept within a very narrow range (<2%) of the target values: temperature $20.4 \pm 0.3^\circ\text{C}$ (mean $\pm$ SD), relative humidity $39.8 \pm 0.1\%$, and air flow rate $1499 \pm 1\text{ m}^3/\text{h}$.

The measurement of the pollen concentrations was performed using the two different detection systems: (1) the laser particle counter (LASAIR), which evaluated data online and (2) the rotating rod sampler (Rotorod), which was evaluated manually (offline) using a light microscope. The two systems were compared and a regression analysis was carried out. Figure 2 shows an excellent correlation between the two methods. The y-intersection at about 400–500 particles/ m^3 represents the particles introduced into the room by the participants. The online measurement of pollen concentrations using the LASAIR was an effective and accurate method.

To test for temporal stability of the pollen concentration in the chamber, several 4 h periods were evaluated in detail. All target concentrations (1000, 2000, 4000, and 8000 grains/ m^3) showed only slight variation as demonstrated in Fig. 3. This indicates good pollen stability in the environmental exposure unit. Concentration data collected in the course of a 28-day exposure study with a constant target concentration of 8000 grains/ m^3 showed only small day-to-day variability (LASAIR 8567 ± 320 grains/ m^3 , Rotorod 8154 ± 453 grains/ m^3).

The spatial distribution of the pollen in the environmental exposure unit is shown in Fig. 4. Here, we have plotted isolines of relative concentrations, i.e. concentrations measured at the grid points normalized to the concentration in the centre of the room where the monitoring instruments are located during the challenge studies. The isoconcentration lines covered a range of 0.8–1.6. A homogenous exposure within a range of $\pm 10\%$ was reached in the seating area (see Fig. 4 left of the dotted line) of the environmental exposure unit. The main pollen distribution mechanism was the ceiling

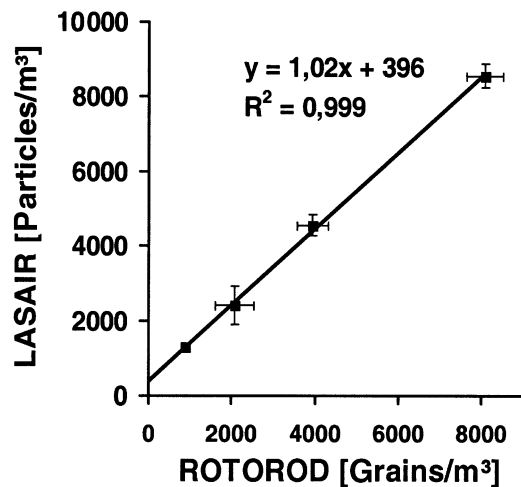


Fig. 2. Correlation between online and offline pollen detection systems: a regression analysis of the results of the LASAIR (online) and Rotorod (offline) systems is shown.

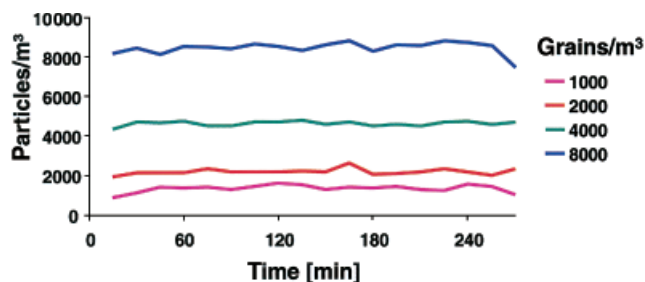


Fig. 3. Stability of pollen concentrations over time: pollen concentrations were measured over a 4 h period using the LASAIR system. Values determined every 15 min were used for evaluation. One representative exposure of each concentration is shown.

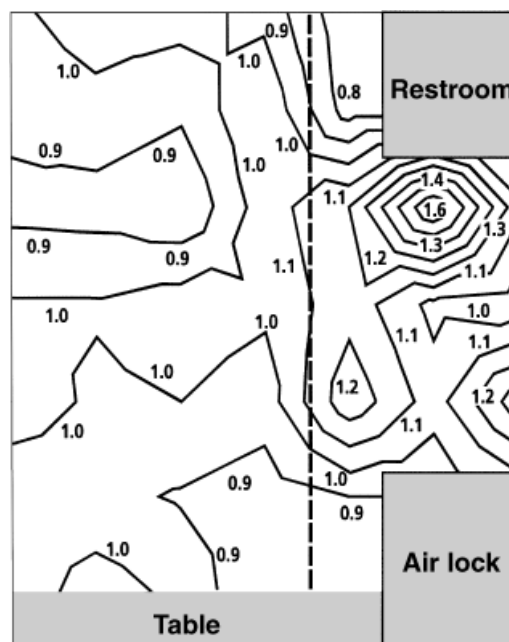


Fig. 4. Spatial distribution of pollen in the environmental exposure unit: pollen concentration distribution was evaluated with a range of size integrating forward scattering particle counters (Fraunhofer Institute development), which counted all particles larger than $15\text{ }\mu\text{m}$. The value measured in the centre of the chamber was defined as 1.0. The participants were seated in the area left of the bold dashed line.

fan, which blows an equal number of pollen grains per time into each section of the room. On the right side of the room, between the airlock and the restroom, the available space was smaller due to blockage by the built-in rooms. This caused higher pollen concentrations. This area was defined as a non-seating area and volunteers were instructed to avoid it.

For technical reasons (complex instrumentation, tubing, and wiring), the homogeneity measurements in the chamber could only be performed without the presence of participants. The distribution was expected to improve with the presence of subjects, because of the increased turbulence generated by movement of the volunteers.

Dose dependency of efficacy and safety parameters

Twenty-three volunteers with SAR were exposed to different pollen concentrations in the environmental exposure unit.

Their TNSS, nasal flow rate, nasal secretion, and lung function were investigated. The TNSS following pollen exposure was significantly higher than placebo in a dose-dependent manner (Fig. 5a; $P < 0.01$; Wilks' Lambda test). A strong increase was already seen after 20 min. The TNSS reached a peak at about 100 min for all pollen concentrations. A significant difference was observed between a concentration of 1000 and 4000 or 8000 grains/m³ and 2000 and 8000 grains/m³ after 120 min and for the remaining course of the experiment ($P < 0.01$; Student's *t*-test).

Using rhinomanometry, the volunteers' nasal flow rate was tested over time at various pollen concentrations. A dose-dependent reduction over time was observed (Fig. 5b; $P < 0.05$; Wilks' Lambda test). A significant decrease in nasal flow rate was seen after 90 min until the end of the experiment (180 min) for 4000 and 8000 grains/m³ compared with clean air ($P < 0.05$; Student's *t*-test). At 150 and 180 min, this was true for all pollen concentrations ($P < 0.01$; Student's *t*-test). At these time points, the highest concentration (8000 grains/m³) caused a significant decrease in the nasal flow rate compared with all other pollen concentrations ($P < 0.05$; Student's *t*-test). This clearly shows that the higher the pollen concentration, the more impaired the nasal flow.

The nasal secretion of participants was significantly increased over time when they were exposed to pollen (Fig. 5c; $P < 0.01$; Wilks' Lambda test). For all time points, there was a significant difference between the 1000 grains/m³ and the 4000 or 8000 grains/m³ pollen concentrations ($P < 0.05$; Student's *t*-test). For a concentration of 2000 grains/m³, the difference was significant at 60 and 180 min.

The FEV₁ was tested in volunteers to assess the pulmonary reaction to the 4 h exposure to 1000, 2000, 4000, 8000 grains/m³, or clean air. There was no significant difference in the values

between pollen exposure and placebo at any time during the experiment regardless of the concentration (Fig. 5d; $P > 0.05$; Wilks' Lambda test). Thus, the pollen concentrations did not have a negative effect on lung function. The peak expiratory flow remained stable from baseline to 8 h after pollen exposure for every pollen concentration, indicating that the pollen exposures had no late-phase effects (data not shown).

Reproducibility of the parameters

Twenty-four volunteers with SAR were exposed to 8000 grains/m³ for 4 h at five different visits. Their TNSS, nasal flow rate, nasal secretion, FEV₁, and peak expiratory flow were evaluated. Figure 6a–c shows that the TNSS, nasal flow rate, and nasal secretion did not differ significantly from visit to visit ($P > 0.05$; Wilks' Lambda test). This demonstrates a comparable course over time for every visit. Figure 6d shows a slight reduction in FEV₁ on visits 2–4, which is statistically significant ($P < 0.01$; Wilks' Lambda test). Further analysis using the Student's *t*-test confirms the significant difference from baseline over time ($P < 0.05$; Student's *t*-test). Interestingly, a reduction in the mean FEV₁ was observed after repeated visits. However, at visit 5, which was 7 days after visit 4, lung function was normal again. This suggests a priming effect, which is statistically confirmed by the Wilks' Lambda test ($P < 0.01$). Although the effect is statistically significant, FEV₁ was reduced by only about 200 mL (<10%), which is not clinically relevant. The peak expiratory flow remained stable from baseline to 8 h after pollen exposure for every visit, indicating that the pollen exposures had no late-phase effects (data not shown).

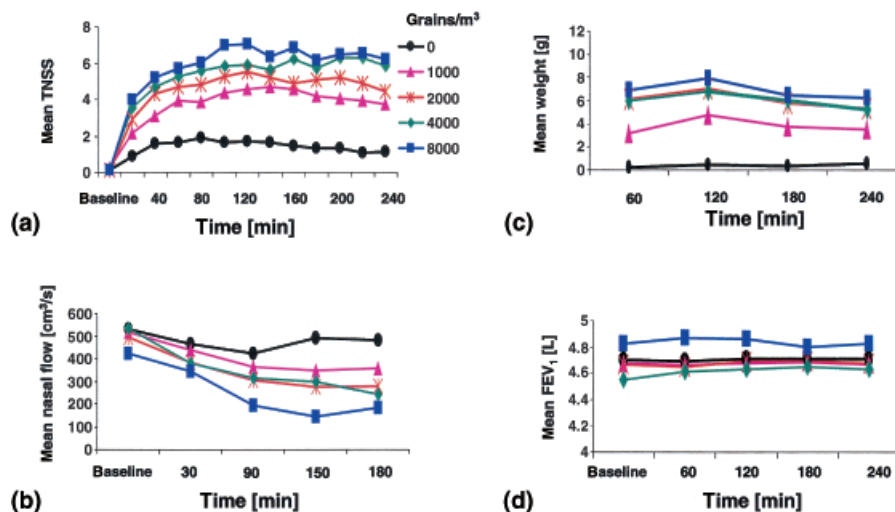


Fig. 5. (a) Dose-dependent total nasal symptom score (TNSS): rhinorrhoea, nasal congestion, sneezing, and nasal itching were self-assessed before and every 20 min during the allergen challenge in the environmental exposure unit according to a four-point severity scale (0 = none; 1 = mild; 2 = moderate; 3 = severe). The TNSS was calculated by adding the four nasal symptoms. A significant concentration effect was seen ($n = 19-23$; $P < 0.01$; Wilks' Lambda test). (b) Reduction of nasal flow rate after pollen exposure: the nasal flow was measured before challenge and at 30, 90, 150, and 180 min during the exposures. A significant concentration effect was seen ($n = 19-23$; $P < 0.05$; Wilks' Lambda test). (c) Increased nasal secretion after pollen exposure: used and unused tissues were placed in a plastic bag, which was collected and weighed every 60 min. A significant concentration effect was seen ($n = 19-23$; $P < 0.01$; Wilks' Lambda test). (d) Lung function normal for all concentrations: forced expiratory volume in 1 s (FEV₁) was measured before and every 60 min during allergen challenge. No significant concentration effect was seen ($n = 19-23$; $P > 0.05$; Wilks' Lambda test).

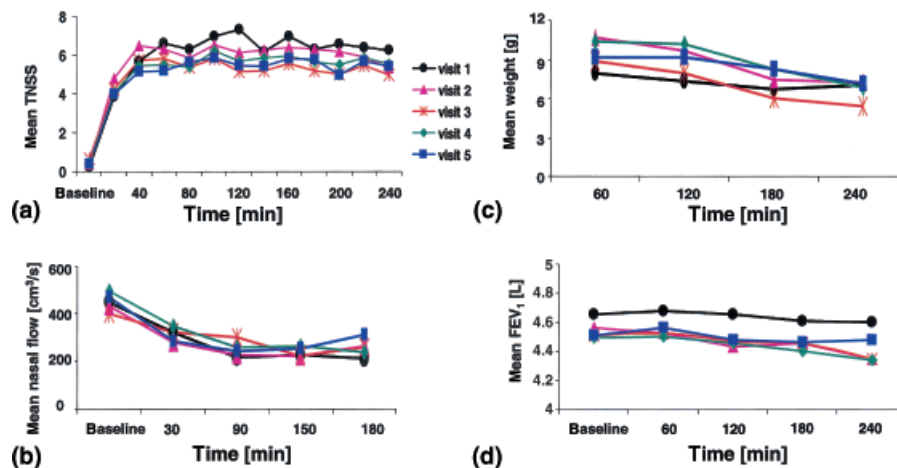


Fig. 6. (a) Consistent total nasal symptom score (TNSS), nasal flow rate, and nasal secretion from day to day: the TNSSs were self-assessed before and every 20 min during allergen exposure. No significant visit effect was seen ($n = 24$; $P > 0.05$; Wilks' Lambda test). (b) Consistent nasal flow rate from day to day: nasal flow rate was measured by rhinomanometry before and 30, 90, 150, and 180 min during pollen exposure. No significant visit effect was seen ($n = 24$; $P > 0.05$; Wilks' Lambda test). (c) Consistent nasal secretion from day to day: used and unused tissues were placed in a plastic bag, which was collected and weighed every 60 min. No significant visit effect was seen ($n = 24$; $P > 0.05$; Wilks' Lambda test). (d) Slight variation of lung function from day to day: forced expiratory volume in 1 s (FEV_1) was measured before and every 60 min during allergen challenge. A significant visit effect was observed, which was not clinically relevant ($n = 45-48$; $P < 0.01$; Wilks' Lambda test).

Discussion

The aim of this study was to validate systematically the induction and reproducibility of different efficacy parameters in the environmental exposure unit used for controlled pollen allergen challenges in patients with SAR. In addition, as a safety parameter, the lung function of participants was evaluated. Mild asthmatics were included in the present study.

Systematic evaluation of the temperature, relative humidity, and volumetric air flow rate showed that they remained stable in the environmental exposure unit. This was also true for various pollen concentrations (1000–8000 grains/m³) over a 4 h period and from day to day. Variations from day to day of about 3.7–5.5% were similar to values of established chambers (5–10% [5]). The spatial distribution of the pollen in our environmental exposure unit was constant and varied only by $\pm 10\%$. This is the first study to evaluate spatial pollen distribution in an exposure unit in detail.

The pollen concentration was measured by two different methods, the offline Rotorod and the online LASAIR systems. The correlation between the two parameters was excellent. The non-specific online monitoring has the great advantage that measurements can be followed immediately, but is limited to relatively high pollen concentrations due to interference with background particles. The pollen-specific Rotorod measurements are retrospective and involve tedious evaluation under the microscope. The environmental exposure unit in Atlanta uses manual evaluation [8], while no specific data have been published for the Vienna challenge chamber [2, 3]. Day et al. [4, 5, 10] applied both an offline and an online method. However, their online system measured the emitted pollen [4] and not the concentration in the room near the participants, as is the case with our unit. Although our online system is very effective, practical, and accurate, it counts particles and not just pollen. Therefore, we are developing a pollen-specific online method. However, even

though this would be an improvement, it does not determine the amount of pollen that each subject actually receives and it does not provide information on the allergenicity of the pollen, which is affected by temperature, humidity, age, and the pollen batch used.

In order to investigate the efficacy of the pollen exposure in the environmental exposure unit, the TNSS, nasal flow rate (rhinomanometry), and nasal secretion were evaluated. Using these three methods, we found a dose-dependent induction of allergic symptoms when participants were exposed to pollen concentrations ranging from 1000 to 8000 grains/m³. These concentrations were within or above the upper range of the naturally occurring grass pollen. The concentration measured in Spain annually did not exceed 2500 grains/m³ [14]. In Austria, Horak and Jager [15] determined a concentration of 1500 grains/m³ at the height of the grass pollen season. In the area around the Fraunhofer Institute in Hannover, Germany, 2100 grains/m³ were measured (in-season; unpublished observation). Values of up to 10 000 grains/m³ (New York, USA) [12] pertain to ragweed pollen, which occurs at higher concentrations than grass pollen.

Since the TNSS is rated by the participants themselves; it is a subjective parameter. Nonetheless, it is recommended by the FDA to be used in industry for drug development (Guidance for Industry). This method is also used for symptom evaluation in the other already established environmental exposure units [8, 9, 16]. In our study, in addition, two objective parameters, rhinomanometry and nasal secretion, were measured to support the TNSS data. Horak et al. and Day et al. also used additional parameters to evaluate anti-allergic medications in their environmental exposure unit [2, 9–11]. Horak et al. [11] showed that after treatment with antihistamines, nasal secretion did not change [9], while nasal air flow improved. Day et al. [10] measured peak nasal inspiratory flow and found that it was increased after treatment with a topical steroid. Further objective parameters are warranted to determine the anti-allergic and the

anti-inflammatory effects of new compounds. In this respect, determination of the skin temperature using thermography [17], measurement of nasal nitric oxide [18], or quantification of blood flow in the nasal mucous membranes are possible markers to evaluate allergic symptoms accurately.

To test the reproducibility of pollen exposure in the environmental exposure unit, participants were subjected to a specific pollen concentration on five different occasions over a 4 h period. The volunteers showed the same course with respect to TNSS, nasal flow rate, and nasal secretion at each visit, which demonstrates that the symptoms in response to allergen exposure in our system are reproducible. This has not been clearly or systematically published for the established environmental exposure units. In a study by Horak et al. [9], patients with allergic rhinitis were challenged on 2 consecutive days with grass pollen (1500 grains/m³) to test the effect of antihistamines. The patients' symptoms, which apart from the ones we used for TNSS included watery and itchy eyes, itchy throat, and coughing, were followed over a 6 h period on each day. When the course of the placebo group was compared, there is a sharper rise in symptom score during the first 2 h on the second day. This indicates a priming effect, which was not observed for the TNSS in our study.

Previous studies have shown that there is a link between allergic rhinitis and asthma [19, 20]. Although pollen grains are assumed to be too large to reach the lower airways, they are able to impair lung function. Several studies have shown that microparticles are most likely responsible [21, 22]. Suphioglu et al. [23] demonstrated that a main allergen of rye pollen is present in intracellular starch granules, which are exposed after the pollen comes in contact with water and bursts. These granules have a diameter of less than 3 µm and, thus, are able to reach the lower airways. Grote et al. [24] and Taylor et al. [25] also described the exposure of cytoplasmic particles, namely starch granules, polysaccharides, and cytoplasmic fragments, in various grass pollens. These findings suggest that lung function could become a problem during allergen exposure. Therefore, FEV₁ and peak expiratory flow rate were measured to determine the safety of pollen exposure in the environmental exposure unit. Our results showed that even at high pollen concentrations (8000 grains/m³), the allergen exposure in the environmental exposure unit was safe. The mean values of all subjects, including mild asthmatics, demonstrated no change in lung function regardless of the pollen concentration during exposure. Lung function has not been tested in detail so far in other established environmental exposure units. Horak et al. [9] report that this parameter was investigated. However, data were not shown.

When lung function (FEV₁) was evaluated after repeated visits a reduction was observed. Although this reduction was not clinically significant, since it was less than 10%, one asthmatic subject developed a drop in FEV₁ below 60% predicted after the second allergen challenge in the second study with a significant late-phase response. He was excluded from the rest of the study. There seems to be a priming effect since the reduction on FEV₁ occurred at visits 2–4. Interestingly, at visit 5, the lung function was back to normal. This was most likely due to the intervals between the visits. For visits 2–4 the interval was 1–4 days, while the interval between visits 4 and 5 was 7 days. It has to be taken into

consideration that the pollen concentration used in these experiments was rather high (8000 grains/m³). As already mentioned, this is above the environmental grass pollen concentration measured in Europe.

We conclude that pollen exposition in an environmental exposure unit is effective, reproducible, and safe. The allergen exposure is also safe for mild asthmatics, because impaired lung function can be determined early by regular spirometry. Future studies must differentiate between rhinitic patients (with and without bronchial hyper-responsiveness) and patients with asthma, in particular, when exhaled and nasal NO measurements are validated as potential new read-out parameters. Furthermore, ongoing studies must clarify the important question of how the exposure unit compares with seasonal natural exposure, or to what extent treatment success with a certain drug in the unit would predict the drug's treatment success under natural conditions. Answers to these questions will help to position the future role of environmental exposure units in clinical allergy research.

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