Curr Allergy Asthma Rep (2016) 16: 21
DOI 10.1007/s11882-016-0599-4

Standardization and Regulation of Allergen Products in the European Union

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Published online: 13 February 2016

Abstract Product-specific standardization is of prime importance to ensure persistent quality, safety, and efficacy of allergen products. The regulatory framework in the EU has induced great advancements in the field in the last years although national implementation still remains heterogeneous. Scores of methods for quantification of individual allergen molecules are developed each year and also the challenging characterization of chemically modified allergen products is progressing. However, despite the unquestionable increase in knowledge and the subsequent improvements in control of quality parameters of allergen products, an important aim has not been reached yet, namely cross-product comparability. Still, comparison of allergen product potency, either based on total allergenic activity or individual allergen molecule content, is not possible due to a lack of standard reference preparations in conjunction with validated standard methods. This review aims at presenting the most recent developments in product-specific standardization as well as activities to facilitate cross-product comparability in the EU.

This article is part of the Topical Collection on *Immunologic/Diagnostic Tests in Allergy*

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Keywords Allergen product · Product-specific standardization · Cross-product comparability · Allergen quantification · Allergoid characterization · EU regulatory aspects

Introduction

Standardization of allergen extracts has been discussed and called for in the field of allergology for decades. The respective activities and achievements have been summarized in several review articles published within the last years [1••, 2, 3, 4••, 5]. However, when catching up on standardization of allergen extracts, one has to realize that there exist several levels to the topic, which require thorough discrimination (Fig. 1).

The first step is product-specific standardization, representing the fundamental prerequisite for consistency between batches of an allergen product. The use of validated analytical methods for control of quality parameters ensures consistency of batches over time resulting in constant quality, safety, and efficacy. This is necessary as well as challenging due to the complex composition of allergen extracts in conjunction with the great variability of the respective natural source materials [6–11]. The level of product-specific standardization and thus knowledge on allergen product composition has greatly advanced in recent years. Not only regulatory requirements but also the pressure exerted by allergy societies, academia, and clinicians are pushing this development forward. This is also reflected in a recent expert position paper on allergen-specific immunotherapy (AIT), stressing that "only standardized extracts should be used in clinical practice because efficacy and safety of AIT depends strictly on extract quality" [12•].

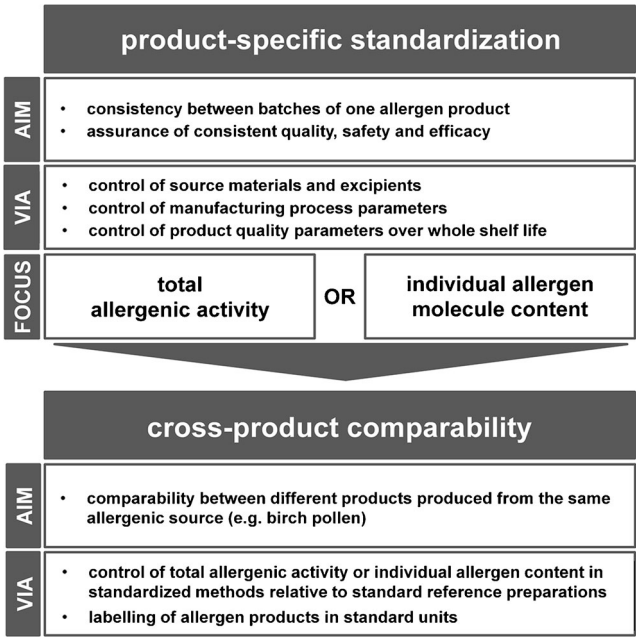

Fig. 1 From product-specific standardization to cross-product comparability

Naturally, the main focus of product-specific standardization is on the quality parameters defining the respective product. Hence, one can differentiate between standardization approaches focusing on total allergenic activity on the one hand and approaches based on the content of individual allergen molecules on the other hand. However, the vast majority of authorized allergen products in the EU are standardized to total allergenic activity by measuring IgE-binding potency. In accordance with the current regulatory requirements in the EU, this parameter is expressed in manufacturer-specific units, determined in manufacturer-specific in vitro assays relative to a product-specific in-house reference preparation (IHRP). Similarly, the control of individual allergen molecules in AIT products is until now to be performed relative to the respective IHRP, in manufacturer-specific immunoassays or other suitable methods.

The second step is cross-product comparability—comparability of products out of the same allergenic source produced by different manufactures. Although both total allergenic activity and individual allergen molecule content can provide the basis for cross-product comparability, the current state of product-specific standardization does not allow for this next level of allergen extract standardization. The prerequisite for comparability is the comprehensive use of a standardized analytical method relative to a standard reference preparation. In the USA, comparability of standardized extracts is demanded by the Food and Drug Administration (FDA) [13••]; but due to the current clinical practice, these allergen extracts do not represent the finished allergen products administered to patients. In contrast, the European strategy is to establish validated allergen-specific immunoassays in conjunction with allergen standards to allow labeling the finished products with the individual allergen content in comparable mass units. Unfortunately, the efforts to enable cross-product comparability in the EU evolved slowly. In the light of several studies showing remarkable differences in total allergenic activity and/or individual allergen content between marketed products [14–18, 19•], cross-product comparability is still regarded as one of the unmet needs in the field of AIT [12•].

Current Regulatory Situation in Europe

In accordance with the European Directive 2001/83/EC, marketing authorization (MA) is required for all industrially produced medicinal products including allergen products for diagnosis and AIT. The regulatory requirements have remained virtually unchanged in the EU between the update of the *Note for Guidance on Allergen Products* in 1996 [20] and the Revision of the *Monograph on Allergen Products* in 2008 [21]. The subsequent revisions of existing as well as the implementation of new guidance documents have been summarized by Kaul et al. [22]. However, in practice, the implementation of the existing regulatory framework for allergen products remains heterogeneous in the EU. For example, the so-called umbrella marketing authorizations are still common practice in several member states [23•]. Moreover, in several member states, AIT products are mainly distributed without marketing authorization as medicinal products manufactured for an individual patient, so-called "named-patient products" (NPPs). However, regarding AIT products for treatment of highly prevalent allergies such as birch pollen or house dust mite allergy distribution as NPPs should no longer represent the regulatory status of choice.

Various national strategies exist to enforce MA of allergen products with state-of-the-art quality and limit the use of non-registered products. In Germany, the Therapy Allergy Ordinance demands MA for all products intended for immunotherapy of highly prevalent allergies [22, 24, 25], restricting the use of NPPs to the treatment of rare allergies. In France, the control of NPPs was tightened by limiting their production to a panel of authorized extracts of clinically relevant allergen sources, which can be used by authorized persons to prepare NPPs according to a patient-specific prescription [26]. In Spain, a proposal has been drafted, establishing different regulatory routes for different types of allergen products (Marcos Timón, AEMPS, personal communication, 22-12-2015). MA application is to be mandatory for in vivo diagnostics, industrially manufactured bulks used in the preparation of NPPs and industrially-manufactured finished products, but will not be required for *bona fide* NPPs. Interestingly, manufacturers applying for MA for diagnostic allergen products or industrially manufactured bulks for NPPs will benefit from strongly reduced fees in Spain. In 2010, the national competent

authority in Italy requested the companies to submit updated parts of the product dossiers for each marketed allergen product. The aim is re-assessing the quality documentation and identifying products which are marketed as NPPs despite their production at serial industrial scale [27]. The assessment of the submitted data is ongoing (Lorenzo Montrasio, AIFA, personal communication, 21-12-2015). In the Netherlands, the Inspectorate started to enforce article 3.17 of the Regulation Medicinal Product Act from October 2009, which strictly regulates the dispensing of non-licensed NPPs to exceptional cases [28] (http://huisartsvandaag.nl/nieuws/27/Nieuws/16200/Beperking-markttoelating-en-vergoeding-allergeenpreparaten). The use of NPPs is evaluated annually to successively enforce the need of product registration and to stop evasion of the obligatory MA. Successively, also the coverage by health care insurers in the Netherlands was omitted for non-registered allergen products, at first for new patients and recently also for patients in treatment [29] (https://www.gipdatabank.nl/infoPagina.asp?naam=02-beleidsmaatregelen&bijlage=maatregelgeneesm_2014). In addition to these intra-EU variations, the regulation of allergen products in the EU and the USA is different [30], complicating the parallel commercialization of products in both markets.

Recent Developments in Allergen Quantification and Allergoid Characterization

Methods for Individual Allergen Molecule Quantification

The European Pharmacopoeia (Ph. Eur.) demands the control of allergen product potency using a validated assay [31••]. As outlined above, this requirement can be fulfilled either by determination of total allergenic activity or by control of individual, relevant allergen molecules. During characterization of an IHRP, both parameters have to be determined wherever possible. Mirroring this demand in conjunction with the high number of potentially clinically relevant allergens, the number of methods for allergen quantification is steadily increasing (Table 1). Many of the methods published in the last 5 years focus on the quantification of single major allergens, which are believed to be of great clinical importance given the definition that IgEs against a major allergen are present in more than half of the allergic patients [32]. However, also the number of assays for quantification of so-called minor allergens is growing. In parallel, various methods for detection of traces of allergenic foods in compound food products have been developed in recent years. Those allowing for quantification of single food allergen molecules on protein level have been included in Table 1, as they might be suitable for standardization of allergen extracts in the future.

Methods for Characterization of Allergoids

In contrast to clinical practice in the USA, the use of chemically modified allergen products, so-called allergoids, is popular in the EU. Chemical modification, e.g., via glutaraldehyde is thought to reduce IgE-mediated side effects in AIT while retaining immunogenicity. Only the underlying allergen extract but not the resulting allergoid can be validly assessed in IgE-based assays or other common analytical methods such as SDS-PAGE [62]. Consequently, allergoid analysis was usually limited to demonstrating the reduction of IgE reactivity. Since 2008, the *Guideline on Allergen Products: Production and Quality Issues* requests that retention of immunogenicity has to be demonstrated during pharmaceutical development of a chemically modified allergen product [63]. Immunogenicity is often verified in vivo via allergen-specific IgG production in either mice or rabbits immunized with the allergoid; a method not only conflicting with the 3R principles [64] (guiding principles for more ethical use of animals—replacement, reduction, and refinement) but also impaired by great variability. The guideline further requests that modified allergens have to be analyzed in a potency test allowing for discrimination of native and modified molecules based on immunoassays or other appropriate test methods. Furthermore, the guideline recommends that other techniques like MS or size exclusion chromatography (SEC) may be used to demonstrate consistency of the modification process. Despite these regulatory requirements, publications on allergoid-specific characterization methods are still proportionally scarce (Table 2).

Standardization of Allergen Products

Standardization Based on Total Allergenic Activity—Up to Date or Outdated?

Changes in the regulatory environment of allergen products have induced a modernization of production processes and promoted product-specific standardization. The current state of the art as well as the changes required due to new or revised regulatory documents have recently been summarized by Cárnes et al. [1••]. Product-specific standardization in the EU still keeps the traditional focus on skin testing for biological standardization in combination with IgE-binding potency for determining total allergenic activity in vitro, irrespective of its drawbacks. Firstly, both approaches depend on a unique patient population and their outcome is influenced by patient selection parameters. In addition, it is well known that the outcome of allergen skin testing is highly variable, depending, for example, on operators and test devices [74, 75]. Moreover, variability between sera pools remains great despite clear requirements for their preparation [63], e.g., due to geographical differences in sensitization patterns. Secondly, the determination of total allergenic activity provides no information on allergenic components or the ratios between them. Thirdly, the

Table 1 Methods for individual allergen molecule quantification published between 2011 and 2015

	Allergen	Allergen source	Method	Reference
WHO/IUIS-accepted allergens	Ara h 1	*Arachis hypogaea* (peanut)	Sandwich ELISA + immunosensor	[33]
	Ara h 1	*Arachis hypogaea* (peanut)	Amperometric magnetoimmunosensor	[34]
	Ara h 6	*Arachis hypogaea* (peanut)	Sandwich ELISA + voltammetric biosensing	[35]
	Asp v 13	*Aspergillus versicolor*	Capture ELISA	[36]
	Bet v 4	*Betula verrucosa* (birch)	Sandwich ELISA	[17]
	Bla g 1	*Blattella germanica* (German cockroach)	Antibody-based multiplex assay	[37]
	Bla g 2			
	Bla g 4			
	Bos d 4	*Bos domesticus* (cattle)	Competitive FLISA	[38]
	Bos d 5	*Bos domesticus* (cattle)	LC/SRM-MS/MS	[39]
	Bos d 10			
	Bos d 11			
	Bos d 12			
	Bos d 11	*Bos domesticus* (cattle)	UPLC-TQ-MS/MS	[40]
	Can f 4	*Canis familiaris* (dog)	Sandwich ELISA	[41]
	Cyp c 1	*Cyprinus carpio* (carp)	Competitive ELISA	[42]
	Gad m 1	*Gadus morhua* (cod)		
	Onc m 1	*Oncorhynchus mykiss* (rainbow trout)		
	Dau c 1.01	*Daucus carota* (carrot)	Sandwich ELISA	[43]
	Dau c 1.02		Competitive ELISA	
	Dau c 4			
	Gly m 4	*Glycine max* (soybean)	2DLC-UV/MS	[44]
	Gly m 4	*Glycine max* (soybean)	Sandwich ELISA	[45]
	Jug r 1	*Juglans regia* (walnut)	Competitive ELISA	[46]
	Pla a 1	*Platanus acerifolia* (London plane)	Competitive ELISA	[47]
	Pen a 1	*Penaeus aztecus* (shrimp)	Mast cell-based electrochemical biosensor	[48]
	Per a 9	*Periplaneta americana* (American cockroach)	Dot-blot ELISA	[49]
	Phl p 1/5	*Phleum pratense* (Timothy grass)	LC-MS/MS	[50]
	Sin a 1	*Sinapis alba* (mustard)	LC-MS/MS	[51]
	Sola l 3	*Solanum lycepersicum* (tomato)	LC-MS/MS	[52]
	Zea m 14	*Zea mays* (maize)	LC-UV/MS	[53]
Putative allergens	Chg47	*Chaetomium globosum*	Capture ELISA	[54]
	Gliadin	*Triticum aestivum* (wheat)	Liposomal fluorescence immunoassay	[55]
	NP24	*Solanum lycepersicum* (tomato)	LC-MS/MS	[56]
	Pch52	*Penicillium chrysogenum*	Capture ELISA	[57]
	Tropomyosin	shellfish (→ conserved IgE epitope peptide)	Sandwich ELISA	[58]
	Tropomyosin	mixture of *Penaeus monodon* (black tiger prawn), *Litopenaeus vannamei* (Vannamei prawn), *Fenneropanaeus merguiensis* (banana prawn), *Metapenaeus macleayi* (school prawn)	Sandwich ELISA	[59]
	Tropomyosin Arginine kinase	*Penaeidae* spec. *Chionoecetes* spec.	LC-MS/MS	[60]
	Xylanase	*Aspergillus niger*	Competitive ELISA	[61]

use of patient IgE limits the significance of the result to a certain set of allergen isoforms. Fourthly, total allergenic activity is quantified in the EU relative to a product-specific IHRP and products are labeled in manufacturer-specific potency units [76] which are incomparable. Last but not least, it needs to be highlighted that it remains unclear for AIT products whether or not there is correlation between IgE-binding capacity and therapeutic efficacy.

Standardization Based on Individual Allergen Molecule Content

The alternative to total allergenic activity and its many drawbacks is a change in focus towards individual allergen molecules. The main advantage is that allergenic proteins can be quantified independent of patient sera, either absolutely via

Table 2 Methods for characterization of chemically modified allergens and extracts published until 2015

Allergen source	Sample	Polymerization	Methods	Reference
Betula verrucosa	Extract	Glutaraldehyde	NanoLC-MS/MS HP-SEC light scattering dynamic light scattering	[65, 66]
Phleum pratense				
Dermatophagoides pteronyssinus				
6-grass pollen mixture	Extract	Glutaraldehyde	IgG inhibition ELISA	[67]
Betula verrucosa				
Dermatophagoides pteronyssinus				
Arachis hypogaea : Ara h 2	Purified allergen	Polyphenol oxidase	IgG inhibition ELISA	[68]
Gadus spec.: Parvalbumin	Purified allergen	Maillard reaction	LC-MS/MS	[69]
Dermatophagoides pteronyssinus	Extract	Glutaraldehyde	LC-MS/MS	[70]
Dermatophagoides farinae				
Dermatophagoides pteronyssinus	Extract	Glutaraldehyde	Mass spectrometry HPLC-SEC free lysine determination fluorescence spectroscopy	[71]
Dermatophagoides farinae				
Dermatophagoides pteronyssinus	Extract	Glutaraldehyde	IgG inhibition ELISA	[72]
Dermatophagoides pteronyssinus	Extract	Glutaraldehyde	IgG inhibition ELISA	[73]

mass spectrometry (MS) or, at present more commonly, relative to a reference standard in immunoassays like ELISA systems based on polyclonal or monoclonal antibodies. Importantly, monoclonal antibodies (mAbs) are available in reproducible quality and theoretically unlimited quantity, presenting a substantial advantage compared to the limited and variable resource of patient sera. Furthermore, determining the content of relevant individual allergens gives information on the ratios between these allergenic components and most immunoassays are indicative of allergen stability. Besides, total allergenic activity and major allergen content were shown to correlate in many allergen extracts [3, 17]. Nevertheless, to date, there are also limitations when standardizing the content of individual allergens based on immunoassays, since manufacturers usually use in-house established allergen-specific immunoassay. Consequently, the results of these different allergen-specific immunoassays are usually not comparable [77]. It will be possible to label product in comparable mass units at the earliest once validated standard immunoassays and standard reference preparations have been established. In addition, the high specificity of some mAbs to certain allergen isoforms presents an unambiguous weakness of immunoassays and may, in contrast to MS methods, result in underestimation of the true allergen content. However, apart from the expensive instrumentation, MS-based techniques require full sequence information of the relevant allergens, their isoforms, and variants in order to identify diagnostic peptides shared by preferably all allergen isoforms. Once this level of knowledge is reached, MS can provide isoform-independent, absolute results [62, 78•, 79–81] whereas allergen quantification in immunoassays is standard-, technique-, and reagent-dependent. Furthermore, it is usually not possible to analyze mixtures of allergen extracts from related species in immunoassays in spite of substantial cross-reactivity.

Finally, standardization of individual allergen molecules conform to the requirements of the Ph. Eur. presents a great challenge as preceding selection of the relevant allergen molecules is necessary. The most obvious candidates in terms of relevance are of course major allergens. However, while the current state of scientific knowledge suggests that, e.g., birch pollen extracts could be standardized only to Bet v 1, multiple major allergens have been described for other allergen extracts such as house dust mite. Moreover, the requirement of the *Monograph on Allergen Products* to control the content of each relevant allergen has deliberately not been limited to major allergens [31••]. However, it is understandable that the topic of potential additional control of minor allergens is commonly avoided. Regulation of allergen products aims at ensuring quality, safety, and efficacy [82], but the influence of minor allergens on either of these parameters is virtually unknown. Nevertheless, minor allergens should not be regarded as irrelevant *per se*. For example, it has been reported that sensitization to certain minor allergens is a risk factor for extensive cross-reactivities as well as for severe symptoms and asthma development [83–85]. Furthermore, it has been shown for several allergen products that their minor allergen content is often highly variable, both between batches of the same product and between products from different manufacturers [14, 15, 17, 86]. The influence of such differences on safety or efficacy is mostly unknown, but for one documented case: In Spain, a high content of the minor olive pollen allergen Ole e 9 has been associated with an increase in adverse events during AIT, leading to the introduction of a specification now

controlling the content of this minor allergen to ensure the safety of the product [86, 87]. However, until further studies provide the basis to estimate the relevance of minor allergens for safety and efficacy of AIT, standardization of individual allergens will focus on major allergens.

Activities Towards Cross-Product Comparability in the EU

As outlined above, the regulatory demand to determine the potency of an allergen product relative to a product-specific IHRP prevents cross-product comparability in the EU, although the need to enable a comparison between products of different manufacturers has been recognized decades ago. The history of standardization of allergen extracts has recently been comprehensively summarized in an entertaining review by Henning Løwenstein [4••]. It describes the first discussions on the topic of cross-product comparability, eventually resulting in the formation of the World Health Organization and International Union of Immunological Societies (WHO/IUIS) Allergen Standardization Committee in 1980. The committees' first major aim was the establishment of International Reference Preparations of allergen extracts. Although the reference extracts originating from this joint activity are still available at the National Institute for Biological Science and Control (NIBSC), they never became broadly accepted in the field, among other reasons due to a refusal of the FDA to adopt them. After this unfortunate start, the focus in the EU shifted away from total allergenic activity. All subsequent activities aimed at standardization based on individual major allergens via the establishment of standard immunoassays in conjunction with allergen standards. The first key project in this field was called "*Development of certified reference materials for allergenic products and validation of methods for their quantification*," in short "CREATE." It was initiated in 2001 and aimed at developing certified reference materials in combination with methods for quantification of allergens representing eight highly prevalent allergies [88–90]. Thus, eight recombinant major allergens were assessed for their suitability to become Ph. Eur. reference standards. For this reason, they were analyzed using various techniques like analytical SEC-HPLC, small-range x-ray scattering, circular dichroism spectroscopy, and tandem MS. In parallel, 21 allergen-specific candidate ELISA systems were investigated in at least three participating laboratories, respectively. For each allergen, at least one ELISA could be identified allowing for accurate quantification of the respective recombinant reference preparation as well as the native allergen in extracts. However, neither the production of reference standards in sufficient amounts nor the validation of the candidate ELISA systems could be completed until the end of CREATE in 2005.

The follow-up project, BSP090, is part of the Biological Standardization Programme of the European Directorate for the Quality of Medicines and HealthCare (EDQM) and centers on the two most promising candidates evaluated in CREATE: the major birch allergen Bet v 1 and the major Timothy grass allergen Phl p 5 [88, 91, 92]. The two recombinant proteins rBet v 1 and rPhl p 5a have been successfully characterized [93, 94•] and were adopted as the first allergen chemical reference substances (CRS) by the Ph. Eur. commission in 2012. Two years later, the two CRS have been introduced to the *Monograph on Allergen Products* [31••]. Given that neither of the two necessary standard ELISA methods has reached the level of full acceptance and public availability yet, the requirements in the monograph to use the two CRS are still rather vague and non-binding. The validation of two candidate Bet v 1-specific ELISA assays has been completed in 2014, after parallel assessment in 13 laboratories in a large ring trial followed by a post-study testing of birch allergen products in order to mimic the use as Ph. Eur. standard method (manuscript in prep.). Based on these results, one ELISA has been selected to be proposed as Ph. Eur. standard method and its commercialization is currently ongoing. Regarding Phl p 5, validation of the candidate ELISA in the large ring trial could unfortunately not be completed successfully. As reasons for the problems observed could not be identified *post hoc*, the Phl p 5-focused activities in BSP090 underwent a complete restart in 2014. Qualification of another candidate ELISA is currently ongoing as part of the preparation of a new ring trial.

Standardization of Allergen Products in the USA and Beyond

Similar to the EU, product-specific standardization in the USA focuses mainly on total allergenic activity. However, the FDA mandates the use of reference extracts in conjunction with standardized procedures, mainly competitive ELISA systems, resulting in the labeling of products in consistent units like bioequivalent allergy units (BAU/ml) or allergy units (AU/ml) [13••, 95–97]. In contrast, cat hair as well as short ragweed pollen extracts have to be standardized towards the major allergens Fel d 1 and Amb a 1 in a radial immunodiffusion assay. So far, 19 reference extracts and test procedures have been established, covering the most relevant allergen sources in the USA. Although this approach could provide the basis for cross-product comparability in the USA, the situation is complicated by the clinical practice. The standardized allergen extracts provided by the manufacturers are mixed, formulated, and diluted by the physician in order to administer a specifically tailored final product to the patient. Therefore, cross-product comparability in the USA is limited to the active substance. Nevertheless, it has been suggested that the adoption of reference extracts has increased the consistency of the available allergen extracts in the USA compared to the European market [2].

It is therefore not surprising that cross-product comparability is also advancing in other countries. For example, an extensive allergen standardization initiative has been launched in Korea in

2009, supported by the Korea Center for Disease Control and Prevention, with the aim to provide standards for the most relevant allergens on the Korean Peninsula [98, 99]. So far, seven allergen extracts have been investigated including house dust mite allergens, Japanese Hop, and German cockroach, but also characterization of recombinant proteins of more East Asian-specific allergens like Asian needle ant are currently in progress. Many other countries like Canada do not have their own allergen standardization initiatives but rather require standardization relative to already existing international reference standards when manufacturers apply for MA [100].

Cross-Product Comparability—An Outdated Topic?

In view of the various standardization activities around the world, it might seem that the general need of comparability between allergen products is beyond controversy, but indeed it has at times been suggested that cross-product comparability will become progressively obsolete [3, 4••, 101•]. The most popular reasons mentioned for this putative development are the advancements in product-specific standardization, the increase in state-of-the-art evidence for the clinical efficacy of single products, and the development of recombinant allergen products.

Undoubtedly, product-specific standardization has greatly improved the quality of allergen products in recent years, but ensuring consistency of quality attributes over shelf life and from batch to batch does not diminish the need for comparability and harmonization between products. Despite the large progress in total allergenic activity determination and quantification of individual allergen molecules, the resulting values remain incomparable. Hence, today, allergists cannot decide for one or the other allergen product based on comparing contents of active ingredients or potency.

This leaves the allergist with the option to decide for a product based on data from clinical studies. Again, the progress in this field in the last years is undisputable: Four allergen products for AIT have gained cross-national marketing authorization in Europe via mutual recognition (expansion of a national marketing authorization in the reference member state to additional EU member states) or decentralized procedures (parallel application in several EU member states for marketing authorization for a product without marketing authorization in the EU with one coordinating member state (reference member state)) so far. Dozens of established allergen products are currently reassessed to either confirm or optimize safety and efficacy in state-of-the-art clinical trials. And a scoring system for evaluation of symptoms and medication use has been recommended in an EAACI position paper [102] to finally move towards harmonization after decades of using various, incomparable clinical outcome measures [103]. It is therefore tempting to conclude that cross-product comparability is no longer needed if an allergist can decide for one or the other product based on sound clinical data collected in trials with harmonized design. However, even if all future clinical studies would use the proposed (but so far not validated) primary endpoint, comparability is still limited due to, e.g., differences in study populations, natural pollen exposure, statistical evaluation, and, last but not least, the definition of clinical relevance. Real comparability of clinical efficacy could be achieved by comparative head-to-head clinical trials in a randomized study population; ideally using allergen products assessed in parallel for major allergen content and/or total allergenic activity. However, such studies are beyond the scope of today's regulatory requirements and consequently scarce. All the more, the rare examples of a clinical head-to-head comparison [104•] in relation to a preceding parallel quantification of major allergen content in the products applied [105] are to be valued highly.

Certainly, cross-product comparability would become virtually unnecessary if biotechnological products like recombinant allergens or peptides were to take over the market of AIT products. Such products are of lower complexity compared to allergen extracts, contain a uniform active substance that is defined on the molecular level, and can be labeled in mass units of the active ingredient. Beyond, the possibility to create hypoallergenic recombinant allergen variants or peptides will eventually turn the regulatory focus away from IgE-based potency testing. Though this (quote) "biotechnology revolution" has been evolving for more than 15 years by now [106, 107], no such product has reached marketability yet and time will tell whether they will eventually replace extract-based allergen products. Until this day, cross-product comparability will remain an important goal to pursue.

Conclusions

In view of the complex composition of allergen extracts, controlling the quality of allergen products is of prime importance for safety and efficacy of allergy diagnosis and AIT. The basis of a standardized allergen product is formed by well-controlled reagents and source materials, followed by processing in a robust manufacturing process and quality control based on validated analytical methods. Promoted by the advancing development of assays for quantification of individual allergen molecules and characterization of allergoids, product-specific standardization has improved significantly in the last decade. However, products in the EU are still standardized with respect to IgE-binding potency determined by methods that differ between manufacturers and make use of product-specific IHRPs, preventing comparability. Cross-product comparability can only be reached by establishing well-characterized allergen references in conjunction with the respective standard methods, irrespective of the focus on individual allergen molecules or total allergenic activity. Although a given allergen product is nowadays much better controlled then in the past, only cross-product comparability

will enable allergists to select a product based on the content of active ingredients.

Compliance with Ethical Standards

Conflict of Interest Dr. Kaul reports non-financial support from European Academy of Allergy and Clinical Immunology and European Directorate for the Quality of Medicines and Health Care and grants from European Directorate for the Quality of Medicines and Health Care.

Dr. Vieths reports personal fees from the Food Allergy Resource and Research Program, Lincoln, NE, USA, personal fees from the Medical University of Vienna, Austria, grants from the Monsanto Company, personal fees from the American Academy of Asthma, Allergy and Immunology, personal fees from the Deutsche Dermatologische Gesellschaft, personal fees from the Westdeutsche Arbeitsgemeinschaft für pädiatrische Pneumologie und Allergologie e.V., Köln, Germany, personal fees from the Gesellschaft für pädiatrische Allergologie und Umweltmedizin, personal fees from the Ärzteverband Deutscher Allergologen, personal fees from the Swiss Society for Allergy and Immunology, personal fees from the Schattauer Allergologie Handbuch, personal fees from the Elsevier Nahrungsmittelaller-gien und Intoleranzen, personal fees from the Karger Food Allergy: Molecular Basis and Clinical Practice, and non-financial support from the German Research Foundation, from the Federal Institute for Risk Assessment, from the European Directorate for the Quality of Medicines and Health Care, from the European Academy of Allergy and Clinical Immunology , from the Deutscher Allergie- und Asthmabund, from the Association Monégasque pour le Perfectionnement des Connaissances des Médicins, from the Federal Office of Consumer Protection and Food Safety , from the German Chemical Society (GDCh), from the AKM Allergiekongress, and from the International Union of Immunological Societies. Dr. Vieths also reports grants from the European Directorate for the Quality of Medicines and Health Care.

Dr. Zimmer reports grants from the European Directorate for the Quality of Medicines and Health Care.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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