POSITION PAPER

Allergen exposure chambers: harmonizing current concepts and projecting the needs for the future – an EAACI Position Paper

O. Pfaar[1,2], M. A. Calderon[3,4], C. P. Andrews[5], E. Angjeli[6], K. C. Bergmann[7], J. H. Bønløkke[8], F. de Blay[9,10], P. Devillier[11], A. K. Ellis[12,13], R. Gerth van Wijk[14], J. M. Hohlfeld[15], F. Horak[16], R. L. Jacobs[5], L. Jacobsen[17], M. Jutel[18,19], S. Kaul[20], M. Larché[21], D. Larenas-Linnemann[22,23], R. Mösges[24], H. Nolte[25], P. Patel[26], L. Peoples[6], R. L. Rabin[27], C. Rather[5], A. M. Salapatek[26], T. Sigsgaard[8], S. Thaarup[28], J. Yang[29], P. Zieglmayer[16], T. Zuberbier[30] & P. Demoly[31,32]

[1]Department of Otorhinolaryngology, Head and Neck Surgery, Universitätsmedizin Mannheim, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany; [2]Center for Rhinology and Allergology, Wiesbaden, Germany; [3]Section of Allergy and Clinical Immunology, Imperial College London, London, UK; [4]National Heart & Lung Institute, Royal Brompton Hospital, London, UK; [5]Biogenics Research Chamber LLC, San Antonio, TX, USA; [6]Ora, Inc., Andover, MA, USA; [7]Allergy-Centre-Charité, Charité Universitätsmedizin Berlin, Berlin, Germany; [8]Department of Public Health, Section for Environment, Occupation and Health, Danish Ramazzini Center, Aarhus University, Aarhus, Denmark; [9]ALYATEC, Strasbourg, France; [10]Chest Disease Department, University Hospital of Strasbourg and Federation of Translational Medicine, EA3072, Strasbourg University, Strasbourg, France; [11]UPRES EA 220, Airway Diseases Department, Hôpital Foch, Université Versailles Saint Quentin, University Paris Saclay, Suresnes, France; [12]Department of Medicine, Queen's University, Kingston, ON, Canada; [13]Environmental Exposure Unit, Kingston General Hospital, Kingston, ON, Canada; [14]Section of Allergology, Department of Internal Medicine, Erasmus MC, Rotterdam, The Netherlands; [15]Department of Clinical Airway Research, Fraunhofer Institute of Toxicology and Experimental Medicine (ITEM), Hannover, Germany, Member of the German Center for Lung Research; [16]Vienna Challenge Chamber, Vienna, Austria; [17]Allergy Learning and Consulting (ALC), Copenhagen, Denmark; [18]ALL-MED Medical Research Institute, Wrocław, Poland; [19]Wroclaw Medical University, Wrocław, Poland; [20]Division of Allergology, Paul-Ehrlich-Institut, Federal Institute for Vaccines and Biomedicines, Langen, Germany; [21]Firestone Institute for Respiratory Health, Department of Medicine, St. Joseph's Hospital Healthcare, McMaster University, Hamilton, ON, Canada; [22]Department of Investigation, Hospital Médica Sur, Mexico City, Mexico; [23]Center for Excellence in Asthma and Allergy, Mexico City, Mexico; [24]Institute of Medical Statistics, Informatics and Epidemiology (IMSIE), University Hospital of Cologne, University at Cologne, Cologne, Germany; [25]Merck&Co, Kenilworth, NJ, USA; [26]Inflamax Research Inc., Mississauga, ON, Canada; [27]Center for Biologics Evaluation and Research, United States Food and Drug Administration, Silver Spring, MD, USA; [28]Mobile Chamber Experts GmbH (MCX), Berlin, Germany; [29]Red Maple Trials, Ottawa, ON, Canada; [30]Global Allergy and Asthma European Network (GA^{2}LEN), Department of Dermatology & Allergy, Charité Universitätsmedizin Berlin, Berlin, Germany; [31]Département de Pneumologie et Addictologie, Hôpital Arnaud de Villeneuve, University Hospital of Montpellier, Montpellier, France; [32]UPMC Paris 06, UMR-S 1136, IPLESP, Equipe EPAR, Sorbonne Universités, Paris, France

To cite this article: Pfaar O, Calderon MA, Andrews CP, Angjeli E, Bergmann KC, Bønløkke JH, de Blay F, Devillier P, Ellis AK, Gerth van Wijk R, Hohlfeld JM, Horak F, Jacobs RL, Jacobsen L, Jutel M, Kaul S, Larché M, Larenas-Linnemann D, Mösges R, Nolte H, Patel P, Peoples L, Rabin RL, Rather C, Salapatek AM, Sigsgaard T, Thaarup S, Yang J, Zieglmayer P, Zuberbier T, Demoly P. Allergen exposure chambers: harmonizing current concepts and projecting the needs for the future – an EAACI Position Paper. *Allergy* 2017; **72**: 1035–1042.

Keywords
allergen exposure chambers; allergic; clinical trials; rhinitis; validation.

Correspondence
Professor Oliver Pfaar, MD, Department of Otorhinolaryngology, Head and Neck Surgery, Universitätsmedizin Mannheim, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany;
and Center for Rhinology and Allergology, An den Quellen 10, Wiesbaden D-65183, Germany.
Tel.: +49-611-308-608-0
Fax: +49-611-308-608-255
E-mail: oliver@pfaar.org

Abstract

Background: Allergen exposure chambers (AECs) are clinical facilities allowing for controlled exposure of subjects to allergens in an enclosed environment. AECs have contributed towards characterizing the pathophysiology of respiratory allergic diseases and the pharmacological properties of new therapies. In addition, they are complementary to and offer some advantages over traditional multicentre field trials for evaluation of novel therapeutics. To date, AEC studies conducted have been monocentric and have followed protocols unique to each centre. Because there are technical differences among AECs, it may be necessary to define parameters to standardize the AECs so that studies may be extrapolated for driving basic immunological research and for marketing authorization purposes by regulatory authorities.

Methods: For this task force initiative of the European Academy of Allergy and Clinical Immunology (EAACI), experts from academia and regulatory agencies

Accepted for publication 22 January 2017

DOI:10.1111/all.13133

Edited by: Thomas Bieber

met with chamber operators to list technical, clinical and regulatory unmet needs as well as the prerequisites for clinical validation.

Results: The latter covered the validation process, standardization of challenges and outcomes, intra- and interchamber variability and reproducibility, in addition to comparability with field trials and specifics of paediatric trials and regulatory issues.

Conclusion: This EAACI Position Paper aims to harmonize current concepts in AECs and to project unmet needs with the intent to enhance progress towards use of these facilities in determining safety and efficacy of new therapeutics in the future.

Environmental exposure chambers (EECs) have been developed to generate controlled exposures to inhaled substances such as allergens, endotoxins, chemicals, dry/humid air, thermal shifts or air pollutants. Some of these EECs are used exclusively for controlled exposure of environmental allergens to subjects with allergic rhinitis, conjunctivitis or asthma. The number of these allergen exposure chambers (AECs) available worldwide is limited because of their technological complexity, and the intricacies of the experimental protocols necessary to perform a valid study.

However, during the last 4 years the number of AECs, as well as the number of clinical trials using AECs, has increased (1). Meanwhile, European regulatory authorities have shown interest in assessing the broader utility of AECs in drug development, specifically for allergen immunotherapy (AIT), based on well-defined criteria and protocols/procedures (2).

In addition, AECs have contributed towards understanding the pathophysiology of allergic rhinitis, local (non-systemic) allergic rhinitis, allergic conjunctivitis, allergic asthma and of the pharmacological properties, efficacy, and onset/duration of action of new therapeutics (1, 3, 4). Because subjects are exposed to defined levels of allergens for a specific length of time, AECs may complement traditional multicentre field trials. As the frequency of mechanistic and therapeutic trials in AECs has increased, it has become apparent that defining characteristics of each AEC in a standardized manner, and harmonizing protocols for these studies will improve the quality of data acquired in all chamber facilities and may allow for better application towards proving the safety and efficacy of novel therapeutics. Therefore, this EAACI Position Paper aims to outline the most relevant unmet needs and prerequisites for validation of current and future AECs.

Abbreviations

AAAAI, American Academy of Allergy, Asthma & Immunology; AEC, allergen exposure chamber; AIT, allergen immunotherapy; EAACI, European Academy of Allergy and Clinical Immunology; EEC, environmental exposure chamber; EMA, European Medicine Agency; FDA, Food and Drug Administration; GMP, good manufacturing practice; IMP, investigational medicinal product; MCID, minimal clinically important difference; NNT, numbers needed to treat; PDCO, Paediatric Committee; PIP, Paediatric Investigation Plan; SOP, standard operating procedures.

Materials and methods

Aims of the task force

Our first aim was to gather precise information from the AEC operators to understand the specifications of each AEC, including their similarities and differences, and potential future improvements. We also aimed to define minimal criteria to perform an acceptable study, including allergen sources, and airborne concentration of the allergens, including spatial and temporal uniformity, and distribution in particle size.

This information will serve towards (i) defining procedures that will lead to well-defined and reproducible allergen exposures; (ii) establishing maximum safety for the subjects, particularly those with asthma; and (iii) defining procedures to aid manufacturers of therapeutics to choose the most adequate challenge method to support regulatory requirements for approval.

Invited participants

To define the role of AECs in the development of new interventions and to give the most suitable recommendations, we followed EAACI standard procedures for running task forces, and invited all European and North American groups working with chamber facilities as well as representatives of regulatory authorities to participate in this project.

Consultation procedure

Direct contacts were initiated by emails. We then made a list of available AECs worldwide (Table 1) and met twice (Zurich May 2015, Barcelona June 2015). After open discussion, we focused on two specific issues: (i) (most relevant) unmet needs for the future and (ii) prerequisites for the clinical validation of AECs currently in use.

Participants were given 3 months to write their views, which were then compiled in a comprehensive draft (*Pfaar/Demoly/Calderon*) and sent for further email discussion, tuning and approval. The final manuscript was approved by EAACI ExCom before submission.

What are the (most relevant) unmet needs in the future of AECs?

Validation process

The task force committee understands the purpose of AECs as a technology in which the allergen exposure is controlled,

Table 1 Overview on Allergen Challenge Chambers (AECs) worldwide (in alphabetic order)

AEC facility	City, Country
Allergen BioCube (ABC), ORA Clinical	Andover, MA, USA
Allergen Challenge Theatre, Red Maple Trials	Ottawa, ON, Canada
Allergen Challenge Chamber (ACC), Osaka Medical College	Osaka, Japan
Allergen Challenge Chamber, Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM)	Hannover, Germany
ALYATEC, Strasbourg University Hospital	Strasbourg, France
Biogenics Research Chamber LLC, Biogenics	San Antonio, TX, USA
Chamber at Department of Public Health, Aarhus University	Aarhus, Denmark
Environmental Challenge Chamber (ECC), Chiba University	Chiba, Japan
Environmental Exposure Chamber (EEC), ALL–MED	Wroclaw, Poland
Environmental Exposure Chamber (EEC), Inflamax Research Inc.	Mississauga, ON, Canada
Environmental Exposure Unit (EEU), Queen's University	Kingston, ON, Canada
Environmental Exposure Unit (EEU)	Wakayama, Japan
Mobile Chamber Experts (MCX)	Berlin, Germany
OHIO Chamber, Kitasato Institute Hospital, Minato-ku	Tokyo, Japan
Vienna Challenge Chamber (VCC)	Vienna, Austria

stable and independent from the season or weather conditions or any other random external physical and environmental factors. AECs contribute towards drug development for upper and lower airway hypersensitivity/allergic diseases and mechanisms to characterize the pathology and natural history of allergic disease, develop subject reporting tools and to validate patient-reported outcomes. Because AECs decrease variability of exposure, the number of subjects required to test a therapeutic or mechanistic hypothesis may be lower than for a standard field trial. In addition, because the exposures occur over a defined period of time, the overall length of each study is much shorter than in field trials. Since the performance of valid trials is dependent on the technology of AEC, the validation process should be harmonized and transparent for every AEC. Without validation and harmonization, it may be difficult to extrapolate from a trial performed in one AEC towards another, and from any AEC trial to field studies.

This process should include the following:

(i) The validation for each allergen used because airborne characteristics may vary among allergens. All factors that may impact on upper and lower airway allergy should be controlled and adapted on demand to consider the following criteria:
- Continuous registration of airborne allergen concentration per m^3 air with the coefficient of variation,
  - **a** Registration of the aerodynamic diameter of the particles carrying the allergens with the coefficient of variation (continuously if material is recirculated),
  - **b** Allergen particle counts (and method),
  - **c** Spatial homogeneity and temporal stability of the exposure,
  - **d** The use of good manufacturing practice (GMP)-grade allergens when processed material is mandated by local regulatory agencies.

(ii) The control of nonallergenic exposure:
- **a** Continuous monitoring of relative humidity and temperature,
- **b** Control of the quality of incoming air, e.g., utilizing HEPA filtering,
- **c** Description of antistatic/nonadhering, easy-to-clean materials (i.e. wall materials, seats),
- **d** Well-defined cleaning procedures for sufficient removal of allergens, dust, human particles and microbes so that each chamber is free of debris accumulated during the previous exposure. Each unit should have all these details formulated and secured for each session by having updated standard operating procedures (SOPs) and by logging the data.

Although there have been publications for different allergens in some AECs (5–11), published specifications are still lacking for some allergen sources.

Box 1: Regarding the validation process of allergen chambers, the group recommends

1. A validation of the allergenic exposure, including at least pollen grain counts and distribution
2. The use of GMP-grade allergens when processed material is mandated by local regulatory agencies
3. Control of the chamber environment
4. Standard operating procedures for cleaning of the facilities after each exposure
5. Standard operating procedures for chamber operation including data collection/management
6. Logging of the above data for each exposure session

Standardization of the challenge procedures and assessments

The challenge procedures and assessments should be standardized with recommendations on how to perform them according to the objective(s) of the study:

1. Duration of exposure and read-out: Duration of exposure may vary according to the study objectives (2, 4, or even more hours, on one or several consecutive days). A stable plateau of symptoms should be reached. The respective read-out should be justified and harmonized.
2. Single-*vs*-serial (daily) challenges: *Priming* in allergic patients occurs in real life (12) and attempts to reproduce this in chambers are published in studies of serial exposure in an AEC to Juniperus pollen (9), house dust mites (7) and cat allergens (13). Therefore, single-*vs*-serial challenge procedures must be justified according to study

objective(s) and in particular, with respect to the intervals between serial challenges and to the potential impact of allergy seasons to the patient's responsiveness to a controlled allergen challenge in the AEC.

3 Allergen exposure level: The purpose of AEC is to expose subjects to levels of allergens that are stable and reproducible. Some investigators try to induce a symptom severity level comparable to that documented in real life. However, comparisons of symptom severity between natural seasonal and chamber exposure to three major pollens revealed that natural seasonal symptoms were more severe due to cofactors (14). Other investigators try to induce maximum levels of symptom severity in all patients. Therefore, low- *vs* high-dose challenge procedures must be justified according to study objective(s) and potential ethical issues.

4 Justification of patient selection and screening should be reported, including specific inclusion and exclusion criteria, and study stopping criteria based on severity of symptoms and in line with the study protocol.

5 All outcome measures in AEC studies should be clinically relevant and standardized.
   - **a** Standardized procedures for symptom scoring should be delineated along with standardized instructions for subjects on how to score. The symptom medication score is the primary outcome for field trials that has been determined by consensus in an EAACI Position Paper (15). Because concomitant anti-allergic medications are not allowed during AEC studies, the most obvious primary outcome for AEC studies is the symptom score on a 0–3 scale [e.g., using the four nasal symptoms' score and if deemed useful according to the allergen employed and the respective protocol, ocular and bronchial symptoms' score (2, 16)]
   - **b** Timing to assess the outcomes for each allergen extract should also be reported. Previous studies of allergen challenges of either grass pollen or HDM show that symptom scores rise over the first 1–2 h and are followed by a plateau phase. The plateau phase indicates that the symptom scores have stabilized, and was therefore considered the more relevant time to analyse the primary outcome (13, 17–19).
   - **c** Along with the evaluation period, the calculation of the effect size (e.g., mean and/or AUC) should be reported and justified in the study protocol prior to performance of the study and data analysis.

6 Chamber-specific *placebo* (development of symptoms in sensitized patients exposed to allergen-free air, the so-called 'sham run') and *nocebo* (development of symptoms due to just seeing chamber subjects with exacerbation of allergic symptoms) effects should be documented as it increases the validity of the exposure tests.

7 Safety measures should be specified in terms of:
   - **a** Selection of subjects suitable for exposure,
   - **b** Prevention of adverse reactions (e.g., by monitoring symptoms and lung function in asthmatic patients),
   - **c** Availability of emergency medical personnel and resuscitation equipment,
   - **d** Availability of nearby emergency room and intensive care facilities,
   - **e** Definition of surveillance procedure following exposure (e.g., late-phase asthmatic reaction),
   - **f** Reporting of all adverse events and their treatment.

Box 2: Regarding the standardization of the challenge and outcomes in allergen chambers, the group recommends

1 Evaluation of the outcomes at the plateau phase of the challenge
2 Justification of single-*vs*-serial challenges
3 Justification of low- *vs* high-dose challenges
4 Disclosure of general patient selection criteria to assure appropriate patient selection and safety
5 The use of standardized and clinically relevant outcomes
6 Reporting and justification of the calculation of the effect size of the intervention
7 Definition of safety measures

Intra-AEC reproducibility

Reproducibility of findings among studies in the same chamber is a critical consideration towards advancing use of AEC. If conditions are tightly controlled, repetition of the same protocol on separate occasions should yield the same results. This reproducibility has been reported with the Vienna Challenge Chamber (VCC) (Vienna, Austria), the EEC (Mississauga, Canada), the Environmental Exposure Unit (EEU) (Kingston, Canada), the Chamber at Department of Public Health, Aarhus University (Aarhus, Denmark), Biogenics Research Chamber LLC (San Antonio, TX, USA), Mobile Chamber Experts (MCX) (Berlin, Germany) and the Fraunhofer Allergen Challenge Chamber (Hannover, Germany) (7, 20–26). Other facilities should publish their reproducibility data.

Inter-AEC variability and reproducibility

Studies conducted to date have been monocentric, and there are technical differences between units, the most palpable being the method of allergen dispersion and the method of monitoring allergen concentration (27–29). It is unclear at present how outcome measures compare between units when identical protocols are used (variability) and how outcomes compare when the same subjects are challenged at different units (reproducibility). Regulatory authorities and other stakeholders would have greater confidence in clinical efficacy (such as pivotal phase 3) studies conducted in multiple AECs across multiple countries once a common standardized protocol and clinical outcomes in one facility are comparable to those in another facility.

Comparability with field trials

One major issue is the comparability of AEC data with data obtained in field trials. For AEC studies, exposure

parameters are precisely defined and reproducible, but for natural exposure they are not. To determine comparability, it would be necessary to sufficiently quantify the natural allergen exposure at the individual level for each subject, which is currently not possible. Regulators and other stakeholders would appreciate data supporting the fact that treatment effects are equal when studied in an AEC compared with a traditional field trial, while the magnitude of measured effects may differ. The solution may be combined studies evaluating subjects in real life, for example pollen season, with additional embedded challenge sessions (2). To date, there is a paucity *of hybrid trials* where the same intervention is investigated 'in the field' as well as in an AEC exposure for direct comparability in the same subject population (8). Simple comparisons of treatment effect sizes between AEC and field trials are not entirely scientifically correct for several reasons. Rescue medications are allowed in the latter but not in the former, and allergen exposure is controlled and quantified in the former but not in the latter. Another aspect is that in AEC trials subjects who develop symptoms at screening as part of fulfilling the inclusion criteria are enrolled, whereas in most cases field trials recruit subjects based only on their history and their sensitization profile.

Box 3: Regarding comparability of allergen chamber trials with field trials, the group recommends that

1. Direct comparison is not possible (as natural allergen exposure cannot be determined and rescue medication is allowed in field trials)
2. Indirect comparison of effect sizes is possible when allergic subjects with comparable clinical characteristics are evaluated
3. Hybrid designs are feasible and may be an option

Paediatric issues

Long-term paediatric, placebo-controlled field trials are difficult to perform and are often inconclusive because there are no objective efficacy parameters for allergenic therapeutics. Safely executed in 5- to 11-years-old children and 12- to 17-years-old adolescents, AEC challenges may hasten development of therapeutics for children and may provide a major step towards changing the required criteria of the Paediatric Investigation Plan (PIP) of the Paediatric Committee (PDCO) of the European Medicines Agency (EMA) or other regulatory agencies (30, 31). Ideally, clinical validation should be done during a short-term efficacy and safety field trial, followed by AEC studies for long-term efficacy in children. For very young subjects, consideration should be given to symptom scoring by an accompanying parent.

Regulatory issues

In 2013, American Academy of Allergy, Asthma & Immunology (AAAAI) and EAACI PRACTALL experts (32) for the first time advocated for validation and acceptance of AEC as suitable surrogates for natural allergen exposure for the study of allergic diseases. Clinical efficacy can be demonstrated for therapeutic agents with fewer subjects and within shorter timelines, meriting higher ethical ground than in traditional trials. An AEC study may even be an option for developing therapeutics for rare allergies, for example those which affect a smaller number of the population which are not developed due to the clinical requirements for a marketing authorization (33, 34).

For dose-finding studies, EMA 2008 guidelines (2) state that '*provocation tests (… or allergen exposure in allergen challenge chambers) and/or clinical endpoints may be used as primary endpoints*'. For confirmatory studies, '*provocation tests in allergen chambers (are) deemed to be a promising tool for the evaluation of efficacy, however the results of such provocations have to be validated in comparison with clinical symptoms by natural exposure*'. For long-term studies, AEC '*might be a helpful marker*', especially in case of low natural pollen counts (2). Regulatory authorities will be interested in the confirmation of phase 3 field trial results in AEC studies (e.g., in '*hybrid*' model as described above) although whether AEC studies are acceptable for pivotal efficacy trials may vary among the various regulatory bodies. However, such models would make a comparison of study results feasible and might support to update the EMA guideline (2).

To do this, there are financial as well as technical issues (size of the investment and number of allergen chambers needed, respectively) that must be considered. This mostly concerns short-term studies, but AEC might also be useful to test sustained and long-term effects. Prerequisites for clinical validation should be developed.

Box 4: Regarding regulatory issues for allergen chambers, the group recommends that

1. If the above (Boxes 1–3) issues are solved, AECs can be used not only for phases 1 and 2 but also for confirmatory phase 3 trials.
2. We all agree on the prerequisites listed below for the clinical validation of AECs.

What are the prerequisites for the clinical validation of AECs?

As outlined above, the validation of clinical trials performed in several chambers, spread in widely disparate geographic areas and using different engineering and technology, would be required to address all the above unmet needs. The prerequisites therefore include definition and standardization (i) of technical parameters as described above and (ii) of clinical SOPs, definition of meaningful and valid clinical and statistical parameters, and reproducible dose response in clinical symptoms. Validation utilizing a standardized protocol demonstrating reproducibility and consistency of results between competing chambers must be accomplished.

Regarding AIT, AEC models have demonstrated to be useful in establishing the maximum effect size without concomitant rescue medication (35, 36), the onset of action (35, 36), the long-term treatment effects (37) and, possibly, the validation of new clinical outcomes (3) and biological markers (38, 39).

Presently, it cannot be predicted which outcomes have to be obtained in order to claim chamber trials as being valid and complementary for marketing authorization. Thus, it should be documented in detail, to what extent the efficacy of an investigational medicinal product (IMP) shown in an AEC setting can predict efficacy during a season or a chronic exposure to a perennial allergen source. To this end, the following two questions will have to be answered: (i) what is the effect size of a therapeutic intervention such as AIT in an AEC trial, (ii) what is the relationship between the effect size in an AEC and that in a field trial (where allergen exposure vary and symptom-relieving medication is permitted)? The kinetics of increase in symptom scores should be determined for each AEC and each allergen source to determine the plateau of symptoms. A thorough discussion and consensus on protocol standardization and/or SOPs for the evaluation of symptom scores in the AEC should be promoted. Other metrics of meaningful clinical outcomes [such as minimal clinically important difference (MCID), numbers needed to treat (NNT)] can be used.

Conclusion

If all AEC operators collaborate and address the issues outlined in this document, the group is confident that chamber studies will rank highly in product development for allergic diseases and in the decisions taken by regulatory authorities with respect to marketing authorizations.

Funding

This work was funded by the European Academy of Allergy and Clinical Immunology (EAACI).

Conflicts of interest

Dr. Pfaar reports grants and personal fees from ALK-Abelló, grants and personal fees from Allergopharma, grants and personal fees from Stallergenes Greer, grants and personal fees from HAL Allergy Holding B.V./HAL Allergie GmbH, grants and personal fees from Bencard Allergie GmbH/ Allergy Therapeutics, grants and personal fees from Lofarma, grants from Biomay, grants from Nuvo, grants from Circassia, grants and personal fees from Biotech Tools S.A., grants and personal fees from Laboratorios LETI/LETI Pharma, personal fees from Novartis Pharma, personal fees from MEDA Pharma, grants and personal fees from Anergis S.A., personal fees from Sanofi US Services, personal fees from Mobile Chamber Experts (a GA^2LEN Partner), personal fees from Pohl-Boskamp, outside the submitted work. Dr. Calderon reports personal fees from ALK, personal fees from HAL-Allergy, personal fees from Merck, personal fees from Stallergenes Greer, outside the submitted work. Dr. Andrews has nothing to disclose. Mr. Angjeli has nothing to disclose. Dr. Bergmann reports personal fees from Mobile Chamber Experts Gmbh, during the conduct of the study; and he is a member of the Mobile Chamber Experts GmbH. Dr. Bønløkke has nothing to disclose. Dr. de BLAY reports grants from STALLERGENES - GREER, grants from CHIESI, personal fees from ALK, personal fees from MUNDIPHARMA, personal fees from NOVARTIS, during the conduct of the study; other from STALLERGENES-GREER, other from NOVARTIS, other from ALK, other from MUNDIPHARMA, other from MEDAPHARMA, other from BOEHRINGER, outside the submitted work. Dr. Devillier reports personal fees from Stallergenes Greer, personal fees from ALK, personal fees from Meda Pharma, outside the submitted work. Dr. Ellis reports other from AstraZeneca, grants and other from Circassia Ltd, grants and other from Merck, grants and other from Novartis, grants and other from Pfizer, other from Takeda, other from Meda, outside the submitted work. Dr. Gerth van Wijk reports personal fees from HAL, personal fees from ALK-Abello, grants from Biomay, grants from DBV, personal fees from Allergopharma, outside the submitted work. Dr. Hohlfeld reports nonfinancial support from European Academy of Allergy and Clinical Immunology, during the conduct of the study; grants from Allergopharma GmbH, grants and personal fees from Leti Pharma GmbH, grants and personal fees from Novartis Pharma AG, grants from ALK-Abelló A/S, outside the submitted work; in addition, Dr. Hohlfeld has a patent DE 10 2008 060 614 issued. Dr. Horak has a patent US 2004/0054262 A1 issued, a patent CA No. 2 416 328 issued, a patent EU No. 1 335 750 issued and a patent AT 411.214 issued. Dr. Jacobs has nothing to disclose. Dr. Jacobsen has nothing to disclose. Dr. Jutel reports personal fees from ALLERGOPHARMA/MERCK AG, personal fees from ANERGIS, personal fees from BIOMAY, personal fees from ALK, personal fees from Stallergenes Greer, outside the submitted work. Dr. Kaul reports employment at the Paul-Ehrlich-Institut, the German Federal Institute for Vaccines and Biomedicines. However, Dr. Kaul contributed to the paper with her personal opinion not necessarily reflecting the view of the Paul-Ehrlich-Institut. Dr. Larché reports grants and personal fees from Adiga Life Sciences, grants, personal fees and other from Circassia Ltd/Circassia Pharmaceuticals PLC, during the conduct of the study; personal fees from UCB, personal fees from Sanofi, personal fees from Aravax Pty, personal fees from Brandon Capital Ventures, personal fees from Air Canada, outside the submitted work; in addition, Dr. Larché is an inventor on 17 patent families (pending or issued) held by Circassia Ltd. Dr. Larenas Linnemann reports grants from UCB, TEVA, GSK, Boehringer Ingelheim, MSD, Pfizer, Senosiain, Almirall, Sanofi, Astrazeneca, Meda, Chiesi, Novartis, ALK.-Abelló, personal fees from UCB, astrazeneca, Meda, Pfizer, Chiesi, Glenmark, Novartis, personal fees from Mitpharma, AZ, Boehringer Ingelheim, Chiesi, Meda, Sanofi, Novartis, DBV, outside the submitted work. Dr. Mösges reports personal fees from ALK, grants and personal fees from BTT, grants and nonfinancial support

from ASIT-Biotech, personal fees from Allergopharma, grants and personal fees from Bencard, grants from HAL, grants and personal fees from Leti, grants and personal fees from Lofarma, grants from Roxall, grants, personal fees and nonfinancial support from Stallergenes Greer, outside the submitted work. Dr. Nolte has nothing to disclose. Dr. Patel reports other from Inflamax Research Inc., outside the submitted work; in addition, Dr. Patel has a patent MOBILE CHAMBER APPARATUSES AND RELATED METHODS pending, a patent METHOD AND CHAMBER FOR EXPOSURE TO NON-ALLERGIC RHINITIS TRIGGER ENVIRONMENTS pending, a patent METHOD AND CHAMBER FOR EXPOSURE TO NON-ALLERGIC RHINITIS TRIGGER ENVIRONMENTS issued, and a patent METHOD, MATERIALS AND APPARATUS FOR INVESTIGATING ASTHMA USING DUST MITE ALLERGEN pending and he is a majority owner, a patent holder and an inventor of chamber models at Inflamax Research Inc. He is an employee of a contract research organization, which provides chamber services to drug development companies. E. Peoples has nothing to disclose. Dr. Rabin states that this position paper reflects the views of the author and should not be construed to represent FDA's views or policies. Mrs. Rather has nothing to disclose. Dr. Salapatek reports other from Inflamax Research Inc., outside the submitted work; in addition, Dr. Salapatek has a patent MOBILE CHAMBER APPARATUSES AND RELATED METHODS pending, a patent METHOD AND CHAMBER FOR EXPOSURE TO NON-ALLERGIC RHINITIS TRIGGER ENVIRONMENTS pending, a patent METHOD AND CHAMBER FOR EXPOSURE TO NON-ALLERGIC RHINITIS TRIGGER ENVIRONMENTS issued, and a patent METHOD, MATERIALS AND APPARATUS FOR INVESTIGATING ASTHMA USING DUST MITE ALLERGEN pending and a patent holder and inventor of chamber models at Inflamax Research Inc. He is an employee of a contract research organization which provides chamber services to drug development companies. Dr. Sigsgaard has nothing to disclose. Dr. Thaarup reports other from Mobile Chamber Experts GmbH (MCX), outside the submitted work; in addition, Dr. Thaarup has a patent B10628 PDE pending and a patent B10659 PDE pending. J. YANG has nothing to disclose. Dr. Zieglmayer reports personal fees from Allergopharma, grants, personal fees and other from Allergy Therapeutics, personal fees from ALK-Abello, grants from Biomay, grants from Calistoga, grants from GSK, grants from HAL, grants from MSD, personal fees from Novartis, grants from Ono, grants from Oxagen, grants from RespiVert, personal fees and other from Stallergenes Greer, personal fees from ThermoFisher Scientific, grants from VentiRX. Dr. Zuberbier reports grants, personal fees and nonfinancial support from AstraZeneca, AbbVie, ALK, Almirall, Astellas, Bayer Health Care, Bencard, Berlin Chemie, FAES, HAL, Henkel, Kryolan, Leti, L'Oreal, Meda, Menarini, Merck, MSD, Novartis, Pharmasquire, Pfizer, Quintiles, Sanofi, Serono, Stallergenes Greer, Takeda, Teva and UCB, outside the submitted work; and Dr. Zuberbier has the following organizational affiliations: Committee member, WHO initiative 'Allergic Rhinitis and its Impact on Asthma' (ARIA); Member of the Board, German Society for Allergy and Clinical Immunology (DGAKI); Head, European Centre for Allergy Research Foundation (ECARF); Secretary General, Global Allergy and Asthma European Network (GA²LEN); Member, Committee on Allergy Diagnosis and Molecular Allergology, World Allergy Organisation (WAO). Dr. Demoly reports personal fees from ALK, personal fees from Circassia, personal fees from Stallergenes Greer, personal fees from Allergopharma, personal fees from Allergy Therapeutics, personal fees from ThermoFisher Scientific, personal fees from Chiesi, personal fees from Menarini, personal fees from Meda, personal fees from AstraZeneca, personal fees from MSD, outside the submitted work.

References

1. Rösner-Friese K, Kaul S, Vieths S, Pfaar O. Environmental exposure chambers in allergen immunotherapy trials: current status and clinical validation needs. *J Allergy Clin Immunol* 2015;**135**:636–643.
2. European Medicines Agency. Committee for medicinal products for human use (CHMP): Guideline on the Clinical Development of Products for Specific Immunotherapy for The Treatment of Allergic Diseases (CHMP/EWP/18504/2006); 2008. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003605.pdf
3. Devillier P, Le Gall M, Horak F. The allergen challenge chamber: a valuable tool for optimizing the clinical development of pollen immunotherapy. *Allergy* 2011;**66**:163–169.
4. North ML, Soliman M, Walker T, Steacy LM, Ellis AK. Controlled allergen challenge facilities and their unique contributions to allergic rhinitis research. *Curr Allergy Asthma Rep* 2015;**15**:11.
5. Ellis AK, Ratz JD, Day AG, Day JH. Factors that affect the allergic rhinitis response to ragweed allergen exposure. *Ann Allergy Asthma Immunol* 2010;**104**:293–298.
6. Ellis AK, Steacy LM, Hobsbawn B, Conway CE, Walker TJ. Clinical validation of controlled grass pollen challenge in the Environmental Exposure Unit (EEU). *Allergy Asthma Clin Immunol* 2015;**11**:5.
7. Jacobs RL, Andrews CP, Ramirez DA, Rather CG, Harper N, Jimenez F et al. Symptom dynamics during repeated serial allergen challenge chamber exposures to house dust mite. *J Allergy Clin Immunol* 2015;**135**:1071–1075.
8. Jacobs RL, Harper N, He W, Andrews CP, Rather CG, Ramirez DA et al. Responses to ragweed pollen in a pollen challenge chamber versus seasonal exposure identify allergic rhinoconjunctivitis endotypes. *J Allergy Clin Immunol* 2012;**130**:122–127.
9. Jacobs RL, Ramirez DA, Andrews CP. Validation of the biogenics research chamber for *Juniperus ashei* (mountain cedar) pollen. *Ann Allergy Asthma Immunol* 2011;**107**:133–138.
10. Krug N, Hohlfeld JM, Larbig M, Buckendahl A, Badorrek P, Geldmacher H et al. Validation of an environmental exposure unit for controlled human inhalation studies with grass pollen in patients with seasonal allergic rhinitis. *Clin Exp Allergy* 2003;**33**:1667–1674.
11. Patel P, Holdich T, Fischer von Weikersthal-Drachenberg KJ, Huber B. Efficacy of a short course of specific immunotherapy in patients with allergic rhinoconjunctivitis to ragweed pollen. *J Allergy Clin Immunol* 2014;**133**:121–129.
12. Connell JT. Quantitative intranasal pollen challenge. II. Effect of daily pollen challenge,

environmental pollen exposure, and placebo challenge on the nasal membrane. *J Allergy* 1968;**41**:123–139.
13. Patel D, Couroux P, Hickey P, Salapatek AM, Laidler P, Larché M et al. Fel d 1-derived peptide antigen desensitization shows a persistent treatment effect 1 year after the start of dosing: a randomized, placebo-controlled study. *J Allergy Clin Immunol* 2013;**131**:103–109.
14. Jacobs RL, Harper N, He W, Andrews CP, Rather CG, Ramirez DA et al. Effect of confounding cofactors on responses to pollens during natural season versus pollen challenge chamber exposure. *J Allergy Clin Immunol* 2014;**133**:1340–1346.
15. Pfaar O, Demoly P, Gerth van Wijk R, Bonini S, Bousquet J, Canonica GW et al. Recommendations for the standardization of clinical outcomes used in allergen immunotherapy trials for allergic rhinoconjunctivitis: an EAACI Position Paper. *Allergy* 2014;**69**:854–867.
16. US Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER). Allergic rhinitis: developing drug products for treatment guidance for industry. Available from: http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm071293.pdf; Revision 1; February 2016.
17. Horak F, Stübner P, Zieglmeyer R, Harris AG. Comparison of the effects of desloratadine 5-mg daily and placebo on nasal airflow and seasonal allergic rhinitis symptoms induced by grass pollen exposure. *Allergy* 2003;**58**:481–485.
18. Badorrek P, Dick M, Hecker H, Schaumann F, Sousa AR, Murdoch R et al. Anti-allergic drug testing in an environmental challenge chamber is suitable both in and out of the relevant pollen season. *Ann Allergy Asthma Immunol* 2011;**106**:336–341.
19. Roux M, Devillier P, Yang WH, Montagut A, Abiteboul K, Viatte A et al. Efficacy and safety of sublingual tablets of house dust mite allergen extracts: results of a dose- ranging study in an environmental exposure chamber. *J Allergy Clin Immunol* 2016;**138**:451–458.
20. Horak F, Stübner UP, Zieglmayer R, Harris AG. Effect of desloratadine versus placebo on nasal airflow and subjective measures of nasal obstruction in subjects with grass pollen-induced allergic rhinitis in an allergen-exposure unit. *J Allergy Clin Immunol*. 2002;**109**:956–961.
21. Day JH, Briscoe M, Rafeiro E, Chapman D, Kramer B. Comparative onset of action and symptom relief with cetirizine, loratadine, or placebo in an environmental exposure unit in subjects with seasonal allergic rhinitis: confirmation of a test system. *Ann Allergy Asthma Immunol* 2001;**87**:474–481.
22. Salapatek AM, Lee J, Patel D, D'Angelo P, Liu J, Zimmerer RO Jr et al. Solubilized nasal steroid (CDX-947) when combined in the same solution nasal spray with an antihistamine (CDX-313) provides improved, fast-acting symptom relief in patients with allergic rhinitis. *Allergy Asthma Proc* 2011;**32**:221–229.
23. Zuberbier T, Abelson MB, Akdis CA, Bachert C, Berger U, Bindslev-Jensen C et al. Validation of the Global Allergy and Asthma European Network (GA2LEN) chamber for trials in allergy: innovation of a mobile allergen exposure chamber. *J Allergy Clin Immunol* 2016; pii: S0091-6749(16)31049-1.
24. Lueer K, Biller H, Casper A, Windt H, Mueller M, Badorrek P et al. Safety, efficacy and repeatability of a novel house dust mite allergen challenge technique in the Fraunhofer allergen challenge chamber. *Allergy* 2016;**71**:1693–1700.
25. Hohlfeld JM, Holland-Letz T, Larbig M, Lavae-Mokhtari M, Wierenga E, Kapsenberg M et al. Diagnostic value of outcome measures following allergen exposure in an environmental challenge chamber compared with natural conditions. *Clin Exp Allergy* 2010;**40**:998–1006.
26. Kenney P, Bønløkke J, Hilberg O, Ravn P, Schlünssen V, Sigsgaard T. Method for a homogeneous distribution of pollens in an environmental exposure chamber. *Clin Exp Allergy* 2016;**46**:1176–1184.
27. Day JH, Horak F, Briscoe MP, Canonica GW, Fineman SM, Krug N et al. The role of allergen challenge chambers in the evaluation of anti-allergic medication: an international consensus paper. *Clin Exp Allergy Rev* 2006;**6**:31–59.
28. Ellis AK, North ML, Walker T, Steacy LM. Environmental exposure unit: a sensitive, specific, and reproducible methodology for allergen challenge. *Ann Allergy Asthma Immunol* 2013;**111**:323–328.
29. Horak F, Jäger S. The Vienna challenge chamber (VCC) – a new method for allergen exposition tests. *Wien Klin Wochenschr* 1987;**99**:509–510.
30. Calderon MA, Gerth van Wijk R, Eichler I, Matricardi PM, Varga EM, Kopp MV et al. Perspectives on allergen-specific immunotherapy in childhood: an EAACI position statement. *Pediatr Allergy Immunol* 2012;**23**:300–306.
31. EMA/PDCO Standard Paediatric Investigation Plan for Allergen Products for Specific Immunotherapy. Revision 4. EMA/PDCO/737605/2009; 2015. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Regulatory_and_procedural_guideline/2009/11/WC500015814.pdf
32. Burks AW, Calderon MA, Casale T, Cox L, Demoly P, Jutel M et al. Update on allergy immunotherapy: American Academy of Allergy, Asthma & Immunology/European Academy of Allergy and Clinical Immunology/PRACTALL consensus report. *J Allergy Clin Immunol* 2013;**131**:1288–1296.
33. Kaul S, May S, Lüttkopf D, Vieths S. Regulatory environment for allergen-specific immunotherapy. *Allergy* 2011;**66**:753–764.
34. Pfaar O, Bachert C, Bufe A, Buhl R, Ebner C, Eng P et al. Guideline on allergen-specific immunotherapy in IgE mediated allergic diseases – S2k Guideline of the German Society for Allergology and Clinical Immunology (DGAKI), the Society for Pediatric Allergy and Environmental Medicine (GPA), the Medical Association of German Allergologists (AeDA), the Austrian Society for Allergy and Immunology (ÖGAI), the Swiss Society for Allergy and Immunology (SGAI), the German Society of Dermatology (DDG), the German Society of Oto-Rhino-Laryngology, Head and Neck Surgery (DGHNO-KHC), the German Society of Pediatrics and Adolescent Medicine (DGKJ), the Society for Pediatric Pneumology (GPP), the German Respiratory Society (DGP), the German Association of ENT Surgeons (BV-HNO), the Professional Federation of Paediatricians and Youth Doctors (BVKJ), the Federal Association of Pulmonologists (BDP) and the German Dermatologists Association (BVDD). *Allergo J Int* 2014;**23**:282–319.
35. Horak F, Zieglmayer P, Zieglmayer R, Lemell P, Devillier P, Montagut A et al. Early onset of action of a 5-grass-pollen 300-IR sublingual immunotherapy tablet evaluated in an allergen challenge chamber. *J Allergy Clin Immunol* 2009;**124**:471–477.
36. Nolte H, Maloney J, Nelson HS, Bernstein DI, Lu S, Li Z et al. Onset and dose-related efficacy of house dust mite sublingual immunotherapy tablets in an environmental exposure chamber. *J Allergy Clin Immunol* 2015;**135**:1494–1501.
37. Couroux P, Patel D, Armstrong K, Larché M, Hafner RP. Fel d 1-derived synthetic peptide immuno-regulatory epitopes show a long-term treatment effect in cat allergic subjects. *Clin Exp Allergy* 2015;**45**:974–981.
38. Baron-Bodo V, Horiot S, Lautrette A, Chabre H, Drucbert AS, Danzé PM et al. Heterogeneity of antibody responses among clinical responders during grass pollen sublingual immunotherapy. *Clin Exp Allergy* 2013;**43**:1362–1373.
39. Zimmer A, Bouley J, Le Mignon M, Pliquet E, Horiot S, Turfkruyer M et al. A regulatory dendritic cell signature correlates with the clinical efficacy of allergen-specific sublingual immunotherapy. *J Allergy Clin Immunol* 2012;**129**:1020–1030.