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GUIDELINE ON ALLERGEN PRODUCTS: PRODUCTION AND QUALITY ISSUES

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This Guideline replaces the Note for Guidance on "*Allergen products*" (CPMP/BWP/243/96).

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GUIDELINE ON ALLERGEN PRODUCTS: PRODUCTION AND QUALITY ISSUES

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EXECUTIVE SUMMARY

This Guideline lays down the quality requirements for allergen products of biological origin, including allergen extracts from natural origin and allergens produced through recombinant DNA technology, used for specific immunotherapy (SIT) or *in vivo* diagnosis of immunoglobulin E (IgE)-mediated allergic diseases.

1. INTRODUCTION (background)

Type I allergy is an adverse and vigorous reaction of the immune system to distinct and normally harmless environmental substances (allergens), which results in inflammatory reactions, including the release of histamine, cytokines and other mediators induced by allergen specific IgE antibodies, and the *de novo* synthesis of inflammatory mediators. Allergies are one of the most prevalent diseases in Europe. While test allergens are an important part of clinical allergy diagnosis, specific immunotherapy with allergen products containing the same antigens is an immuno-modulatory treatment option which is intended to generate persistent relief from allergy symptoms.

The previous Note for Guidance on "*Allergen Products*" (CPMP/BWP/243/96) as well as the Ph. Eur. Monograph on Allergen Products (07/2007:1063) address the technical quality of allergen products that are based on allergen extracts. In recent years, more and more allergens have been generated by using recombinant DNA technology. Such recombinant proteins have been evaluated as novel therapeutic products in clinical trials on specific immunotherapy.

2. SCOPE

This document provides principles and guidance for the manufacturing and quality control of allergen products of biological origin, including allergen extracts from natural origin and allergens produced through recombinant DNA technology, used for specific immunotherapy (SIT) or *in vivo* diagnosis of immunoglobulin E (IgE) mediated allergic diseases. It applies to all allergen products and their intermediates manufactured by a method involving an industrial process as defined by Directive 2001/83/EC, as amended.

Allergen products are obtained from allergen extracts, allergoids, conjugates or allergens manufactured using recombinant DNA technology. This guideline does not cover allergenic preparations consisting of synthetic peptides, DNA or RNA constructs and/or cell preparations or low molecular weight chemical allergens.

This document also provides guidance on the establishment and use of in-house reference preparations (IHRP), which are used for quality control including the analysis of batch-to-batch consistency. Moreover, criteria for the preparation of the serum pools used for potency measurements are defined.

3. LEGAL BASIS

This Guideline should be read in conjunction with the introduction and general principles and Part II of Annex I of Directive 2001/83/EC, as amended.

4. MAIN GUIDELINE TEXT

4.1. General concepts

4.1.1. Homologous groups

Due to the high number of allergens in an allergen extract or in an allergen extract mixture and the cross-reactivity of the individual components, it is impossible to determine all relevant parameters for the allergens within a given extract or a defined allergen extract mixture. Therefore, in the previous Note for Guidance on "*Allergen Products*" (CPMP/BWP/243/96), extrapolation of stability data among members of taxonomic families were defined in a very broad sense and used by applicants. The concept of homologous groups introduced here replaces the concept of taxonomic families. This new concept limits the extrapolation to groups defined and justified by scientific criteria, restricts extrapolation to a few parameters while at the same time it retains the flexibility needed.

Allergen extracts prepared from different species, different genera or different families, and finished products which are derived from these allergen extracts and for which clinical experience already exists may be grouped into homologous groups.

The grouping should be based on the following criteria:

- Comparable physicochemical and biological properties of the source material
- Cross-reactivity/structural homology of the allergens
- Identical formulation of the finished product
- Identical production process of the allergen extract and of the finished product.

One member of a homologous group is selected as the representative allergen. This choice should be justified, taking into consideration for example geographical differences in the sensitization patterns and other relevant factors.

To a limited extent data on stability, safety and efficacy can be extrapolated from the representative allergen to other members of the homologous group. For allergens that cannot be included into one homologous group, the data for quality, safety and efficacy have to be provided on a single-product basis.

Detailed safety studies are only requested for the representative allergen, while post-marketing safety reports will be requested for non-representative allergens of the same group. Extrapolation of clinical data is addressed in the separate EWP guideline[1].

Accepted homologous groups are listed in Annex I. If justified, the applicant may define other groups or introduce new members into an existing group provided the above-mentioned criteria are fulfilled.

4.1.2. Allergen extract mixtures

Allergen extract mixtures have to be prepared from individual extracts from single source materials. Therefore, the different source materials should not be mixed prior to extraction. Since extracts are considered as active substances (see section 4.2), each individual extract should be considered as an active substance of its own. Potency testing should be performed for each individual active substance prior to mixing or on the first homogeneous mixture if it is not possible at the finished product level. If the testing of the individual active substances in the finished product is not possible due to cross reactivity of the constituents, the total potency of the finished product should be determined by an IgE-inhibition test.

The number of allergen extracts in a mixture should be kept to a minimum regardless of homology and cross-reactivity of the individual allergens. Combinations containing more than five individual allergen extracts should be justified. If in a mixture the allergens do not belong to the same homologous group, the combination of the components has to be justified.

The following issues should be taken into consideration:

- Allergens with proteolytic activities should not be used in mixtures unless justified.
- Perennial and seasonal allergens should not be mixed.
- Hymenoptera venoms should not be mixed with any other allergens. Venoms from different species should not be mixed.

4.1.3. Comparability

The development of an allergen product might involve changes in the manufacturing process which have impact on the finished product. Given its complex nature, it is particularly important that all stages of the development process are fully evaluated and tracked within the dossier where applicable. Applicants should take into consideration the step-by-step approach according to EU guidance[2] considering not only the characterisation studies at the level of the active substance, but also the validation of the manufacturing process as well as in-process controls and stability data.

Comparability studies must be performed if the changes have been introduced after initiation of the critical studies (for example stability, non-clinical or pivotal phase II/III studies). If the changes were introduced at very early stages of development, no comparability studies have to be performed.

4.2. ACTIVE SUBSTANCE

4.2.1. General information

The active substance can be an unmodified allergen extract, an allergoid, a conjugate as well as a purified natural or recombinant protein. Preferably, the active substance is a stable preparation at the latest step before mixing or formulation. In general, adsorption and addition of excipients are considered as formulation steps.

Allergen extracts mainly consist of proteins and glycoproteins and contain various major and minor allergens as well as non-allergenic components. Because of the intrinsic variability of the natural source material, concentrations of individual allergens in such extracts may vary and standardisation is therefore very important. Active substances obtained by recombinant DNA technology consist of pre-defined allergenic polypeptides, for example a major allergen, or a mixture of defined polypeptides. The quantity and structure of these polypeptides can be determined and these products should be standardised like other biological products consisting of purified proteins.

4.2.2. Manufacture

4.2.2.1. Manufacture of the active substance derived from natural source materials of biological origin

The production process including pre-treatment, extraction, filtration, dialysis or concentration should be described in detail and validated. The in-process control methods including the corresponding acceptance criteria should be reported. For better illustration, a step-by-step diagram (flow-chart) indicating all process steps, including the relevant in-process controls, should be presented. If aseptic precautions are introduced, these should also be indicated in the flow-chart. In case of modified allergen extracts such as allergoids or conjugates, the modification processes should be described. Intermediates in the process should be identified and controlled.

4.2.2.2 Manufacture of recombinant allergens

In contrast to allergen preparations obtained from natural source materials of biological origin, the quality of individual allergen batches obtained by recombinant DNA technology does not vary according to the properties and quality of the individual source materials, but depends on the cell systems used, fermentation processes and purification procedures. Therefore, a detailed characterisation of the cell lines used and the manufacturing process is required. This includes but is not restricted to a detailed examination of the expression constructs in the cell lines as described in the relevant regulations.

For the production of recombinant allergens, all guidelines for products derived from recombinant DNA technology (e.g.[3-6]) have to be taken into consideration.

4.2.3. Control of materials

This section includes starting (source) materials (for example natural source materials from biological origin for allergen extracts and cell substrates for the production of recombinant allergens) and raw materials (for example solvents and diluents for extraction, media for the cultivation of mites, moulds and media and reagents for production of recombinant allergens). When substances of animal or human origin are used as source materials or as raw materials, viral safety[7, 8] and compliance with TSE requirements[9] should be demonstrated. Traceability should be provided for these materials.

4.2.3.1. Control of source materials for allergen extracts

The name(s) and address of the supplier(s) of the allergenic source material should be stated. The description of the allergenic source materials should contain all relevant details. The name (scientific name, for example genus and species as well as any common name), and type (e.g. pollen, insect venoms, pelt, dander, saliva) of the allergenic source material(s) should be stated. Details concerning the cultivation, collection, pre-treatment and storage should be supplied for each separate source material. Whenever purification steps (for example defatting) or other treatments are performed by the supplier of the source material, these activities have to be mentioned and justified; moreover

acceptance limits have to be defined. The quality control of source materials should be documented. Acceptance criteria and control methods for the source material(s) should be included. They should encompass requirements and control methods relating to identity and purity. The acceptance criteria should ensure the consistency of the allergenic source material from a qualitative and quantitative point of view. The source materials should be stored and transported under controlled conditions justified by stability data. If source materials from different suppliers and deliveries are mixed to achieve uniform source material batches, the underlying concept should be described. Uniformity of the source material from different origins should be justified.

Each individual source material has to be qualified regardless of whether it belongs to the same homologous group.

Additional requirements for specific source materials:

Pollens

Geographic location and nature of the fields and seeds used, field characteristics, treatments, visual control, way of collection and random sampling procedures should be described. The variety of the plants used should be given including transgenic plants if used. The use of transgenic plants has to be justified. The principles of the Guideline on Good Agricultural and Collection Practice (GAPC) for Starting Material of Herbal Origin [9] and other relevant guidelines for the source material of plant origin should be followed where applicable[10, 11, 12]. Tests methods and acceptance criteria for the identification and determination of the content of impurities such as foreign pollen, mould spores, extraneous plant material from the same species and non-related contaminations should be included.

The content of relevant pesticides, heavy metals and solvents should be monitored in order to demonstrate that their level is kept at a minimum in the allergenic source material.

The content of pollen from other species should be limited to 1% of mixed pollens and 0.5% of one individual pollen as determined by a microscopic particle count. Detectable mould spores should not exceed 1%[13]. The contamination with particles of plant origin, other than pollen, should be kept to a minimum. The maximum allowed contamination should be justified.

Moulds

The strain(s) of moulds used should be specified. Morphology and other parameters for characterisation and identification (for example biochemical or genetic properties) as well as the cultivation method and the kind of source material harvested (mycelium and/or spores) should be specified. The cultivation method should be validated and evidence should be provided that no mycotoxins are produced. Details on the composition of the cultivation medium and the media components should be submitted. Synthetic media i.e. media free of animal-derived material or allergen-free media should be preferably used.

Strains which produce mycotoxins such as aflatoxins or ochratoxins should not be used unless justified and their mutagenic potential should be evaluated. In this case, the amount of relevant mycotoxins should be quantified before processing and its removal through processing should be implemented and validated. Appropriate measures have to be implemented to avoid contamination by other mould strains.

Mites

The strain of mite should be specified. Morphology and other parameters (for example biochemical or genetic properties) for the identification of the mites should be specified.

The cultivation method and the composition of the cultivation medium as well as the media components should be described. Details on the composition of the cultivation medium and the media components should be submitted. Synthetic and consequently free of animal derived material allergen-free media should be preferably used. The conditions of culture and the time of harvest must be validated. It should be indicated which part of the culture is used for further processing, e.g. mites, mite faeces only or the whole mite culture.

Animal Allergens

Only healthy animals should be used. When killed animals are used, the source materials should be collected within a few hours after death and the dead animals should be stored under conditions that maintain the quality of the source material. Morphology and other parameters used for the identification of the allergenic source material should be described.

The composition of the source material (for example hair, pelt, epithelium, saliva or urinary fluid) should be indicated. Any possible contamination with mites and moulds for example should be addressed and avoided. The collector should certify that the animals used have not recently been treated with antiparasitics or other drugs.

The collection of hair and dander must be performed without injuring the skin of the animal. Methods employing the grinding of whole skin and /or pelts must not be used.

The storage conditions should be described.

Hymenoptera Venoms

The morphological characteristics and other parameters of characterisation should be specified. The collection method of venom from hymenoptera species should be described. The content of relevant pesticides should be monitored and acceptance criteria should be defined.

4.2.3.2. Control of source materials used for the manufacture of recombinant allergens

For recombinant allergens, all relevant guidelines have to be considered.

4.2.3.3. Control of raw materials

For each raw material, the specifications, information on its source and justification for its use should be provided.

If any allergenic components are used in the culture medium, their removal should be demonstrated

4.2.4. Characterisation and control of the active substance

4.2.4.1. Characterisation and control of allergen extracts

Characterisation and quality control of allergen extracts should be performed on the active substance level. If certain control tests cannot be applied to the active substance, for example allergoids, testing at intermediate stages rather than at the active substance stages may be appropriate and acceptable if justified. In such circumstances, quality specifications should be defined for the product just prior to the modification or dilution step and test results should be considered as in-process acceptance criteria and included in the specifications of the active substance. Generally, the following tests and acceptance criteria are applicable: appearance and description, identity, purity and impurities, potency, and quantity.

The allergens relevant for the product have to be defined by the manufacturer. It should be demonstrated that the manufacturing process is able to maintain these allergens by proving their presence using appropriate methods such as antibody based techniques or mass spectrometry. The content of relevant allergens should be measured by validated assays using certified reference standards or biological reference preparations and assays validated in international standardisation programmes whenever possible. The protein profile should correspond to that of the IHRP and the presence of the relevant allergen components be verified wherever possible. The choice of the relevant allergen components subject to determination must be justified. If safety concerns arise from other (for example minor) allergens, these have to be quantified as well.

The manufacturer should demonstrate its capability to obtain batch-to-batch consistency and provide a justification for the selected and validated test procedures.

4.2.4.2. Characterisation and control of recombinant allergens

Emphasis should be put on the structural integrity, post-translational modifications and the consistency of folding since these factors may influence the immunogenic properties and safety in specific immunotherapy. The intact biological function (for example physiological function as plant enzyme) of an allergenic protein derived from recombinant DNA technology may serve as an indirect indicator

of structural integrity but is not an essential property determining allergenicity or immuno-modulating activity. Therefore, the demonstration of biological function may not be necessary for recombinant allergens.

Special attention should be given on impurities from the media or host cell components. These impurities should be identified and quantified and their potential in giving rise to undesirable and potentially allergic reactions should be estimated.

Recombinant allergens should be characterised and quantified by techniques appropriate for recombinant proteins. The content should be expressed in weight per volume whenever possible. The correlation between the quantity of the individual recombinant allergens and the corresponding biological (for example allergenic) activity should be shown in validation studies. For recombinant allergen molecules, ELISA methods with specific animal antibodies may be used as potency assays as long as a correlation with the IgE binding has been demonstrated. For recombinant allergens with a reduced IgE reactivity, potency tests should preferably consist of a discriminatory test to distinguish between molecules with high and low IgE binding capacities (for example by quantification in ELISA systems) and an assay to determine the lack of IgE reactivity.

For mixtures of different recombinant allergens, the content of the individual allergens should be determined by adequate quantification methods, for example ELISA just prior to mixing and in the mixture, unless otherwise justified.

4.2.4.3. Characterisation and control of modified allergen preparations

For modified allergens (for example denatured or chemically-modified allergoids or conjugates), antibody-based assays or other appropriate test methods have to be established to identify the relevant allergens in the modified form. Other assays should be used to analyse the expected modification of the allergens and for the characterisation of the modified allergens, and to demonstrate consistency of the modification process, for example by peptide mapping by mass spectrometry, or size-exclusion chromatography to determine the degree of polymerisation.

4.2.4.4. Potency assays

The following potency tests should be performed for the different kinds of active substances:

- For allergen extracts and purified allergens without structural modification, the IgE inhibition test should be performed.
- Relevant individual allergens may be determined by immunological methods (for example ELISA) with specific animal antibodies as long as a correlation with the IgE binding has been demonstrated.
- For allergoids, potency tests should consist of a discriminatory test to distinguish between native and modified molecules (for example by quantification in ELISA systems or mediator release assay) and an assay to determine the lack of IgE reactivity. As an alternative to a discriminatory immunoassay, other techniques (for example mass spectrometry) may be used to demonstrate the presence of the relevant allergens.
- For conjugates, the potency testing should consider the immuno-modulating properties of the specific modifications.

In addition to the potency, the relevant individual allergens should be identified and the content of major allergens should be measured whenever possible using certified reference standards or biological reference preparations and approved assays. If safety concerns arise from other (for example minor) allergens, these have to be measured as well.

4.2.5. Stability

If the active substance is stored, stability data should be obtained according to the relevant guidelines to provide information concerning the allowed maximum storage period. The general principles of the ICH Q1A guideline[14] are valid for allergen products. For well-characterized proteinaceous allergens (for example recombinant allergens) ICH Q5D guideline is applicable. The stability studies should be based on real time studies.

Regarding the homologous groups, a full set of data should be presented for the "representative" allergen of the particular homologous group. For the "non-representative" allergens, stability studies may be performed on an ongoing basis for the overall shelf life of the active substance. If these data are not available at the time of submission of a marketing authorisation application, a commitment should be made to continue the stability studies after approval. The marketing authorisation application should contain a detailed protocol of the stability studies of the "non-representative" allergens. If justified, some stability data may be extrapolated from the "representative" allergen. The extrapolation of the results from the "representative" allergen" should be discussed and justified, taking into account data concerning the activity of those enzymes (such as proteases) which might impact on the structure of the individual molecules. In case of mixtures of members of different homologous groups, an extrapolation is not acceptable.

4.3. STANDARDS AND REFERENCE MATERIALS

In-House Reference Preparations (IHRP):

Individual company's established IHRP should only be used as long as no official standards with confirmed and monitored content of the major allergens and potency are available.

Allergen extracts are potentially different between manufacturers and, due to the variability of biological source materials, may even vary to a certain extent from one batch to another within a single company. These characteristics represent a problem with regard to any further harmonisation between products from different manufacturers. At least an appropriate batch-to-batch consistency has to be reached by a company within its production runs by introducing an IHRP, which should be used as internal reference preparation, and using a number of biological and analytical procedures. The IHRP is derived from a production run following the manufacturing process as defined in the dossier; it establishes a reference point against which extracts from all future production runs will be compared. Therefore, the qualitative and quantitative composition of regular production batches should match the IHRP.

The IHRP should be characterised using available relevant methods and its specific allergenic activity shall be established. Data should be provided on protein and, whenever possible, carbohydrate composition. The relevance of glycoproteins for the IgE-binding should be considered. The presence of all relevant allergens in the IHRP should be demonstrated.

Some of the following methods should be applied for biochemical and structural characterisation: isoelectric focusing, determination of the distribution of molecular weight by SDS-PAGE, capillary electrophoresis, chromatographic techniques, mass spectrometry or other appropriate techniques. As far as possible, individual allergens should be identified using specific antibodies or other techniques and the internationally accepted allergen nomenclature should be used.

The concentration of the relevant individual allergens should be determined if possible using certified reference standards or biological reference preparations and assays validated in international standardisation programs.

Information regarding the allergenic properties of the proteins in the IHRP may be obtained from experiments involving immunoblotting techniques using patient pool serum. Sera from individual patients can be used to obtain an allergogram. The potency of the IHRP should be determined by immuno-assays (for example IgE-inhibition or cellular mediator release assays) and expressed in terms of units of biological activity. In addition, the T-cell proliferation capacity may be analysed.

The IHRP should be biologically standardised by appropriate methods on the basis of skin test reactivity [Turkeltaub[15] and Nordic Council of Medicines[16]]. In case not enough patients are available for *in vivo* standardisation, standardisation of the IHRP by *in vitro* methods may be justified. The stability of the IHRP and the storage conditions should be documented.

If a new batch of an IHRP for the same allergen has to be established, both the old and the new IHRP should be tested in parallel to avoid a shift of the biological activity. This standardisation should be performed using a predefined set of *in vitro* methods, but *in vivo* standardisation procedures may also be included. A detailed description of the protocol should be provided. In general, the results of the quantitative assays should be in agreement with the results of the previous batch of the IHRP. In case

of deviating results, a correlation factor should be established. Trending analysis has to be performed to avoid a shifting of quality parameters.

Sera Pools:

Specific sera pools should be established for batch control and for the qualification of individual IHRP. The problem of geographically different sensitisation patterns should be taken into consideration in the preparation of the pools. For the used sera, the frequency of IgE-recognition of different allergens as well as the content of allergen-specific IgE antibodies and the clinical relevance of sensitisation should be taken into account when preparing the pool. The pool should be composed of sera from 10 to 15 individuals. Sera recognising carbohydrate epitopes and sera from patients who had a previous SIT treatment with the respective or cross-reactive allergen should not be included in the pool. In addition, sera containing IgE antibodies against bovine serum albumin, milk proteins or gelatin could cause experimental problems and should therefore be avoided in the pool.

Specifications should be set for the sera pool, including criteria for the reactivity profile of the pool. Prior to use, the adequate quality of the pooled sera should be demonstrated in appropriate control experiments. This should include the demonstration that the relevant allergens are recognised by the pools.

4.4. FINISHED PRODUCT

4.4.1. Description and composition of the finished product

A detailed description of the finished product should be given. If the finished product consists of a mixture of active substances, a complete list of all the active substances used should be given. In general, adsorption and addition of excipients are regarded as formulation, and these steps should be described in the manufacturing process of the finished product.

4.4.2. Manufacture

The production process should be described in detail, including process scale. A step-by-step diagram (flow-chart) should be presented, indicating all process steps and including the relevant in-process controls. If aseptic precautions are introduced, this should also be described and indicated in the flow-chart. Any allowed process holding times should be identified and justified. Description, documentation, and results of the validation of the manufacturing process should be provided.

If further adsorption or modification steps are performed, these manufacturing steps have to be described in detail and reported in the flow-chart. The purpose of these steps should be explained. In addition, tests should be carried out to demonstrate the success of these activities and the consistency of production.

In case human plasma derived materials are used during the manufacturing process or as excipients / stabilizers in the formulation of allergen finished products, the quality and safety with respect to transmissible agents have to comply with the current guideline on plasma-derived medicinal products[17].

4.4.3. Control of finished product

Appropriate specifications should be set for the finished product. If any of the control tests (e.g. potency tests) cannot be performed on the finished product, specifications should be defined for the intermediate at the latest stage prior to the modification step. In such cases, test results should be considered as acceptance criteria and included in the specifications of the finished product. In addition, aspects described in previous parts of this guideline that are also relevant to the control of the finished product should be taken into account.

Control of non-modified allergen preparations:

IgE-binding tests are required for the standardization and batch control of finished products containing non-modified allergens. Consequently, the labelling should be indicated in potency units. If test systems validated in international standardisation programs are available for the quantification of individual allergens, these should be applied. In that case, the content in weight per volume of the

individual allergens should be included in the specifications of the finished product and should be indicated in the SPC in addition to potency. If safety concerns arise from individual minor allergens, these have to be measured as well.

Control of allergen mixtures:

For allergen mixtures, potency testing should be performed for each individual allergen active substance in the mixture. If the testing of the individual active substances in the finished product is not possible due to cross reactivity of the constituents, the total potency of the finished product should be determined by an IgE-inhibition test.

Control of adsorbed products:

For adsorbed products, the stability of the adsorption has to be determined by measuring the amount of total soluble protein and/or the presence of IgE-binding components in the supernatant or by using other relevant methods at release and at the end of the shelf life period.

Control of recombinant allergens:

Finished products containing recombinant allergens have to comply with the guideline ICH Q6B[6]. The content of the purified protein (for example major allergen) and the potency as described in chapter 4.2.4.4 should be determined.

Non-standardised allergen extracts:

Certain allergens cannot be fully standardized because sufficient numbers of patients are not available for biological standardisation and to create an appropriate sera pool, such as in rare allergies. In this case, a range of *in vitro* methods such as determination of an antigen profile, protein profile and the content of total protein and individual allergens may be applied for the control of the finished product.

4.4.4. Container closure system

The container closure system(s) used for the various strengths should be described in detail. Additionally, all other parts of the final medicinal product including for example solvents for reconstitution or syringes have to be described.

4.4.5. Stability

Stability testing should be performed as real-time stability studies as indicated in the relevant guidance documents (e.g. [4, 14]). For allergen extracts belonging to the same homologous group, a full set of stability data has to be provided for the representative allergen. For the non-representative allergens some stability data may be extrapolated from the "representative" allergen. Therefore, only a limited number of parameters may be tested in these studies. The applicant should justify the choice of these parameters. The extrapolation of the results from the "representative allergen" should be discussed and justified. This justification should include experimental evidence that the relevant enzymatic pattern of the non representative allergens is equivalent to the enzymatic pattern of the representative allergen. The data for the non-representative allergens may be obtained in ongoing real-time stability studies after granting of a marketing authorisation. If the data are not available at the time of submission of a marketing authorisation, a commitment should be made to continue the stability studies after approval. The marketing authorisation application should contain a detailed protocol of the stability studies of the "non-representative" allergens.

If the finished product consists of a mixture of allergen extracts not belonging to the same homologous group, stability studies have to be performed for the mixture considering each individual active substance. If the individual extracts in a mixture belong to the same homologous group and therefore cross-reactivity occurs between the relevant allergens, it may not be possible to determine the activity of the individual active substances. In such cases (for example a mixture of grass pollen extracts), an overall potency testing by IgE inhibition may be appropriate. The selected testing strategy should be described in detail and justified by the applicant.

For allergen extracts, stability studies of finished products manufactured with active substance at the end of its shelf-life should be performed on an ongoing basis after marketing approval.

For adsorbed products, the stability of the adsorption and /or modification has to be proven at the end of the shelf life by testing the total amount of soluble protein in the supernatant and/or by determining the presence of IgE-binding components in the supernatant or by using other relevant methods. In order to prove the stability of products containing native and modified allergens, mediator release assays (for example with mouse IgE and rat basophil leukaemia cells) may be considered as potency tests.

If it is not possible to perform potency tests, for example in the case of adsorbed material, *in vivo* immunogenicity tests or validated alternative *in vitro* tests should be performed at the beginning and end of the stability study to provide evidence on the stability of the product.

DEFINITIONS

- An **allergen** is a molecule capable of inducing an IgE response and/or a Type I allergic reaction.

- **Allergen extracts** are extracts from **natural** biological source materials containing a mixture of allergenic and non-allergenic molecules.

- **Allergen products** are medicinal products containing allergens or derivates of allergens for the purpose of *in vivo* diagnosis or treatment of allergic diseases.

- **Major/minor allergens** are allergens, against which at least 50% (major allergens) or less than 50% (minor allergens) of the patients tested have allergen-specific immunoglobulin E (IgE) antibodies.

- **Relevant allergens** are allergens causing a clinically relevant effect in a significant proportion of the allergic patients.

- **Allergoids** are allergens which are chemically modified to reduce IgE reactivity.

- **Conjugates** are allergens, which are covalently coupled to other molecules to modulate their immunological properties.

- **Homologous groups:** Allergen extracts prepared from different species different genera or different families and finished products derived from these allergen extracts may be grouped in homologous groups based on the composition and the physiochemical biochemical properties of the source material, the cross-reactivity/structural homology of allergens, the formulation of the finished product and the production process of the allergen extract and of the finished product.

REFERENCES (scientific and / or legal)

Some of the Guidelines for biological and biotechnology derived products mentioned below and other relevant Guidelines are available at the following address on the EMEA website:

http://www.emea.europa.eu/htms/human/humanguidelines/biologicals.htm

(1) Draft Guideline on the Clinical Development of Products for Specific Immunotherapy for the Treatment of Allergic Diseases (CHMP/EWP/18504/2006)

(2) Note for Guidance on Biotechnological/Biological Products Subject to Changes in their Manufacturing Process (ICH Q5E; CPMP/ICH/5721/03)

(3) Note for Guidance on Quality of Biotechnological Products: Analysis of the Expression Construct in Cell Lines Used for Production of r-DNA Derived Protein Products (ICH Q5B; CPMP/ICH/139/95)

(4) Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products (ICH Q5C; CPMP/ICH/138/95)

(5) Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products (ICH Q5D; CPMP/ICH/294/95)

(6) Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products (ICH Q6B; CPMP/ICH/365/96)

(7) Note for Guidance on Quality of Biotechnological Products: Viral Safety Evaluation of Biotechnology Products derived from Cell Lines of Human or Animal Origin (ICH Q5A; CPMP/ICH295/95)

(8) Note for Guidance on Virus Validation Studies: The Design, Contribution and Interpretation of Studies validating the Inactivation and Removal of Viruses (CPMP/BWP/268/95)

(9) Note for Guidance on Minimising the Risk of Transmitting Animal Spongioform Encephalopathy Agents via Human and Veterinary Medicinal Products (EMEA/410/01)

(10) Guideline on Good Agricultural and Collection Practice (GAPC) for Starting Materials of Herbal Origin (EMEA/HMPC/246816/2005)

(11) Guideline on Specifications: Test Procedures and Acceptance Criteria for Herbal Substances, Herbal Preparations and Herbal Medicinal Products/Traditional Herbal Medicinal Products (CPMP/QWP/2820/00 Rev 1)

(12) Guideline on Quality of Herbal Medicinal Products/Traditional Herbal Medicinal Products (CPMP/QWP/2819/00 Rev 1)

(13) Ph. Eur. monograph on Allergen Products (07/2007:1063)

(14) Note for Guidance on Stability Testing: Stability Testing of New Drug Substances and Products (ICH Q1A (rev. 2); CPMP/ICH/2736/99)

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(17) Note for Guidance on Plasma-Derived Medicinal Products (CPMP/BWP/269/95)

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ANNEX 1

Accepted homologous groups [Lorenz[18]]

1. Tree pollen

The 'birch group'

*Betula verrucosa = B. pendula** = *B. alba* European white birch

Alnus glutinosa Alder

Carpinus betulus Hornbeam

Corylus avellana Hazel

Quercus alba Oak

Suggested representative allergen source: Birch[+]

* Correct taxonomic name according to NCBI taxonomic database

The group of *Oleaceae*

Olea europaea Olive

Fraxinus excelsior Ash

Ligustrum vulgare Privet

Syringa vulgaris Lilac

Suggested representative allergen sources: Olive or Ash[+]

The group of *Cypressaceae*

Juniperus sp. Cedar

Cupressus sp. Cypress

Suggested representative allergen sources: Cedar or Cypress[+]

Non-grouped species within tree pollen species. Justification required.

Fagus sylvatica European beech

Acer sp. Maple

Platanus sp. Plane tree

Populus sp. Poplar

Robinia pseudoacacia False acacia, Locust tree

Salix sp. Sallow / Willow

Tilia sp. Linden / Lime tree

Ulmus sp. Elm

Cryptomeria japonica Japanese Cedar

2. Grass and cereal pollen

The group of sweet grasses of the *Poaceae* (*Gramineae*) family, subfamily of *Pooideae*

Anthoxanthum odoratum Sweet vernal grass

Avena sativa Oat
Dactylis glomerata Orchard grass/Cocksfoot
Festuca sp. Meadow fescue
Holcus lanatus Velvet grass/Yorkshire fog
Hordeum vulgare Barley
Lolium perenne Perennial ryegrass
Phleum pratense Timothy grass
Poa pratensis Kentucky bluegrass
Secale cereale Cultivated rye
Triticum aestivum Cultivated wheat
Suggested representative allergen sources: Timothy grass, Orchard grass or Kentucky Bluegrass[+]

Additional grass species belonging to the homologous group of *Pooideae* with reservations
Agropyron sp. Couch grass, Crested wheatgrass
Agrostis sp. Bent grass
Alopecurus pratensis Meadow foxtail
Arrhenatherum elatius False oat
Bromus sp. Brome grass
Suggested representative allergen sources: Timothy grass or Kentucky bluegrass[+]

Non-grouped grass pollen species. Justification required.
Cynodon dactylon Bermuda grass
Cynosurus cristatus Dogstail

3. Weed pollen
The group of weed pollen species
Ambrosia artemisiifolia, Ambrosia trifida Ragweed
Artemisia vulgaris Mugwort
Parietaria judaica, Parietaria officinalis Pellitory
Suggested representative allergen sources: Ragweed or Mugwort, but not Pellitory[+]

Non-grouped weed species. Justification required.
Plantago sp. Plantain

4. Mites
The group of house dust mites of the *Dermatophagoides* genus
Dermatophagoides pteronyssinus
Dermatophagoides farinae

Non-grouped mite species. Justification required.

Acarus siro flour mite

Glycyphagus domesticus house mite

Lepidoglyphus destructor house mite

Thyreophagus entomophagus flour mite

Tyrophagus putrescentiae storage mite

5. Insect venoms

No homologous groups formed. Justification required.

6. Allergen extracts derived from vertebrates

Extracts such as animal epithelia, hair, dander.

No homologous group formed. Non-grouped species. Justification required.

Canis familiaris Dog

Felis domesticus Cat

Cavia porcellus Guinea pig

Cricetus cricetus Hamster

Equus caballus Horse

Mus musculus Mouse

Oryctolagus cuniculus Rabbit

Rattus sp. Rat

7. Moulds

No homologous group formed. Justification required; in case of justification of grouping of mould species, special emphasis on similar stability is necessary.

$^{+}$: The relevance of geographical differences should be taken in consideration for the individual products