SPECIFIC REQUIREMENTS FOR THE PRODUCTION AND CONTROL OF ALLERGEN PRODUCTS

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Additional Notes	This note is intended to provide guidance on the type of data which should be included in applications for marketing authorisation for allergens. It is intended to supplement Directive 81/852/EEC, as amended by Directive 92/18/EEC, and must be read in conjunction with that Directive and the notes for guidance on *General Requirements for the Production and Control of Live Mammalian Bacterial and Viral Vaccines for Veterinary Use* and *General Requirements for the Production and Control of Inactivated Mammalian Bacterial and Viral Vaccines for Veterinary Use.*

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This document is intended to provide guidance on the type of data which should be included in applications for marketing authorisation for allergens. It is intended to supplement Directive 81/852/EEC, as amended by Directive 92/18/EEC, and must be read in conjunction with that Directive and the General requirements for live and inactivated mammalian vaccines (GRLMV; GRIMV).

For the purpose of this note for guidance, allergen products are divided into two categories:

a) industrially produced allergen products containing either a single allergen or defined mixtures manufactured in batches and placed on the market as medicinal products either for:
   - the purposes of *in vivo* diagnosis or for
   - treatment of allergic disease;

b) allergen products prepared from raw materials in accordance with the requirements of an individual prescription for a specific animal and for products produced for use in that animal only.

This note for guidance only refers to industrially produced allergen products placed on the market as medicinal products for the purpose of *in vivo* diagnosis or for treatment of allergic disease (point a above).

DEFINITION OF ALLERGEN PRODUCTS AND GROUPING OF ALLERGENS

DEFINITION OF ALLERGEN PRODUCTS

Allergen products falling under the scope of Directive 81/851/EEC are industrially produced products placed on the market as:

1. Finished products for therapeutic use (hereafter abbreviated as Ther.).
2. Products for *in vivo* diagnosis (hereafter abbreviated as Dg.).

GROUPS AND FAMILIES OF ALLERGENS

Allergen products are derived from several groups of allergen source materials such as pollens, mites, food substances, chemicals etc. Such groups are not necessarily homogeneous. In certain required testing programmes data obtained with one representative allergen product may be extrapolated to another. However, this is only acceptable if a close relationship exists between their active components, particularly with respect to the properties being examined. This may necessitate subdividing such groups of allergens into smaller families. A tentative classification in groups and families, with justification where appropriate, is given below.

1. Pollens

   Since the stability of pollen extracts may be influenced by various differences e.g. in the amount of different secondary metabolites, the following families should be distinguished on the basis of plant taxonomy: gymnosperms; monocotyledonous angiosperms; dicotyledonous angiosperms.

2. Fungi (moulds): Several families like aspergillus, ustilago, yeasts, etc.

3. Mites and housedust: mites, housedust

4. Animal allergens (for each animal species): epithelials (dander); hair and pelt; feathers; fleas

5. Hymenoptera venoms: one family

6. Food allergens: flour/meal, bran; fruits; nuts; vegetables; meat; fish and seafood; milk and milk products; eggs; spices

7. Pharmaceutical and industrial chemicals, secondary bacterial and plant metabolites (e.g. sesquiterpene lactones): due to the nature of these products no families (such as photochemicals) can be established. Antibiotics for allergen testing should be used from therapeutic pharmacological preparations.

8. Bacterial and parasitological allergens (tuberculin, brucellin, toxoplasma, echinococcus, etc.): no families.

GENERAL REQUIREMENTS

The name (scientific name, e.g. genus and species as well as any common name), and type (e.g. pelt, dander, saliva) of the allergenic source material(s) should be stated. In the case of modified or adsorbed allergen products, this and the agents used in the modification procedures should be described. For all the excipients, the names, grades and quantities should be given. This also includes the composition of any separately packaged dilution or reconstitution fluid to be used with the product.

Whenever possible, the potency of the active ingredient should be expressed in units of biological activity, and the unit system used should be unambiguously indicated.

1. STARTING MATERIAL

1.1 General requirements

The allergenic active ingredients should be described in as much detail as possible. Details concerning collection, including country of origin, pre-treatment and storage should be supplied for each separate allergen. The name and the address of the supplier shall be stated. Specifications and control methods applied by the supplier for the source material(s) (including allergen preparations) should be provided. The specifications should ensure that the qualitative and quantitative composition of the material is as uniform as possible from one delivery to another. They should encompass requirements and control methods relating to identity and purity. Quality control of source materials should be documented. The source materials should be stored under controlled conditions.

All substances of animal origin should be either sterilised or subject to an inactivation procedure by a suitable validated method or tested to be free from extraneous agents (see, GRLMV, GRIMV, table.

1.2 Additional requirements

For specific active ingredients each family or subgroup has to be tested separately.

1.2.1 Pollens

The manner of collection of pollens should be stated. Tests for content of foreign pollens, spores, extraneous plant material from the same species and non-related contamination should be included. Control tests should be applied to confirm the maximal acceptable amount of pesticides, and where relevant heavy metals, detected by a suitable and validated method. Thresholds for these contaminants should be established.

The pollen content from other species should be limited to 1% of mixed pollens and 0.5% of a single pollen as determined by a microscopic particle count. Detectable spores should not exceed 1%. Contamination by particles of plant origin, other than pollen, should not exceed a total of 10% in terms of microscopic count. Justification should be given if these standards cannot be complied with.

1.2.2 Moulds: several families like aspergillus, ustilago, yeasts etc.

The strain or strains of moulds should be specified. The cultivation method should be described. Details of the composition and preparation of the cultivation medium should be submitted and information provided on all substances of animal origin included in it and the absence of risk of transmission of infectious diseases should be demonstrated.

Strains which produce mycotoxins such as aflatoxins or ochratoxins should not be used unless justified. In this case, the source material should be tested for mycotoxins; the source materials should be tested for mutagenicity before processing unless the removal of mycotoxins has been validated.

Synthetic and consequently allergen-free media shall preferably be used. Morphological characteristics (mycelium and spores/spores only/mycelium only) as well as the cultivation method for preparation of the active ingredients should be specified. Conditions of culture must be validated to provide evidence that mycotoxins are not produced.

1.2.3 Mites

The cultivation method and the composition and preparation of the cultivation medium should be described. When substrates of animal origin are used in the culture medium, information should be given on all of these and the absence of risk of transmission of infectious diseases should be demonstrated. To this end, the manner of collection of these substrates should be described in detail together with details of processing and testing of batches for the absence from extraneous agents. Any allergenicity of the medium should be as low as possible in order to avoid any non-specific reactions of the finished product. Therefore, the use of animal dander or any animal protein in the culture medium should be avoided. It should be indicated whether for further processing, mites only or the whole mite culture is used.

1.2.4 Animal allergens: epithelials (dander), hair and pelt, feathers, fleas

The species of the animal and its country of origin should be stated. An account should be given of the health examination of the animals from which the raw materials are collected. Materials should be collected from animals that do not exhibit overt infections at the time of collection. The collector should certify that the animals used are overtly healthy and have not recently been treated with antiparasitics or other drugs.

Epidermal material obtained from killed animals should be collected within a few hours of death. After killing, animals must be stored under specified conditions which will ensure that post mortem decomposition does not affect the epithelium. Collection of hair and dander must take place using methods which provide a good epithelial harvest without injuring the skin of the animal. Methods employing the grinding of whole skin/pelts must not be used. The composition of the final source material (e.g. hair, dander, pelt, saliva, [urinary fluid]) should be indicated. For exclusion of extraneous agents see 1.1.

1.2.5 Donor animals

Animals from which source materials are derived shall be shown to not have been recently treated with heavy metals, insecticides, antibiotics, and hormones (or appropriate testing of the collected material shall be documented).

1.2.6 Hymenoptera venoms: one family

The method of collection of venom from the venom sacs of hymenoptera species should be described and should be such as to ensure that the raw material is of a suitable quality.

1.2.7 Food substances

For food substances their origin (e.g. geographical area, farms) shall be indicated. Suppliers and the origin of the materials should be maintained constant. Food substances shall be shown to be free from potentially toxic contaminants (see above) and preservatives. Where such conditions cannot be realised this should be justified. Where appropriate, the means of pre-treatment (e.g. for flour, spices) shall be indicated. For meat, fish, and seafood the veterinary and microbiological controls to which the animal and/or material was subjected should be stated and documented; the part(s) of the animal's body used for further processing, the means of its isolation and treatment shall be stated.

1.2.8 Other substances

For materials such as industrial chemicals and certain food substances where manufacture and quality/purity is subject to variation, the methods taken to compensate for this (e.g. by manufacturers) shall be indicated.

1.2.9 Fungal and bacterial allergens

For fungal and bacterial organisms, the strain, its origin, cultivation and passage history, and the controls to which it was subjected at various stages shall be described and results of tests provided.

1.3 Control of starting materials

1.3.1 Starting materials described in a pharmacopoeia

Probably only pharmaceuticals and certain industrial chemicals will fall under this category. Documentation on compliance with pharmacopoeial requirements shall be provided.

1.3.2 Starting materials not described in a pharmacopoeia

a) Source materials for active ingredients

The recommendations on source materials included in section 1.1 and 1.2 shall apply.

b) Method of preparation and testing of starting materials

The recommendations given in section 1.1 and 1.2. shall apply. The following details should be provided.

For allergens of bacterial and fungal origin, where production should be based on the seed lot system, the procedure for the establishment of the seed lot, the controls to which it was subjected and the appropriate specifications shall be described in detail. Documentation shall include production and control protocols in the relevant part of the dossier. As an essential part of the documentation the means of characterising the allergen extract or, where appropriate the purified material derived from it and used to prepare the finished product, shall be described in detail. For products of biological origin testing should cover the identification characteristics, and the estimation of total allergenic potency. A series of appropriate methods for identification/quantitation is indicated in section 3.1.

Data should be obtained by means of at least two methods, preferably isoelectric focusing and crossed immunoelectrophoresis, or SDS-PAGE and Western blot analysis. The characteristics of the in-house reference preparation and the identification/quantification criteria and specifications for the product shall be indicated and documented, with indication of the relevant markers. For the determination of major allergens and of total allergenic potency tests such as quantitative immunoelectrophoresis or radioimmunodiffusion, and RAST, respectively, should be used. Where certain immunological methods are considered as inapplicable, this should be justified.

1.4 Stability

The stability of starting materials shall be documented for each individual product. Appropriate maximal storage times shall be proposed. The absence of such tests has to be justified.

1.5 Description of the production process

The production process should be described, step by step with a diagram (flow-chart) indicating the principles of the process, accompanied by an explanatory text. The different stages of the production process, such as grinding, extraction, filtration, clarification, dialysis, concentration, fractionation, sterilisation, lyophilisation etc. should be clearly defined. The description should state the stage at which aseptic precautions are introduced. Intermediate or bulk products in the process should be identified and the in-process controls performed at these or other stages of production reported. The principle of the purification and fractionation methods should be defined, and it should be clearly apparent at which step in the process special biochemical techniques are used.

Where purification procedures critical for the characteristics of the finished product are used, validation data should be provided.

The manufacturer should demonstrate his capability of obtaining batch to batch consistency as mentioned under 2.4.

2. FINISHED PRODUCT – ASSAY RESULTS REQUIRED IN THE APPLICATION FOR MARKETING AUTHORISATION

2.1 Safety testing

See GRLMV and GRIMV

Safety data must be generated using the maximum concentration and dose of product (single or mixture of allergens) which is to be recommended for administration at one time. Safety of repeated administration is particularly important. As much information as possible should be provided on use of the product in allergic animals and in animals of the youngest and/or most susceptible species.

For safety testing, the concept of taxonomic family may be applied and data obtained on one member of the family may be extrapolated to another member of that family providing the manufacturing procedures applied are the same. In the case of mixtures of members of different taxonomic families, extrapolation is not acceptable.

For any additives see Directive 81/852/EEC as amended, Annex Title II Part 7 C. 7 and Council Regulation (EEC) 2377/90/EEC.

Efficacy data are required to support the claims being made using the product administered in accordance with the proposed recommendations for use. Consideration should be given to conducting allergen challenge tests, such as determination of concentration for desensitisation ("hyposensibilisation") treatments.

Details of clinical trials performed should be provided.

For the performance of clinical trials, the concept of taxonomic family may be applied and where justified data obtained on one member of the family may be extrapolated to another member of that family providing the manufacturing procedures applied are the same. In the case of mixtures of members of different taxonomic families, extrapolation is not acceptable.

Where extrapolation to other families or the whole group is considered as justified, this should be appropriately documented. Scientific literature can play an essential part in the documentation of efficacy provided it is relevant to the product to be marketed. Where such publications do exist, they may be used as supporting evidence of efficacy.

Documentation of pharmacodynamic properties is particularly important for Ther. products containing mixtures of crossreacting allergens. Available information should demonstrate that the allergenic potency of each component of the final mixture is similar to that of the appropriate individual product.

For Dg. products of biological origin an approach similar to that described for Ther. products (i.e. extrapolation) is acceptable. Special consideration should be given to the documentation of safety, and of both the specificity and the sensitivity of the testing procedure. The evaluation of these parameters should be based on clinical and laboratory data (e.g. RAST).

Data from studies carried out with Prick tests may be extrapolated to intracutaneous tests; this may not be applicable to provocation tests, unless appropriately justified.

For Dg. products containing pharmaceutical or industrial chemicals, efficacy data shall be provided for each individual product. They shall cover the safety as well as the specificity and the sensitivity of the testing procedure. Documentation based on scientific publications is acceptable, if appropriate. Since only a very small number of a given species may be allergic to certain Ther. and Dg. products case reports may be accepted, as supporting evidence of efficacy. Data obtained with individual allergens cannot be extrapolated to mixtures of crossreacting allergens.

Any data available on post-marketing experience should be provided by the applicant, particularly on adverse reactions reported, relative to doses sold.

2.3 Stability

It is accepted that in some circumstances, it is impossible or difficult to evaluate fully the allergenic potency and other characteristics of the finished product. In these cases, such as adsorbed-modified or adsorbed- unmodified allergens, it would be acceptable to carry out stability tests on materials stored at the stage just before applying the modifying treatment.

In addition, the stability of adsorption should be monitored over the proposed shelf life, since free allergens can cause immediate (anaphylactic) reaction.

For stability data, the concept of taxonomic family may be applied and data obtained on one member of such a family may be extrapolated within that same family. This extrapolation should be discussed and justified. In the case of mixtures of members of different taxonomic families, extrapolation is not acceptable.

The stated minimal allergenic activity and the required efficacy of the product should be maintained to the end of shelf life. This must be demonstrated for 3 batches for 3 months beyond the required shelf life.

Where storage recommendations specify clear temperature limits, the documentation can be limited to real time studies for the recommended storage temperature as indicated in the Ph. Eur. Stability data shall be provided for at least three batches of product. Data obtained with one product may be extrapolated to products derived from other allergens of the same family contained in the same composition of excipients. Where extrapolation to other families or the whole group is considered as justified, this should be appropriately documented. Where stability studies using immunological methods are not possible due to reagents not being available (e.g. antisera), this should be justified. In this case other methods should be used to give information about the stability of the allergen extract. A shelf life longer than 12 months is only acceptable with stability studies obtained by immunological methods.

For non-adsorbed/non-modified (mainly Dg. products) the proposals for shelf lives shall be based on stability data referring to the total allergenic potency (RAST inhibition). Additional data obtained by means of the testing program outlined C above should be provided.

For adsorbed/modified products stability shall be documented with data obtained with the appropriate material prior to modification/adsorption. For the finished product the stability of adsorption shall be documented.

2.4 Batch to batch consistency

Since allergen products are generally a complex mixture of allergenic and non-allergenic components, they cannot be easily standardised and each component cannot be defined, with a few exceptions, in a quantitative way. Although some international reference preparations or standards are available, it is commonly accepted that allergenic extracts cannot all be standardised in the same manner as the other biological products.

An extract will possibly be different from one batch to another. These characteristics represent a real problem. It must be stressed that batch to batch consistency has to be reached by a company within its production by introducing in house reference preparations (IHR) which should be used as internal reference preparation and using a number of biological and analytical methods. The IHR is derived from a production run following the manufacturing process defined in the dossier. The IHR establishes a reference point against which extracts from all future productions runs will be compared. Thus the qualitative composition of regular production batches should match the IHR.

Consistency of production shall be documented on five production runs (intermediate materials and finished product).

2.4.1 Characterisation of the in house reference preparations (IHR)

The IHR shall be characterised using available methods and its specific allergenic activity shall be established, and data should be provided on protein and, whenever possible, carbohydrate composition. Some of the following methods may be applied: crossed-immuno-electrophoresis (CIE), isoelectric focusing, electrophoresis in polyacrylamide gel, determination of the distribution of molecular weight by SDS-PAGE analysis, HPLC, gel electrophoresis and quantitative determination of total protein. Information regarding the allergenic specificity of the proteins in the IHR may be obtained from experiments involving combinations of electrophoretic methods and immunoblotting techniques or crossed-radio-immuno-electrophoresis (CRIE). Sensitivity spectra (allergograms) derived from such experiments on individual patients' sera should be included in the basic documentation, thus identifying the major, intermediate and minor allergens. The presence of all relevant allergens in the IHR shall have been demonstrated in comparative studies involving several batches of extract. As far as possible, the individual allergens should be identified using internationally accepted nomenclature or the correspondence with allergens described in the scientific literature should be given, including literature reference. The potency of this IHR should be judged by immuno-assay (e.g. IgE-inhibition, ELISA-techniques, immunofluorescence techniques) or/and skin prick test and expressed in terms of units of biological activity. When a product consists of one or a few well characterised allergenic components, potency can be assayed by means of alternative relevant techniques, such as single radial immunodiffusion, quantitative immuno electrophoresis or other quantitative techniques. All these methods and the immunological reagents mentioned above should be in accordance with the scientific knowledge at the time of application.

The stability of the IHR and storage conditions should be documented.

2.4.2 Use of the IHR

The characterised and standardised IHR for a given allergen product should be used to prove batch consistency, by using relevant methods already employed in the characterisation and standardisation of the IHR. The choice of the methods used must be justified and limits for variations should be defined and documented.

3. FINISHED PRODUCT – BATCH TESTING

See requirements of GRIMV/GRLMV

3.1 Control tests carried out at an intermediate stage of the manufacturing process of the finished product

Control tests carried out at intermediate stages of manufacture should be defined. When certain control tests cannot be applied to the finished product, for instance in the case of chemically modified, precipitated or adsorbed allergen preparations, quality specifications should be defined for the product just prior to the modification, dilution, etc.

Where allergen extracts are modified by, and/or adsorbed to, a chemical compound (as in the case of most Ther. products) the appropriate procedure shall be described. The efficiency of the modifying procedure and the identification characteristics of the modified material (allergoid) shall be documented.

3.2. Control tests carried out on the finished product

Measurements of the total allergenic activity of individual batches of an allergen extract should be undertaken preferably by IgE inhibition or by direct IgE-binding or other immuno-assay, all methods having to be suitably validated.

The estimated potency derived from the assay of total allergenic activity should be not less than 50% and not more than 200% of the stated potency.

The characteristics of the finished product should ideally be documented for all strengths (dilutions). Where appropriate testing is not possible due to methodological limitations, this should be justified.

For adsorbed/modified products, where measurement of allergenic activity is not possible on the finished product, documentation of adsorption should be provided.

The characteristics of non-adsorbed/non-modified finished products (mainly Dg. products) shall be documented. This documentation can be based on data from RAST inhibition, direct RAST, or another immuno-assay. For adsorbed/modified products, where measurement of allergenic activity is not possible, documentation of adequate adsorption, i.e. the limit of free protein shall be provided.

A sterility test should be performed in accordance with the Ph. Eur.