The Applicability of Scientific Guidelines to Allergens of Biological and Biotechnological Origin

A User Manual

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Table of contents

List of tables

Annex

Abbreviations

aaaai	American Academy of Allergy, Asthma & Immunology
aaapc	Academy of Allergy & Asthma in Primary Care
APCs	Antigen presenting cells
ADCC	Antibody-dependent cellular cytotoxicity
AIT	Allergen Specific Immunotherapy
API	Active pharmaceutical ingredient
BSE	Bovine spongiform encephalopathy
CCL	Continuous mammalian cell line
CMC	Chemistry, Manufacturing, and Controls
CPP	Critical Process Parameter
CQA	Critical Quality Attributes
CTD	Common Technical Document
DCs	Dendritic Cells
EDQM	Directorate for the Quality of Medicines & HealthCare
EEA	European Economic Area
ELISA	Enzyme Linked Immunosorbent Assay
EM(E)A	European Medicines Agency
EU	European Union
GA^2LEN	Global Allergy and Asthma European Network
GACP	Good Agricultural and Collection Practice
GACVS	Advisory Committee on Vaccine Safety (WHO)
GDP	Good distribution practice
GMP	Good manufacturing practice

HCP	Host cell protein
HIC	hydrophobic interaction chromatography
ICH	International Council (Conference) on Harmonization
IEC	Ion exchange chromatography
IgE/M/G	Immunoglobulin E/M/G
IHRP	In-house reference preparation
IUIS	International Union of Immunological Societies
kD	kilo-Dalton
MA	Marketing authorization
MAA	Marketing authorization application
MCB	Master cell bank
MHC	Major histocompatibility complex
NPP	Named-patient product
PEI	Paul-Ehrlich-Institute
QbD	Quality by Design
QTPP	Quality Target Product Profile
rDNA	Recombinant deoxyribonucleic acid
rpm	Rounds per minute
SEC	Size exclusion chromatography
TSE	Transmissible spongiform encephalopathy
WAO	World Allergy Organization
WCB	Working cell bank
WHO	World Health Organization
JP	Japanese Pharmacopoeia

US	United States (of America)
USP	United States Pharmacopeia
US-FDA	United States Food and Drug Administration

1 Objectives

There are only few allergen-specific guidelines available which provide advice on chemistry, manufacturing, and controls (CMC) issues concerning allergen extracts. Therefore, all further and in depths advice needs to be drawn from a multitude of regulatory documents.

For recombinant allergens the allergen specific regulatory documents as well as all documents for biotechnological active substances and products can be applied.

In total there will be a considerable number of regulatory documents applicable to allergen extracts and recombinant allergens.

The objective of this master thesis is to provide guidance on the applicability of CMC-related existing regulatory documents to allergen extracts and recombinant allergens for diagnosis and immunotherapy of Type I allergies in humans.

The master thesis will reflect on regulatory documents concerning the development, the manufacturing, the control and the stability of allergens while excluding all clinical issues from its scope. Considering the number of documents available, this reflection will not be comprehensive and application of regulatory documents to the individual product always should be considered case-by-case.

The reader can find a table in the Annex which lists the documents discussed in this review and a brief indication on their applicability. Therefore, the reader may use this thesis as a "user manual" to navigate through the range of regulatory documents in order to find the guidance she/he may find useful.

Notes to the reader:

There is no clear difference defined between the terms "biotechnical" and "biotechnological". Therefore, only the term "biotechnological" will be used.

References to the list of literature will be displayed in upright brackets [XX]. References to the annex will be displayed in italics as follows: *[REX]:* Regulations, *[GX]:* guidelines and supportive documents/information, *[EPX]:* Ph. Eur. monographs and chapters, *[DBX]:* data bases.

Guidelines are displayed according to the EMA Reference numbers.

2 Introduction

2.1 Allergies

2.1.1 Clinical aspects of allergies

Allergies are diseases inducing exaggerated specific responses of the immune system after exposure to otherwise harmless substances. The allergic symptoms and their severity differ considerably, involve different organ systems and can be separated into different types based on their immunopathological mechanism. The classification of allergies in four groups as proposed by Coombs and Gell in 1963 [1] still is the most commonly used classification of allergies. In the following a brief introduction to allergies is provided.

Type I allergies: IgE mediated (immediate) hypersensitivity

Type I allergies develop in two steps. During the first step (the sensitization phase) the allergen binds to dendritic cells (DC) which present the processed allergens in combination with their MHC-class II molecules as MHC-antigen complex to naive T-cells which are located in the lymph nodes. The recognition of the MHC-antigen complex by the T-cells "activates" the cells to differentiate predominantly into T_{H2}-cells. T_{H2}-cells trigger B-cells to proliferate and to produce antigen specific IgE molecules. The IgE molecules bind to the FcεRI receptor of mast cells which are mostly located in the skin and mucosa of the respiratory system and the intestines, thereby "sensitizing" the mast cells to the allergen. In parallel memory T-cells and B-cells develop.

The second phase (the allergic phase) starts upon re-exposure to the allergen. IgE coated mast cells release preformed proinflammatory substances like proteoglycans (e.g. histamin: causes itching) and proteases (e.g. Tryptase: stimulates smooth muscle cells, recruits eosinophilic and basophilic granulocytes) from their granules by degranulation. Furthermore they release e.g. prostaglandines (cause vasodilation), leucotrines (cause the contraction of smooth muscles of the bronchioles), and cytokines (attract cells of the immune system like B-cells, macrophages etc.). As a result inflammatory symptoms like redness of skin, itching, difficulty breathing, drop of blood pressure etc. can occur within a few seconds or minutes (early phase of the allergic reaction). During the late phase of the allergic reaction cells of the immune system such as macrophages, T-cells, basophilic and eosinophilic granulocytes infiltrate the

concerned tissue (e.g. the skin or the bronchial mucosa). These cells themselves release inflammatory mediators which directly damage the tissue in their environment. The late phase of the allergic reaction starts about six to twelve hours after the early phase.

Allergic reactions of type I may affect different organ systems like the skin (e.g. urticaria, pruritis, flush), the eyes (e.g. conjunctivitis), the ears (e.g. otitis), the upper respiratory system (e.g. rhinitis), the lower respiratory system (e.g. asthma) the intestines (pain, diarrhea), the vascular system (e.g. anaphylactic shock). If exposure to the allergy inducing agent is permanent, the inflammation may become chronic (e.g. asthma, sinusitis etc.).

The most type I allergy inducing agents are proteins e.g. from pollen, mites, animal dander and outgrowth, moulds, food. Chemical substances like medicines or metals salts also may trigger type I allergies.

Type I allergies can be treated by avoiding the allergy inducing agent. Medical treatment is either symptomatically (e.g. antihistamines, mast cells stabilizers, leukotriene inhibitors, corticosteroids) or causative by immunotherapy (hyposensitization).

Hyposensitization in order to induce tolerance to the allergen may avoid the development of further allergies and the continuous increase in the severity of allergic symptoms as well as the switch to further organs (e.g. development of asthma in addition to rhinitis), the “allergic march”. [2].

Type II allergies (Antibody-dependent cytotoxic hypersensitivity)

IgG and IgM antibodies bind to cell-surface antigens or to foreign molecules which are attached to the cell surface thereby triggering three mechanisms which underlie type II allergies. The first mechanism is the cytolysis of the antigen carrying cells. In this mechanism antigen bound IgG and IgM trigger the complement cascade which ends with the formation of the membrane attack complex (C5b-9) which lyses the cells. In parallel other intermediates of the complement cascade, the anaphylatoxins (C5s and C3a) direct leucocytes (e.g. neutrophilic granulocytes and monocytes) to the place of event where they unfold their inflammatory activity by releasing e.g. lysosomal enzymes, reactive oxygen metabolites, leukotrienes, prostaglandines and cytokines. The second mechanism is phagocytosis of opsonized cells (target cells which are tagged by intermediate peptides of the complement

cascade, the opsonin (C3b)) by monocytes and neutrophils. Additionally, thrombocytes, eosinophils, NK-cells etc. are attracted to the opsonized target cells and attack them by releasing cytotoxic substances. The third mechanism is the antibody-dependent cellular cytotoxicity (ADCC). Antibody coated target cells are identified by e.g. NK-cells, monocytes, neutrophils which then attack the target cells. This attack is supported by the effect of opsonin (C3b) which also is bound to the target cells.

Type II allergies may be triggered by cell (e.g. ABO blood incompatibility) or tissue incompatibilities (e.g. rejection of graft). Other type II reactions are autoimmune reactions. One example is the Goodpasture syndrom in which autoantibodies (IgG) bind to components of type IV collagen and thereby activate the complement which leads to serious glomerulonephritis. Chemical substances such as some medication my trigger e.g. hemolytic anemia.

In case the allergic response is triggered by exposure to chemicals or metals further exposure should be avoided. Non-steroidal anti-inflammatory drugs, corticosteroids and other immunosuppressive therapy may be used depending on the nature and severity of the disease.

Type III allergy (Immune complex mediated hypersensitivity)

The type III allergies resemble type II allergies in their mechanism except for the antigenic trigger. In the case of type III allergies the inducing agents are not bound to cellular surfaces but are soluble. The allergens develop an antigen-antibody complexes with IgG and IgM which triggers the complement system. Under non-pathogenic conditions the complement avoids further growth of antigen-antibody complexes and may also reduce their size. Erythrocytes in the blood vessels bind the complexes via a complement receptor and carry them to the liver where the complexes are eliminated by phagocytes. Under pathogenic conditions the antigen-antibody complexes are not eliminated sufficiently. Surplus complexes are deposited in the blood vessel membranes from where they can migrate into the surrounding tissue after mast cells and basophils increased membrane permeability of the vessels. Immune complexes can primarily be found in the blood vessels of the skin, in joint tissues and in the kidney. Within the tissue the same cascade as described for the type II allergic responses takes place.

Type III allergies may be caused by extraneous sources such as persistent viral and bacterial infections (e.g. post-streptococcal nephritis). Also continuous exposure to e.g. moulds (farmers lung) and exposure to foreign proteins (serum disease) may trigger a type III allergy. Furthermore, type III allergies may be the result of auto-immune responses (e.g. rheumatoid arthritis, Lupus erythematodes).

Depending on the severity the disease non-steroidal anti-inflammatory drugs, corticosteroids and other immunosuppressants are available for treatment.

Type IV allergy (Cell mediated (delayed) hypersensitivity)

This type of allergy is the result of a solely cell mediated reaction whose mechanism is not yet fully understood. Antigen presenting cells (APCs) present the antigen to T-cells thereby sensitizing the T-cells. Upon re-exposure to the antigen in combination with MHC-class II carrying APCs the T-cells are activated and release a variety of cytokines which attract further cells of the immune system to the site thereby triggering the allergic reaction.

Type IV allergic reactions may be directed against bacterial products. Examples are the development of granuloma due to bacterial infections (e.g. leprosy) or as response to natural exposure to bacterial products in the gut (Crohn´s disease).

Another source of type IV allergies are molecules of the body e.g. thyroglobulin (Hashimoto´s thyroiditis) or proteins of the myelin protein (Multiple sclerosis). In this case the allergy causes an auto-immune disease.

The best known type IV allergy to the public is the contact dermatitis which is triggered by haptens (small molecules which only develop their allergic potential if combined with a protein or other molecules of the body). Haptens may be small molecule chemicals like chromates, nickel salts or urushiol, the poison of poison ivy.

There is no cure for type IV allergies. Haptens as source of allergies may be avoided or symptoms treated e.g. by corticosteroids. In case of autoimmune diseases the treatment is aimed at reducing the symptoms and avoiding further progression of the disease.

Table 1 Types of allergies, mode of action and examples

Class	Mode of action	Example
Type I	IgE mediated (immediate) hypersensitivity	Anaphylaxis, allergic conjunctivitis and rhinitis, allergic asthma
Type II	Antibody-dependent cytotoxic hypersensitivity	ABO incompatibility, drug induced hemolytic anemia, Goodpasture syndrom
Type III	Immune complex mediated hypersensitivity	Purpura Schönlein-Henoch, Lupus erythematodes, Rheumatoid arthritis
Type IV	Cell mediated (delayed) hypersensitivity	Contact dermatitis, Crohn´s disease, Hashimoto´s thyroiditis

2.1.2 Socio-economic aspects of allergies

Allergic diseases have continuously risen over the last decades. The World Allergy Organization (WAO) estimates that currently about 10-40% of the world population may suffer from some type of allergy [3]. Allergies can severely impact the living quality of the patients and trigger considerable health care costs and overall economic costs due to reduced productivity/time lost from work. Information on the incidence of allergies and its impact on the economy within the US can be found at the homepage of the American Academy of Allergy, Asthma & Immunology (aaaai) [4] and a publication of the Academy of Allergy & Asthma in Primary Care (aaapc) [5], respectively.

Within the EU an estimated 44 to 76 million subjects of the about 217 million working people (employees or self-employed) suffer from allergic disease. Zuberbier et al. [6] assume that provided 10% of these subjects are treated optimally and the others insufficiently or not at all, the costs to the economy due to absenteeism and "presenteeism" in total ranges from €54.9 billion to €150.8 billion per year depending on the prevalence of the disease (10% to 35%) and the level of impairment (10% to 20%). The authors assume that if treated optimally the EU economy may save between €50 billion to €142 billion per year.

Considering the rising incidence of allergies world-wide and its consequences, causative treatment (inducing tolerance to the allergen) is increasingly important. Immunotherapy can

reduce or eliminate the allergic symptoms for many years, avoid a worsening of the condition (called the “atopic or allergic march”) and thereby improve the living quality and productivity of the patients and reducing long term health care costs and overall costs to the economy.

2.2 Allergens

Allergens are antigens which can

1. “sensitize” the immune system by inducing the immune cells to produce specific antibodies directed against the allergen (particularly IgE) and
2. trigger an allergic reaction (allergic symptoms) or
3. can trigger an allergic reaction by binding IgE which is directed against a similar allergen (cross-reactivity).

The most type I allergy causing allergens are proteins. The so far known type I allergy inducing proteins are distributed over about 190 protein families of the in total 16,230 known protein families (as of May 2015) listed in the Pfam protein family database (http://pfam.sanger.ac.uk) *[DB1]* [7]. The allergen nomenclature which is maintained by the International Union of Immunological Societies (IUIS) under the auspices of the WHO reflects the Linnean system and the nature of proteins. All allergens with a name assigned by the WHO/IUIS can be found in a data base (http://www.allergen.org) *[DB2]*.

The nomenclature is exercised using *Betula verrucosa* (white birch) as an example.

< name genus > < name species > < protein function > < . > < isoallergen > < isoform >

< 3-4 digits* > < 1-2 digits* > < 1-3 digits** > < . > < 2 digits > < 2 digits >

< Bet > < v > < 1 > < . > < 01 > < 01 >

Bet v 1.0101:

- name genus: “Bet” refers to *Betula*
- name species: “v” refers to *verrucosa*
- protein function/class: “1” refers to “pathogenesis-related protein”
- isoallergen: “01” refers to the first isoallergen of the “pathogenesis-related protein”
- isoform (variants): “01” refers to the first isoform of the isoallergen “01”

*:

The variability of the digits for the genus and species name allows for unambiguous identification of the species, as in some cases the first three digits of the genus name and the first digit of the species name may be identical for two different species.

**:

The number depends on the number of the protein families which harbor allergens and which currently ranges up to three digits. Initially the number did not refer to the protein function but to the sequence of the allergen discovery (e.g. the *Betula verrucosa* allergen which was discovered first received the number 1). The adjustment of the number to the protein function/class is not performed consistently in case of already well known allergens whose names were assigned according to the previous nomenclature in order to avoid inconsistencies with previous publications. Yet, renaming is performed by the WHO/IUIS as recently was the case for some *Betula verrucosa* allergens [8].

Isoallergens are allergens of a single species which show > 67% sequence identity with each other, have similar molecular masses and similar biochemical functions. Isoforms (variants) are allergens with > 90% amino acid sequence identity. If the amino acid sequence is identical and only the nucleotide sequence varies, they are considered to be the same variant. The

numbers provided are intended for guidance only as they were set arbitrarily as is emphasized by Radauer *et al.* [8]. In the same publication it is explained that allergenic proteins (allergens) with the same function often are called “group” irrespective of the proteins belonging to the same species. E.g. the pollen allergens from *Phleum pratense* Phl p 1 (Timothy grass), *Lolium perenne* Lol p1 (Rye), and *Cynodon dactylon* Cyn d 1 (Bermuda grass), are ß-expansins and are called the “group one grass pollen allergens” although this terminology is not part of the official terminology for allergens.

As mentioned at the beginning of this chapter, allergens have the potential to induce IgE production and to trigger allergic symptoms (Type I allergy). The term “major allergen” was defined by the WHO/IUIS nomenclature committee by clarifying that the term relates to an allergen against which at least 50% of the patients show specific IgE [9]. This definition was adopted by the guideline EMEA/CHMP/BWP/304831/2007 *Guideline on allergen products: production and quality issues [G1]*. This definition only refers to the frequency of IgE production irrespective of the IgE level and irrespective of the biological activity of the allergen. From a “major” allergen, however, it might be expected that either many patients show clinical symptoms and/or that such symptoms are strong (clinical relevance). This is discussed by Aalberse *et al.* [10]. The guideline EMEA/CHMP/BWP/304831/2007 *[G1]* takes this perspective into account by introducing the term “relevant allergens” which is defined as “...allergens causing a clinically relevant effect in a significant proportion of the allergic patients”. The applicant of a marketing authorization needs define and justify the choice of an allergen as a “relevant allergen”. Breitender and Chapman [11] suggest a more detailed definition of the term “major” allergen and introduce the “Eight Criteria for Defining Allergens That Make a Difference”. These criteria are:

1. “A sensitization rate of > 80% (> 2 ng allergen-specific IgE/ml) in a large panel of allergic patients.
2. A significant proportion of total IgE (> 10%) can be allergen-specific.
3. Removal of an allergen from the source material significantly reduces the potency of the extract.

4. Absorption of a serum with a purified allergen significantly reduces the specific IgE to the allergen extract.
5. An allergen accounts for a significant proportion of the extractable protein in the source material.
6. An allergen can be used as a marker for environmental exposure assessment.
7. Both antibody and cellular responses to an allergen can be measured in a high proportion of allergic patients.
8. Allergens have been shown to be effective as part of allergy vaccines."

It can be assumed, that the definition of "major" allergen will experience further change in the wake of growing scientific knowledge about allergens and improving methodology of allergen production and analysis.

3 Regulatory and scientific framework for allergen products

The basic legal framework for allergen products is the directive 2001/83/EC *Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use [RE1]* which states that a medicinal product which is either manufactured industrially or which at least includes an industrial manufacturing process (Dir. 2001/83/EC, article 2) may only be placed on the market if a marketing authorization (MA) is granted by the competent authority of the concerned EU/EEA member state (Dir. 2001/83/EC, article 6). Allergen products are considered to be immunological medicinal products and in article 1 of the said directive are defined as "any medicinal product which is intended to identify or induce a specific acquired alteration in the immunological response to an allergizing agent". Therefore, therapeutic and diagnostic allergen products irrespective of them being of biological or biotechnological origin require a MA before being placed on the market.

In Germany a medicinal product manufactured for an individual patient based on a prescription or manufactured in a pharmacy to not more than 100 packages per day and delivered to individual patients based on a prescription (so called "named-patient product,

NPP"), is allowed to be released to these patients without a MA based on the German Medicinal Products Act, section 21(2) 1 and 1g [12]. This provision helps to ensure the supply of allergen products to patients with rare allergies and allergy patterns, as the manufacturing of such products often is not feasible for the pharmaceutical industry from an economical perspective. In order to assure the quality, safety and efficacy of therapeutic allergen products as of the 14. Nov. 2008 the Therapeutic Allergen Ordinance came into force. This ordinance extends the obligation to acquire a MA to NPPs if they are prepared from pre-fabricated packages and include certain "common" allergens which are listed in the annex of the ordinance. Further details on the implementation of the Therapeutic Allergen Ordinance are provided by Bonertz *et al.*, 2014 [13].

The scientific framework for medicinal products including allergen products is based on Dir. 2001/83/EC annex I, part I, chapter 3.2.1.1 b) *[RE1]* third sub-paragraph which states that a biological medicinal product requires "…for its characterisation and the determination of its quality a combination of physico-chemical-biological testing, together with the production process and its control." Allergen products are considered immunological medicinal products and therefore fall under the scope of the term "biological medicinal product" as clarified in the second part of the third sub-paragraph.

The Guideline CHMP/BWP/304831/07 *Guideline on allergen products: production and quality issues [G1]*, is the key guideline providing advice for the manufacturing and quality control of industrially manufactured allergen products for the diagnosis and therapy of IgE mediated allergies. The products may be manufactured from allergen extracts from natural sources or by recombinant DNA technology. The guideline *inter alia* provides information on the concept of homologous groups and introduces homologous groups as proposed by Lorenz *et al.* [14] in its annex I. It provides information on the manufacturing and quality control as well as on stability requirements for the active substance and the finished product. The sequence of information resembles the Module 3 CTD structure. A more detailed discussion of the guideline with respect to the preparation of the quality part of a dossier for marketing authorization application (MAA) is provided by A.S. Limpert in her master thesis [15].

The Ph. Eur. general monograph *Allergen Products (1063) [EP1]* only applies to allergen products derived from extracts of naturally occurring sources while excluding recombinant

allergen products. It introduces the requirements for source materials as there are pollen, moulds, mites, animal epithelia and outgrowth and/or dander, hymenoptera venom and food including pretreated source materials. It also informs about the in-house reference preparation (IHRP) characterization requirements (e.g. protein and allergen profile and the biological potency) and tests to be performed for the quality control of the finished product with reference to the according monographs as far as these are available. It is specified that the tests should be performed as late as possible during the manufacturing process thereby clarifying that not all tests may be performed at the finished product level (e.g. lack of a proper analytical procedure). For NPPs the monograph specifies that the quality control should be performed either at the active substance level or an intermediate level between the active substance stage and the finished product.

Currently the allergen product monograph is under revision. The initially single monograph will be separated into one overarching monograph and allergen-specific monographs *[EP2 to EP7]*. The overarching monograph will resemble the currently valid monograph with respect to quality control of the finished product or the latest possible stage prior to the finished product level. The information on the different source materials (pollen, moulds, mites, animal epithelia and outgrowth and/or dander, hymenoptera venom) will be removed and transferred into individual complementary allergen-specific monographs. These source material specific monographs will present more detailed information on the manufacturing of the source material (e.g. the culture of mites) and the quality requirements. The draft monographs will specify the tests to be performed and refer to the according monographs on analytical procedures where available. The required tests refer to source material and include e.g. the acceptable amount of foreign pollen or the amount of foreign matter like plant pieces or soil and tests to determine the batch to batch consistency (e.g. the allergen profile, the major allergen content and the total allergenic activity). The draft monographs leave it open to the manufacturers to decide on the tests to be applied (justification required) and the methods used for testing in order to achieve batch-to-batch consistency. Therefore, the source material test results from different manufacturers may vary considerably.

Lorenz *et al*. [14] propose a concept of “homologous groups”. Allergens are assigned to particular homologous groups based on 1) comparable physico-chemical (e.g. DNA sequence)

and biological properties (source material from comparable tissues with comparable proteins, carbohydrates etc.) and 2) on cross-reactivity of the allergens or their source material. Each homologous group is represented by one or more allergens based on scientific information about the allergens and their cross-reactivity with other members of the same homologous group. Data may be extrapolated from these representative allergens provided 1) their manufacturing process and 2) the formulation of the finished products are identical. This concept was adopted by guideline CHMP/BWP/304831/07 *[G1]*. According to that guideline information on process validation for the non-representative allergens may be extrapolated from the process validation data of the representative allergen of a homologous group. Some stability data may also be extrapolated if justified. However, the vast majority of allergens does not belong to a homologous group. These allergens require full-scale process validation and a full set of stability data. An alternative approach to industrial scale validation is discussed in chapter 4.1 of this thesis. Based on literature Heath *et al.* [16] suggested to add Beech and Bermuda grass to the "Birch group" and the "group of sweet grasses", respectively.

The allergen-specific guidelines are supplemented by a variety of further guidelines and notices which are concerned with all aspects of the manufacturing of medicinal products. Some of these documents include allergen products in their scope, whereas others explicitly exclude allergen products partially or completely. In the following the applicability of such documents to allergen products will be discussed.

4 Applicability of scientific guidelines to allergen products

In this chapter the applicability of guidelines, notices to applicants, and further supportive information to allergen products (products from allergen extracts and recombinant allergen products) is discussed. In the annex a table is provided which indicates the applicability of the regulatory documents to allergen products with respect to:

- Development
- Manufacture
- Quality Control

- Comparability
- Stability
- Primary packaging

4.1 Development

4.1.1 Development tools

ICH Q8(R2) *Pharmaceutical development [G2]* and ICH Q11 *Development and manufacture of drug substances (chemical entities and biotechnological / biological entities) [G3]* are the key guidelines providing advice on the development of pharmaceutical products. The guidelines introduce the "enhanced approach" on product development.

All activities which are indicated as "enhanced" in their sum can contribute to a development approach which is called the "quality by design" (QbD). QbD is based on a comprehensive understanding of the relationships of all possible variables (scientific knowledge) within the manufacturing process and on risk assessment for the manufacturing steps which allows to "design" the manufacturing process in order to reproducibly manufacture a product with the desired quality.

Both guidelines are complemented by ICH Q9 *Quality risk management [G4]* and ICH Q10 *Pharmaceutical quality system [G5]* which will not be discussed further in the scope of this master thesis as they describe general concepts of risk assessment and the quality system which can be applied to any type of product.

The guidelines are joined by groups of supportive and explanatory documents:

1. the *ICH Quality Implementation Working Group Points to Consider (R2), ICH-endorsed guide for the implementation of ICH Q8/Q9/Q10* from 6th December 2011 *[G6]*
2. the *ICH guideline Q8, Q9 and Q10 - questions and answers, volume 4 [G7]* which is derived from the "Quality Implementation Working Group an Q8, Q9 and Q10, Questions & Answers (R4)" from 11th November 2010
3. training material for ICH Q8, Q9, and Q10 *[G8]*

The scopes of ICH Q8(R2) *[G2]* and ICH Q11 *[G3]* are identical to the scope of ICH Q6A: *Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical substances [G43]* and ICH Q6B*: Test procedures and acceptance criteria for biotechnological/biological products [G9]*. ICH Q6A is not applicable to any biological/biotechnological substances and products. However, ICH Q6B which applies to such substances and products emphasizes that allergenic extracts are outside its scope while stating at the same time that "the principles outlined...may also apply to other product types such as proteins and polypeptides isolated from tissues and body fluids". ICH Q11 *[G3]* also provides an "opening clause" and advises to consult with the regulatory authority for the applicability in the individual case. The guideline CHMP/BWP/304831/07 *[G1]* refers to ICH Q6B *[G9]* only with respect to recombinant allergens while at the same time applying the definition of "potency" as provided in ICH Q6B also to allergen extracts (see definition of potency in the guideline CHMP/BWP/304831/2007)

A brief introduction to the complex concepts of ICH Q8(R2) *[G2]* and ICH Q11 *[G3]* is presented below.

The development of a medicinal product can be separated into different steps:

1. Defining the Quality Target Product Profile (QTPP) of the product (finished product). A QTPP defines e.g. the route of administration, the dosage form, strength and stability and considers quality, safety and efficacy of the product.
2. Identifying Critical Quality Attributes (CQAs). CQAs are any physico-chemical or biological attributes which are important to the quality of the finished product. CQAs are established by either the "traditional" approach (CQA are set for the active substance and finished product) or by the "enhanced" approach (CQA are also set for excipients, reaction aides etc.).
3. Establishing a manufacturing process. This can be done by:

   the "traditional approach": the process may be established based on experience and uni-variant experiments. Target values and/or process parameter ranges (set points) and material attributes for each manufacturing step are established.

the “enhanced approach”: the manufacturing process is established based on multi-variant experiments and modeling and can be supplemented by experience. This includes the following aspects (but may not be limited to them):

- the identification of process parameters of e.g. culture conditions of recombinant cell lines, purification steps which may influence the CQAs of the finished product, the “Critical Process Parameters” (CPPs) and their interactions.
- the identification of material attributes (physico-chemical and biological properties) of e.g. active substance, excipients, reaction aides etc. which may influence the CQAs of the finished product and their interactions with each other.
- the identification of the interactions of material attributes and process parameters not only among themselves but also between the material attributes and the process parameters. The sum of the interactions is the basis for setting up a “design space” (please see glossary).

4. Risk assessment as described in ICH Q9 *[G4]* in order to identify CPPs and critical material attributes.

5. A control strategy as described in ICH Q10 *[G5]* in order to control CPPs and critical material attributes. Two control strategies can be applied:

   the “traditional” approach: includes the following subjects:

   - the control of active substance and finished product
   - in-process controls as in process tests
   - control of the sequence of the manufacturing steps

   the “enhanced” approach: this approach encompasses several aspects of the manufacturing process:

   - control of the CQAs of input parameters (upstream process) as there are e.g. source materials, cell culture media or cultivation substrates, excipients etc.).

- control of critical process steps (unit operations) which have an impact on the downstream process or the CQAs of the product such as extraction and purification steps or removal of host cell protein.
- control of the sequence of the manufacturing steps as already described above.

6. Validation/verification of the manufacturing process can differ, depending on the process development approach:

   the “traditional approach”: during the development the acceptable process range and the material attributes (if required) for each manufacturing step (unit operations) are determined and confirmed during validation according to ICH Q7 *Good manufacturing practice for active pharmaceutical ingredients [G10]*.

   the “hybrid” approach as introduced in EMA/CHMP/CVMP/QWP/BWP/70278/2012 *Guideline on process validation for finished products - information and data to be provided in regulatory submissions [G11]* which includes validation steps according to ICH Q7 *[G10]* and other steps performed by the continuous process verification.

   the “enhanced” approach by continuous process verification: Prerequisite for this approach will be a sound data base obtained during development, as the verification needs to confirm the conclusions drawn during development (the conclusions *per se* are realized e.g. as design space).

Product-specific considerations on process validation are provided in chapter 4.2. of this thesis.

4.1.2 Further considerations on development

4.1.2.1 General considerations

Some general considerations with regard to the development of pharmaceuticals are provided in the Note for guidance CPMP/QWP/155/96 *Note for guidance on development pharmaceutics [G12]* which refers primarily to chemical-synthetic finished products. The guideline points out that for some biological products “alternative approaches may be appropriate” without excluding biologics from its scope. The guideline discusses issues of formulation development, packaging, and critical steps of manufacturing such as sterilization.

Annex CPMP/QWP/054/98 *Decision trees for the selection of sterilisation methods (CPMP/QWP/054/98) Annex to note for guidance on development pharmaceutics (CPMP/QWP/155/96) [G13]* to the guideline offers a decision tree for sterilization for products which cannot be terminally sterilized (as is the case for all biological products including allergens). Currently a revision of the annex on the selection of the sterilization method is considered (concept paper EMA/CHMP/CVMP/QWP/128000/2014). The annex CPMP/BWP/328/99 *Development pharmaceutics for biotechnological and biological products (CPMP/BWP/328/99) Annex to note for guidance on development pharmaceutics (CPMP/QWP/155/96) [G14]* focuses *inter alia* on the development of the drug formulation with respect to the stability of the complex biological molecules and compatibility with the primary packaging and is applicable to any type of allergen product. **][**

4.1.2.2 Starting material

The scientific considerations for the choice of a starting material is part of the development. ICH Q11 *[G3]* provides some considerations for the choice of starting materials for chemical-synthetic and semi-synthetic drugs and refers to ICH Q5A(R1), Q5B and Q5D (see below) for biological/biotechnological source and starting material. The considerations relate to starting material attributes which may be considered the CQAs of these materials. Information is also provided by the draft guideline EMA/CHMP/QWP/96664/2015 *Draft guideline on the chemistry of active substances [G15]* which will update and consolidate data from the still valid guidelines 3AQ5a *Chemistry of active substances [G16]* and CPMP/QWP/130/96 *Guideline on the chemistry of new active substances (CPMP) [G17]*. However, 3AQ5a and the draft guideline exclude any type of biological products from their scope. Information is also provided in the *Reflection paper on the requirements for selection and justification of starting materials for the manufacture of chemical active substances* EMA/448443/2014 *[G18]*. The information here is of interest in case of a biotechnological product which may include synthetic components. However, such aspects will not be discussed in this master thesis.

Starting material - Recombinant allergens

ICH Q5D: *Derivation and characterisation of cell substrates used for production of biotechnological/biological products [G19]* can be applied to biological/biotechnological

medicinal products where a cell banking system is used. The guideline provides information on the derivation and characterization of “cell substrates” which are either cells of microbial origin or animal (metazoan) origin. The guideline is also applicable to primary cell lines (appendix 1 of the guideline).

The guideline applies to recombinant allergen products as for the development of such a product a parent cell line is selected for manipulation and a clone of the manipulated cells is selected for establishment as the master-cell bank. Aspects for the choice are e.g. the proof of the identity of the cells, their purity with respect to contamination with other cells, microbial and viral contamination etc. as well as e.g. yield of the desired product and the ability to resume production after storage (freezing). Viral contamination is of particular concern. The following virological aspects my influence the acceptability of a master cell bank: the nature of the contaminating virus (pathogenicity to humans), the clinical use of the finished product (severity of the disease and route of application) and the risk-benefit aspects derived thereof, as well as the viral clearance capacity of the manufacturing process.

Starting material - Allergen extracts

The guideline EMEA/CHMP/BWP/304831/2007 *[G1]* provides requirements for the starting material (source material) of allergen extracts. More detailed information on the source material is provided in the Ph. Eur. general monograph *Allergen Products (1063) [EP1]* and to an even more extent in the allergen-specific draft monographs for the allergen source materials.

4.2 Manufacturing

4.2.1 Validation

Proteins are complex molecules which often cannot be completely characterized and whose properties can easily be modified by their environmental conditions. Therefore, the manufacturing process is considered part of the characteristics of the molecule and part of its quality. Consequently, the validation of the manufacturing process is essential.

Validation - Recombinant allergens

ICH Q7 *[G10]* describes in its chapter 12 the “traditional” approach to process validation of active pharmaceutical ingredients (API) up to the point of sterilization. The guideline applies to APIs which are “... manufactured by chemical-synthesis, extraction, cell culture/fermentation, by recovery from natural sources, or by any combination of these processes”. This includes allergen extracts and recombinant allergens. The information in this guideline is deepened by the draft guideline EMA/CHMP/BWP/187338/2014 *Guideline on process validation for the manufacture of biotechnology-derived active substances and data to be provided in the regulatory submission [G20]* which provides valuable information on the upstream and downstream manufacturing process and clarifies that certain manufacturing steps may be validated as scale down spiking experiments (e.g. removal of process-related impurities) if the process is (up-) scalable and representative of the full-scale process. The guideline refers to recombinant proteins and related products as defined in ICH Q6B *[G9]* for its scope and to ICH Q8(R2) *[G2]* and ICH Q11 *[G3]* for the continuous process verification as an alternative to the “traditional” approach. ICH Q8(R2) and ICH Q11 also refer to ICH Q6B for their scope.

From this it is clear, that the “traditional” approach and the “enhanced” approach on process validation are applicable to recombinant allergen active substances and products. The “enhanced” approach on manufacturing process development and validation may allow e.g. to compensate for reduced agitation of culture medium (reduced rpm) by increasing the aeration rate to keep the partial oxygen pressure unchanged without exceeding design space/action limits.

Validation - Allergen extracts

The “traditional” approach to process validation is the established type of process validation for allergen extracts. However, considering the opportunities of a design space which allows the movement within the design space without having to file a variation application as gets clear when looking at the *Guidelines of 16.05.2013 on the details of the various categories of variations, on the operation of the procedures laid down in Chapters II, IIa, III and IV of Commission Regulation (EC) No 1234/2008 of 24 November 2008 concerning the examination of variations to the terms of marketing authorisations for medicinal products for*

human use and veterinary medicinal products and on the documentation to be submitted pursuant to those procedures [RE2] could be of interest for some allergen extracts even for individual steps of the manufacturing process.

All guidelines concerned with the "enhanced" approach on process validation refer to ICH Q6B *[G9]* for their scope thereby excluding allergen extracts from that scope. This may be due to the fact, that the guidelines which determine the quality of recombinant allergens (e.g. the guideline EMEA/CHMP/BWP/304831/2007, the ICH Q6B and ICH Q5B) request detailed information e.g. on the nucleic acid sequence of the construct, on the conformation of the molecule, on quantification and activity of the recombinant allergens to an extent which is not achievable for allergen extracts. However, for some allergen extracts knowledge of the major and minor allergens, their size (kD), their sequence has improved considerably since the late 90s of the last century, when some of the quality guidelines came into force. This is due to the improvement of analytical procedures and preparative methods such as IEC, HIC, and SEC which allow the removal of many co-extracted proteins and the targeted enrichment of the desired allergen from the extract. Therefore, at least individual manufacturing steps for some well-known allergen extracts possibly could be validated using the "enhanced" approach on validation. The draft guideline EMA/CHMP/BWP/187338/2014 *[G20]* on process validation although referring to ICH Q6B for its scope informs, that the principles of the guidelines also may be applied to biologicals such as "vaccines and blood products". Traditional vaccines as immune-modulators which are extracted from a biological source (e.g. cell culture or bird embryos) are purified in a downstream process which resembles the extraction/purification of allergen extracts (e.g. cross-flow filtration, ultrafiltration and SEC). Therefore, it is concluded that the principles of the draft guideline may be applied to allergen-extracts as well. In case the enhanced approach or hybrid approach is aspired, the responsible authority should be consulted.

4.2.1.1 Further considerations on process validation

The guideline EMA/CHMP/CVMP/QWP/BWP/70278/2012 *[G11]* provides further information on process validation/verification. The principles of the guideline, although primarily referring to chemical-synthetic finished products also can be applied to active substances and biological substances on a case-by-case evaluation. The guideline discusses

the three validation process approaches (traditional process validation, the continuous process verification and the “hybrid approach”).

The guideline informs that in case the “traditional” validation approach is implemented for biological/biotechnological medicinal products industrial scale validation of three consecutive batches should be available at the time of MAA (annex II). This is aggravated by the fact that most allergen products are manufactured using non-standard manufacturing procedures such as e.g. lyophilization and aseptic processing which *per se* already require industrial scale validation in case the “traditional” validation process is applied.

Some facilitation is provided for the validation of the manufacturing process. The guideline EMA/CHMP/CVMP/QWP/BWP/70278/2012 *[G11]* permits “bracketing” which may be applied to different strengths, batch sizes and pack sizes. As the principles of the guideline may be applied to biological and biotechnological products the approach may be applied to allergen extracts and recombinant allergens products. However, the guideline cautions and suggests deciding on a case-by-case basis. Another facilitation is the use of the homologous groups concept for allergen extracts as developed by Lorenz *et al.* [14] and adopted by guideline CHMP/BWP/304831/2007 *[G1]*. A reduced validation program can be applied to the non-representative allergens of the homologous group provided that the allergens are produced using an identical manufacturing process and provided that the finished products are of identical formulation. Data from representative allergen(s) of the homologous group can be extrapolated to the non-representative members of the group whereas critical process steps should be validated for all members of the homologous group. Still numerous allergen extracts are not members of homologous groups thereby requiring industrial scale validation. However, based on justification new allergens may be added to an already existing homologous group or new homologous groups may be established based on the requirements for the establishment of homologous groups as presented in guideline CHMP/BWP/304831/2007 *[G1]*.

In order to combine the homologous group concept and the concept of “bracketing” comprehensive justification is required. E.g. members of the same homologous group may show different behavior at particular steps of the manufacturing process as compared to the representative allergen. Therefore, the applicability of identified CPPs and CQAs during the

manufacturing process need to be justified for every allergen for which extrapolation from the representative allergen is intended. Furthermore, bracketing with respect to batch sizes should only be applied if the batch sizes selected for validation are meaningful for all other batch sizes of the allergens involved in the validation scheme. Extensive documentation of the manufacturing process and batch sizes of the representative allergen should be available for extrapolation.

4.2.1.2 Virus validation/TSE validation

CPMP/BWP/268/95 *Note for guidance on virus validation studies: the design, contribution and interpretation of studies validating the inactivation and removal of viruses [G21]* provides information on the principles of virus removal/inactivation during the manufacturing process and its validation. The guideline refers to "all categories of medicinal biological products" which includes products derived from cell lines, biological fluids, organs and tissues thereby encompassing all types of allergen products with the potential to harbor viruses. The guideline CPMP/BWP/269/95 *Note for guidance on plasma-derived medicinal products [G22]* which is applicable to plasma-derived products provides many valuable technical advises beyond those provided in the CPMP/BWP/268/95 on removal and inactivation steps which can be generally applied to biological/biotechnological products obtained from cell culture. Therefore, the guideline CHMP/BWP/304831/2007 *[G1]* refers to this guideline for advice.

The guideline CHMP/BWP/457920/2012 *Guideline on the use of bovine serum in the manufacture of human biological medicinal products [G23]* is concerned with the detection of viral contamination and its inactivation in bovine serum irrespective of the serum being used prior to or during the manufacturing process of biological medicinal products, thereby also including allergens. The guideline provides information on virus detection of specific (bovine) viruses and on viral inactivation. For further details on viral inactivation studies the guideline refers to CPMP/BWP/268/95 *[G21]*.

With respect to risk of transmissible animal spongiform encephalopathy (TSE) the guideline CHMP/BWP/457920/2012 refers to the guideline EMEA/410/01 *Note for guidance on*

minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products [G24] the text of which is identical to the Ph. Eur. chapter *5.2.8 Minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products [EP8]*. The guideline provides information on the TSE-relevant animal species and tissues and on materials and substances derived thereof with respect to their use for the manufacture of any type of medicinal product. The Certificate of Suitability of the EDQM for confirmation of TSE compliance of materials obtained from TSE-relevant species (e.g. gelatin or bovine serum) is based on this guideline/monograph.

The guideline/Ph. Eur. chapter also refer to guideline CPMP/BWP/CPMP/5136/03 *Guideline on the investigation of manufacturing process for plasma- derived medicinal products with regard to vCJD risk [G25]* for information on clearance studies (inactivation/removal of TSE). However, this guideline only refers to plasma-derived medicinal products and therefore, does not apply to allergens.

Virus validation/TSE validation - Recombinant allergens

The guideline CPMP/BWP/268/95 refers to “all categories of medicinal biological products”, thereby including recombinant allergen products.

Guideline CPMP/BWP/269/95 *[G22]* concerns the manufacturing of plasma-derived medicinal products and plasma derivatives which are used e.g. as excipients and does not concern the manufacture of products which use such substances e.g. as excipient. Therefore, this guideline does not provide requirements for the manufacture e.g. of recombinant allergens which use plasma derivatives at any time-point during manufacturing. However, as mentioned above, the guideline provides good technical advice for virus safety studies.

Similarly, CHMP/BWP/457920/2012 *[G23]* informs about testing of adventitious agents (including viruses) in the serum itself. The testing may be performed by the provider of the serum or by the user of the serum e.g. the manufacturer of the recombinant allergen product.

EMA/410/01 *[G24]* states that vaccine antigens, biotechnology-derived products as defined in Reg. (EC) No 726/2004 *Regulation (EC) No 726/2004 of the European Parliament and of the Council of 31 March 2004 laying down Community procedures for the authorisation and supervision of medicinal products for human and veterinary use and establishing a European*

Medicines Agency [RE3] and any other type of medicinal product derived from seed lots or cell banks lie within its scope. This includes allergens manufactured accordingly. The applicability of EMEA/410/01 is clarified in the explanatory note EMEA/CPMP/BWP/498/01 *Joint Committee on Proprietary Medicinal Products / CVMP note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products: Explanatory note for medicinal products for human use on the scope of the guideline [G26]*. Attention should be paid to the fact, that the guideline EMA/410/2001 and the according monograph only apply to master-seeds and cells banks which were submitted for marketing authorization after 1. July 2000.

Virus validation/TSE validation - Allergen extracts

The Guideline CPMP/BWP/268/95 *[G21]* refers to any biological product thereby including allergen extracts. As already mentioned, the guideline CPMP/BWP/269/95 *[G22]* provides good technical information and is also referred to in the guideline EMEA/CHMP/BWP/304831/2007. Therefore, the guideline should be considered for allergen extracts. CHMP/BWP/457920/2012 *[G23]* applies as far as serum is used for the manufacture of the product. The same is true for the guideline EMA/410/01 *[G24]*.

4.2.2 GMP manufacturing

The Note for Guidance ICH Q7 *[G10]* is of fundamental importance for the manufacturing of active substances with respect to GMP requirements. The note discusses general GMP issues such as the quality management, personnel, buildings and facilities, record keeping etc., as well as GMP requirements which need to be considered from a product-related perspective. Such GMP perspectives are e.g. the production, in-process controls and the validation of the manufacturing. Some aspects of GMP manufacturing will be discussed.

4.2.2.1 Start of GMP manufacture

In chapter 4.1.2.2.of this thesis the choice of starting material with respect to its quality attributes was discussed. In this chapter the question at which time point manufacturing according to GMP requirements is necessary is discussed for allergens. In order to do so, some definitions are provided:

Dir. 2001/83/EC *[RE1]* defines the term “starting material” as “any substance of biological origin such as micro-organisms, organs and tissues of either plant or animal origin, cells or fluids (including blood or plasma) of human or animal origin, and biotechnological cell constructs (cell substrates, whether they are recombinant or not, including primary cells).” The terms “source material” and “starting material” are applied as synonyms in some guidelines, e.g. the allergen guideline EMEA/CHMP/BWP/304831/2007 *[G1]*. Therefore, both of the terms relate to e.g. pollen, hair or epithelia for the manufacture of allergen extracts and e.g. to cell substrates for the manufacture of recombinant allergens.

A “raw material” is defined as “Any other substances used for manufacturing or extracting the active substance(s) but from which this active substance is not directly derived, such as reagents, culture media, foetal calf serum, additives, and buffers involved in chromatography, etc....” Dir 2001/83/EC *[RE1]*. The allergen guideline EMEA/CHMP/BWP/304831/2007 *[G1]* provides as examples “solvents and diluents for extraction, media for the cultivation of mites or moulds and media and reagents for production of recombinant proteins”.

GMP – recombinant allergens

ICH Q7 *[G10]* does not comprise all steps of the GMP manufacturing as is indicated in its scope. Therefore, table 1 of the guideline does not indicate all manufacturing steps where GMP is required for allergens. According to table 1 of Eudralex Vol 4, annex 2 *Manufacture of Biological active substances and Medicinal Products of Human Use [G27]* in case of biotechnological products such as recombinant allergens the assembly of the expression system and the selection of the clone to be established into the MCB should be performed following the principles of GMP and should be well documented. GMP requirements start with the establishment of the MCB and WCB and encompasses all further steps such as the maintenance of the cell banks. Cell growth for protein production and the downstream process need to fulfill full GMP requirements (validated procedures) as is clarified in part B5 of the annex. Further information is provided in Eudralex Vol 4, Part II *Basic Requirements for Active Substances used as Starting Materials [G28]*.

Table 2 Copy of table 1 from Eudralex Volume 4, annex 2

Table 1. Illustrative guide to manufacturing activities within the scope of Annex 2.

Type and source of material	Example product	Application of this guide to manufacturing steps shown in grey			
1. Animal or plant sources: non-transgenic	Heparins, insulin, enzymes, proteins, allergen extract, ATMPs immunosera,	Collection of plant, organ, tissue or fluid[9]	Cutting, mixing, and / or initial processing	Isolation and purification	Formulation, filling
2. Virus or bacteria / fermentation / cell culture	Viral or bacterial vaccines; enzymes, proteins	Establishment & maintenance of MCB[10], WCB, MVS, WVS	Cell culture and/or fermentation	Inactivation when applicable, isolation and purification	Formulation, filling
3. Biotech-nology - fermentation/ cell culture	Recombinant. products, MAb, allergens, vaccines Gene Therapy (viral and non-viral vectors, plasmids)	Establishment & maintenance of MCB and WCB, MSL, WSL	Cell culture and / or fermentation	Isolation, purification, modification	Formulation, filling
4. Animal sources: transgenic	Recombinant proteins, ATMPs	Master and working transgenic bank	Collection, cutting, mixing, and / or initial processing	Isolation, purification and modification	Formulation, filling
5. Plant sources: transgenic	Recombinant proteins, vaccines, allergen	Master and working transgenic bank	Growing, harvesting[11]	Initial extraction, isolation, purification, modification	Formulation, filling
6. Human sources	Urine derived enzymes, hormones	Collection of fluid[12]	Mixing, and/or initial processing	Isolation and purification	Formulation, filling
7. Human and / or animal sources	Gene therapy: genetically modified cells	Donation, procurement and testing of starting tissue / cells[14]	Manufacture vector[13] and cell purification and processing,	Ex-vivo genetic modification of cells, Establish MCB, WCB or cell stock	Formulation, filling
	Somatic cell therapy	Donation, procurement and testing of starting tissue / cells[14]	Establish MCB, WCB or cell stock	Cell isolation, culture purification, combination with non-cellular components	Formulation, combination, fill
	Tissue engineered products	Donation, procurement and testing of starting tissue / cells[14]	Initial processing, isolation and purification, establish MCB, WCB, primary cell stock	Cell isolation, culture, purification, combination with non-cellular components	formulation, combination, fill

See Glossary for explanation of acronyms.

[9] See section B1 for the extent to which GMP principles apply.
[10] See section on 'Seed lot and cell bank system' for the extent to which GMP applies.
[11] HMPC guideline on Good Agricultural and Collection Practice - EMEA/HMPC/246816/2005 may be applied to growing, harvesting and initial processing in open fields.
[12] Principles of GMP apply, see explanatory text in 'Scope'.
[13] Where these are viral vectors, the main controls are as for virus manufacture (row 2)
[14] Human tissues and cells must comply with Directive 2004/23/EC and implementing Directives at these stages.

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ICH Q5B: *Analysis of the expression construct in cell lines used for production of r-DNA derived protein products [G29]* instructs on the information about the expression construct such as its history, assembly prior to being integrated into the MCB. It is not stated, that the assembly should be performed according to GMP requirements, however, the guideline clarifies, that the according information is required.

If a recombinant allergen is combined with a chemical-synthetic component, information on GMP requirements of the chemical-synthetic component can be found in ICH Q7 *[G10]*, ICH Q11 *[G3]* and in the EMA reflection paper EMA/448443/2014 *[G18]*.

GMP – allergen extracts

Eudralex Vol 4, annex 2 *Manufacture of Biological active substances and Medicinal Products of Human Use [G27]* indicates in its table 1 and in its allergen-specific part B2 that the collection of non-transgenic animal and plant material for products such as allergen extracts already fall under the scope of GMP requirements. Therefore, the collection of plant material such as pollen according to EMEA/HMPC/246816/2005 *Guideline on good agricultural and collection practice (GACP) for starting materials of herbal origin [G30]* as well as the setting of acceptance criteria such as e.g. the acceptable amount of contaminating foreign pollen in the collected material is required. Detailed information on the regulatory requirements concerning information on the source material are provided in chapter 4.2.3.1 of the allergen guideline CHMP/BWP/304831/2007 *[G1]* and in the currently valid allergen Ph. Eur. general monograph *Allergen Products (1063) [EP1]* and its drafts *[EP2 to EP7]*. As the drafts are not in force yet, they are currently not considered to be mandatory requirements.

4.2.2.2 Hold times

One issue for the manufacturing of allergens are hold times. Two “types” of hold times can be distinguished as there are 1) hold times due to the manufacturing operation as intermediates of the active substance or finished product may be stored prior to further processing and 2) hold times in order to allow allergenicity to drop to the desired level of the active substance prior to further processing.

Hold times as manufacturing operation – recombinant allergens and allergen extracts

The allergen guideline EMEA/CHMP/BWP/304831/2007 *[G1]* requests any hold time to be identified and justified. However, no further information is provided. The currently valid guideline CPMP/QWP/486/95 *Note for guidance on manufacture of the finished dosage form [G31]* which does not "pertain" to *inter alia* allergens and biotechnological products also does not discuss hold times. The draft guideline EMA/CHMP/QWP/245074/2015 *Guideline on manufacture of the finished dosage form [G32]* for which consultation is planned to be closed in January 2016 will replace CPMP/QWP7/486/1995 and suggests that prolonged storage time during the manufacture of sterile products means a storage for more than 24 hours. Any storage beyond this hold time should be justified and stability of the stored bulk be ensured by process validation studies or stability studies.

The principles of the draft guideline are also applicable to any type of allergen product. As hold times may influence the overall shelf-life of the active substance or finished product. The draft guideline advises to perform stability testing to justify the hold time. In case bulk material is stored the guideline suggests to calculate the product shelf-life according to CPMP/QWP/072/96 *Note for guidance on start of shelf-life of the finished dosage form (annex to note for guidance on the manufacture of the finished dosage form [G33]*. The guideline CPMP/QWP/072/96 advises to calculate the shelf life from the date of batch release unless that day exceeds 30 days from the "production date" of the batch. In such a case, the date of production should be the date from which the shelf life is calculated, with the production date being the date where the API for the first time comes into contact with other ingredients or the day of filling (if no other ingredients are added). The guideline however excludes *inter alia* allergens and biotechnological medicinal products. In fact the allergen guideline EMEA/CHMP/BWP/304831/2007 *[G1]* with reference to ICH Q5C *Stability testing of biotechnological/biological products [G34]* and ICH Q1A(R2) *Stability testing of new drug substances and products [G35]* expects real-time stability data.

Hold time as intended decay period of allergenicity – allergen extracts

It was observed that the allergenic activity of some allergens (e.g. some pollen allergens) decrease over time and finally levels off (personal communication). The reason for this observation is not known. However, protease activity is suspected. Proteases of pollen were

already discussed as potential allergens by e.g. Gunawan et al. [17]. Recently the cysteine protease Amb a 11 was identified as a major allergen of short ragweed [18]. In order to avoid allergenic activity to fall below the finished product specification within a short period of time, the allergen extract is allowed to decay until the plateau is reached prior to standardization and further processing. This hold time may be considered part of the manufacturing process as this step fulfills a particular purpose. As part of the manufacturing process the hold time should be validated. Appropriate testing must be in place (e.g. at drug product stage) to make sure that the product is still compliant with all relevant specifications.

4.2.2.3 Blending

Blending does not include the combination of different source materials (e.g. a mix of allergens from different species) in order to perform co-extraction as is clarified in the allergen guideline CHMP/BWP/304831/2007, chapter 4.1.2 *[G1]* and in Eudralex Vol 4 annex II part B2. Allergen extract mixtures need to be prepared from individual extracts each of which needs to fulfill the specification requirements.

4.3 Quality Control

The allergen guideline EMEA/CHMP/BWP/304831/2007 *[G1]* provides an introduction to the quality requirements for recombinant allergens and allergen extracts. The requirements cover the complete manufacturing chain from the source material, active substance, to the finished product and the requirement on the quality of the finished product up to the end of its shelf-life. The guideline refers to a variety of guidelines for further information. The applicability of some of these guidelines to recombinant allergens and allergen extracts will be discussed.

4.3.1 Expression system

The guidelines ICH Q5D *[G19]* and ICH Q5B *[G29]* refer to “cell substrates” which require a cell-banking systems of animal sources (metazoan) and microbial sources (bacteria, fungi, yeast, other unicellular life forms) and to the expression construct, respectively. Both guidelines were already briefly discussed in chapters 4.1.2.2. (Q5D) and 4.2.2.1 (ICH Q5B)

with respect to the choice of a cell clone for development into the MCB and the choice of the expression construct.

The quality of the MCB with respect to its identity (correct cell line, correct bacterial strain, correct expression vector) and purity (absence of e.g. bacteria, fungi, contaminating cells, viruses, in case of metazoen cells and absence of adventitious bacteria, bacteriophages etc. in case of microbial cells) needs to be demonstrated. Other quality aspects are the stability and productivity of the cell substrate in order to obtain the desired product in a reproducible manner with sufficient yield, and the ability of the cell line to resume production after storage. The guideline provides some hints towards the analytical procedures without going into any details. The WCB usually is submitted to a limited testing.

The guideline ICH Q5B *[G29]* lists in more detail the analytical procedures which are expected in order to demonstrate the stability of the expression system at MCB and WCB level such as the correct nucleic acid sequence, amino acid sequence and post-translational modifications. The stability of the protein coding sequence needs to be demonstrated during the complete production period of the WCB (start of cell culture until end of production or beyond). Both guidelines only apply to recombinant allergens. In case an allergen product is manufactured from primary cells, attachment 1 of the ICH Q5D *[G19]* applies.

4.3.2 Reference standards (IHRP)

Reference standards are used for the identification of the allergen products (protein pattern and major/minor allergen pattern), and their activity/potency. If available, an international or national reference standard with known content of the major allergens and the allergenic activity should be used as reference for the control of allergen products. If no official standard is available, an IHRP should be established.

IHRP – allergen extracts

The allergen guideline EMEA/CHMP/BWP/304831/2007 *[G1]* and the allergen Ph. Eur. general monograph *Allergen Products (1063) [EP1]* provide detailed information on the characterization of the IHRP to be used as reference for allergen extracts. The IHRP characterization includes among others the determination of the protein content and profile and the detection of the relevant allergens and their content if possible. The potency should be

determined as pharmacologic potency in patients (e.g. skin-prick testing) or if this is not possible as the biological activity in biological assays.

IHRP – recombinant allergens

The IHRP for analysis of recombinant allergens should be established according to the provisions of ICH Q6B *[G9]* which states that an "appropriately characterized in-house reference material" should be used without providing further information on the characterization requirements. However, detailed information on the characterization of active substance and product are provided including examples of appropriate analytical techniques. Therefore, it can be assumed that the IHRP needs to at least fulfill all the requirements of an active substance/finished product. The biological activity of the IHRP using according biological assays should be established and linked to the clinical response (pharmacologic potency).

The allergen guideline EMEA/CHMP/BWP/304831/2007 *[G1]* as well as ICH Q6B *[G9]* expect the IHRP to be manufactured with the same process as is used for the batches for which they serve as reference. By doing so product-related substances and product/process-related impurities can be compared thereby providing information on the quality of the manufacturing process.

During the "CREATE" project the attempt was made to develop purified or recombinant reference standards which are applied to the standardization of allergen products (recombinant and extracts) in order to obtain comparable allergen products with respect to their composition and potency [No 19] [No 20] [No 21]. This project which meanwhile focuses on the development of recombinant standards for application to recombinant allergens and allergen extracts is continued by the European Directorate for the Quality of Medicines & HealthCare (EDQM) under the name "BSP090". Up to now only two recombinant allergens fulfill the requirements of the EDQM (Phl p 5 and Bet v 1). The standards can be found in the EDQM reference standard list *[DB3]* [https://crs.edqm.eu/db/4DCGI/web_catalog_CRS and https://crs.edqm.eu].

The USP provides the reference standard list of the US *[DB4]* [http://www.usp.org/reference-standards/find-reference-standard].

The WHO provides a list of five WHO international reference preparations for allergens *[DB5]* [http://www.who.int/bloodproducts/catalogue/en/], all of which are extracts. Criteria for the development of biological standards are provided in the *WHO Technical Report Series*, No. 932, 2006 [22].

4.3.3 Specifications

The guideline EMEA/CHMP/BWP/304831/2007 *[G1]* with respect to the control of recombinant allergens refers to several guidelines relevant for recombinant medicinal products. The guideline ICH Q6B *[G9]* is the central guideline for setting of specifications, acceptance criteria and analytical methods and test procedures for biological and biotechnological products.

Guideline ICH Q6B *[G9]* applies to "proteins and polypeptides, their derivatives, and products of which they are components (e.g., conjugates). These proteins and polypeptides are produced from recombinant or non-recombinant cell culture expression systems and can be highly purified and characterized using an appropriate set of analytical procedures." Although the guideline may be applied to proteins and polypeptides which are isolated from tissues or body fluids, allergen extracts as well as some other products are excluded from the scope of the guideline.

For allergen extracts the allergen guideline EMEA/CHMP/BWP/304831/2007 *[G1]* refers to the allergen Ph. Eur. general monograph *Allergen Products (1063) [EP1]* as well as to the herbals guidelines EMA/CPMP/QWP/2820/00 *Guideline on specifications: test procedures and acceptance criteria for herbal substances[1], herbal preparations[2] and herbal medicinal products[3]/traditional herbal medicinal products [G36]* and EMA/CPMP/QWP/2819/00 *Guideline on quality of herbal medicinal products[1]/traditional herbal medicinal products [G37]*.

In the following, aspects of the setting of specifications for recombinant allergens and allergen extracts are discussed.

4.3.3.1 Identity and purity

Any expected modifications of an active substance (e.g. post-translational modifications of recombinant allergens, or naturally occurring allergen isoforms of allergen extracts) are

considered to be part of the active substance. Process-related modifications of the target molecule, if still showing the desired properties of the active substance are defined as product related substances. Both, active substance and finished product-related substances are the objects of identity, heterogeneity, and purity determination.

Identity and purity - Recombinant allergens

According to ICH Q6B *[G9]* e.g. the protein and allergen profile, isoform pattern, chromatographic patterns (e.g. SEC) but also detailed structural information such as the amino acid sequence, peptide maps etc. of recombinant allergens should be shown in order to demonstrate the identity, homogeneity and the purity of the active substance/finished product. The structural integrity of the recombinant allergen is also stressed in the guideline EMEA/CHMP/BWP/304831/2007 *[G1]*. Furthermore, ICH Q6B *[G9]* specifies for recombinant products that epitopes of the target molecule should be recognized “if feasible”. Therefore, in case of recombinant allergens bearing allergen epitopes of different species, these epitopes should be detected if possible.

Identity and purity - Allergen extracts

The allergen Ph. Eur. general monograph *Allergen Products (1063) [EP1]* provides only little information with respect to source material testing. “Identification” which refers to the identification of the correct species (e.g mite species, plant species from which the pollen was collected) and “purity” which refers to the contamination with foreign species and foreign matter (e.g. pollen of another than the intended species and e.g. soil and plant particles) are requested. As already mentioned in chapter 3 of this review the draft monographs on source material *[EP2 to EP7]* additionally request testing in order to demonstrate batch to batch consistency of the source material. This includes the analysis of e.g. total protein content, the protein and allergen profile, the major allergen content and the total allergen activity. However, the draft monographs state that in order “to establish batch-to-batch consistency, one or more of the following tests are used. The choice of tests must be justified.” Therefore, the extent of analysis and the test methods applied (based on justification) lie in the discretion of the manufacturers. As not all parameters may be tested and different test methods may be applied, it is very likely that the information on batch data will vary from one source material manufacturer to another.

The guideline EMEA/CHMP/BWP/304831/2007 *[G1]* lists the tests and acceptance criteria for the active substance which include testing of identity (protein profile and allergen profile including the presence of relevant allergens if possible) in comparison with an IHRP, purity, impurities as well as the total allergenic activity and the major allergen content.

For finished product testing the guideline EMEA/CHMP/BWP/304831/2007 *[G1]* states that "appropriate specifications should be set for the finished product". With respect to the Ph. Eur. general monograph *Allergen Products (1063) [EP1]* this includes (just as for the active substance) the testing of the protein and allergen profile by comparing the product with an IHRP or a representative batch if no IHRP is available. Alternatively, an allergen-specific reference standard may be used. Guideline EMEA/CHMP/BWP/304831/2007 *[G1]* emphasizes that the presence of relevant allergens should be verified if possible and explains: "The choice of the relevant allergen components subject to determination must be justified. If a significant part of the total allergenic activity or safety concerns arise from other (for example minor) allergens, these have to be measured as well." Furthermore, testing of relevant allergen content and total allergenic activity is required (please see discussion below). All tests should be performed as late as possible during the manufacturing procedure. The guideline EMEA/CHMP/BWP/304831/2007 *[G1]* refers also to the herbals guidelines EMA/CPMP/QWP/2819/00 *[G37]* and EMA/CPMP/QWP/2820/00 *[G36]* in its references. However, these guidelines do not add further information regarding specifications.

4.3.3.2 Quantity and biological activity/potency

Quantity and biological activity/potency - Recombinant allergens

According to the ICH Q6B *[G9]* and the guideline EMEA/CHMP/BWP/304831/2007 *[G1]* the quantity of recombinant allergens should be expressed in "mass" (mass/volume) on the basis of the physico-chemical characteristics of the molecule.

The biological potency (e.g. as allergenic activity) should be evaluated using a biological assay (e.g. animal biological response to the product, cell culture based biological assays, biochemical assay) as is exemplified in ICH Q6B chapter 2.1.2. *[G9]* and can be expressed in units or in mass. The guideline EMEA/CHMP/BWP/304831/2007 specifies that assays other

than IgE binding assay may be used (e.g. ELISA) if the assay used shows correlation with IgE binding.

For expression in “units” the biological assay activity of the finished product is compared to the according activity of an international of national reference standard if available. Otherwise, an IHRP should be established and the potency is expressed as “in-house unit”.

The expression of potency in “mass” is possible if the mass of the recombinant molecule (the immunogenic entity) is known and can be correlated with a specific biological assay activity.

Quantity and biological activity/potency - Allergen extracts

According to guideline EMEA/CHMP/BWP/304831/2007 *[G1]* determination of relevant allergen content, total allergenic activity and, in case the product is defined on the basis of a relevant allergen, the activity of the relevant allergen should be determined and correlated with the total allergic activity.

The guideline explains in its chapter 4.2.4.1: “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 total potency (“total allergenic activity”) of allergen extracts and purified allergens (recombinant allergens) should be determined by a competitive IgE-binding test. This applies to allergens without structural modifications.

Furthermore, the guideline EMEA/CHMP/BWP/304831/2007 *[G1]* states that if the finished product is determined by a relevant allergen, the activity of this allergen and the correlation of its activity with the total allergenic activity should be determined. The Ph. Eur. general monograph *Allergen Products (1063) [EP1]* allows for flexibility of the choice of method by stating that “.a validated test measuring the potency (total allergenic activity, determination of individual allergens or any other justified tests) must be applied.”

4.3.3.3 Impurities and contaminants

ICH Q6B *[G9]* explains which kind of impurities may be seen in biological/biotechnological active substances and finished products. Any modified active substance molecules which do

not show the desired property are considered product-related impurities. Some of these impurities may have intrinsic causes, due to mutations, deletions etc. which may occur naturally in plants and animals or which are due to instabilities of the expression construct in case of recombinant allergens (product-related impurities which appear during upstream processing). Other product-related impurities such as truncations, polymerizations of the molecule may appear during manufacture and storage (product-related impurities which appear during downstream processing).

Other impurities may develop during manufacturing and storage (process-related impurities) without direct relationship to the target molecule and may concern both recombinant allergens and allergen extracts. Furthermore, unintended contaminants may be introduced during the manufacturing process. Process-related impurities and contaminants can be divided in

- upstream-derived impurities as there are a) cell substrate-derived impurities (e.g host cell protein, host cell DNA) and b) cell culture-derived impurities (e.g. cell culture medium constituents such as salts, growth factors, sera, antibiotics).
- downstream-derived impurities which arise during isolation and purification of the allergen (e.g. solvents, salt, and any other kind of processing agent).
- upstream and downstream-derived contaminants which are introduced unintentionally (e.g. adventitious viruses, mycoplasms, endotoxins, pesticides).

ICH Q6B *[G9]* provides a list of process-related impurities and contaminants in its chapter 6.2.1. The examples relate to cell culture-derived products. However, to some extent parallels to allergen extracts may be drawn. E.g. contamination with cultivation media of natural allergen sources (e.g. mites and moulds) correspond to cell culture-derived impurities. The same applies to downstream-derived impurities. E.g. defatting of pollen using light petroleum may result in contamination of the medicinal substance or product. However, the manufacturing process may likely be capable to remove them (e.g. multiple washing steps and lyophilization).

Neither endogenous nor adventitious viruses are a concern for plant-derived allergen extracts. However, if the growth media e.g. for mites contain components of animal origin, their viral

safety should be considered as viral contamination may occur. For animal sourced allergens (e.g. epithelia) viral as well as TSE contamination also should be considered.

The guidelines ICH Q3A(R2) *Impurities in new drug substances [G38]* and ICH Q3B(R2) *Impurities in new drug products [G39]* refer to new chemical-synthetic active substances and finished products, respectively. Both guidelines exclude biological/biotechnological products from their scope. The ICH Q3A(R2) addresses impurities (organic, inorganic impurities and solvents) which develop during synthesis, purification and storage of new chemical-synthetic drug substances, thereby excluding impurities which were introduced unintentionally. ICH Q6B refers to impurities in the finished product resulting from the degradation products of new synthetic APIs. Therefore, none of these guidelines apply to allergens.

ICH Q3B(R2) *[G39]* is also not applicable in any way as this guideline exclusively discusses impurities of chemical-synthetic products as the consequence of degradation processes.

Guidelines and monographs applicable to allergens which are concerned with a variety of impurities are discussed below.

Impurities – Host cell protein and DNA

The CPMP position paper CPMP/BWP/382/97 *CPMP position statement on DNA and host cell proteins (HCP) impurities, routine testing versus validation studies [G40]* suggests to perform process validations or to test for contamination during manufacturing or to combine both approaches. The guideline provides information of conditions under which testing of HCP and host cell DNA during manufacturing is required. Host cell DNA of continuous cell lines was suspected to be tumorigenic. Meanwhile such contaminating DNA is considered a common impurity and should be dealt with accordingly. The position paper refers to the *WHO Weekly Epidemiological Record* for further information [23].

The position paper explains that HCPs irrespective of the manufacturing system being bacteria, yeasts, continuous mammalian cell lines (CCLs) show a potential immunogenic effect and therefore need to be controlled for each batch. Analytical procedures and validation approaches to ensure removal during the manufacturing process need to be established on a case-by-case basis. The ICH Q6B *[G9]* suggests to establish reference materials (if required) to address product and process-related impurities (such as co-extracted protein) if required.

Contaminating proteins (co-extracted proteins) in allergen extracts, are not addressed as impurities in the guideline EMEA/CHMP/BWP/304831/2007 *[G1]*. However, the guideline expects the protein profile to correspond to the profile of the IHRP. The emphasis of the guideline lies with the presence of the relevant allergens.

Impurities – residual solvents

The guideline on residual solvents ICH Q3C(R5) *Impurities: Guideline for residual solvents [G41]* introduces the classification of residual solvents (class 1, 2 and class 3 solvents), the setting of exposure limits, analytical procedures, reporting levels and gives more detailed information on specific impurities. The guideline is also reflected in the general Ph. Eur. chapter *5.4. Residual solvents [EP9]* where the ICH guideline is presented as *Impurities: Guidelines for Residual solvents (CPMP/ICH/283/95).* The ICH Q3C(R5) applies to all types of active substances, finished products and excipients thereby encompassing all types of allergens. The allergen Ph. Eur. general monograph *Allergen Products (1063) [EP1]* which is concerned with allergen extracts only requests the determination of residual solvents by referring to Ph. Eur. chapter *2.4.24. Identification and control of residual solvents [EP10]* (which itself again refers to Ph. Eur. chapter *5.4. Residual solvents).*

Recombinant allergens and allergen extracts both may contain residual solvents as solvents are commonly used for the extraction of allergens. Therefore, guideline ICH Q3C(R5) *[G41]* as well as the Ph. Eur. chapter *5.4. Residual solvents* and Ph. Eur. chapter *2.4.24. Identification and control of residual solvents [EP10]* apply to recombinant allergens and allergen extracts.

Impurities – elemental impurities and heavy metals

Recombinant allergens:

ICH Q3D *Guideline for elemental impurities [G42]* is concerned with elemental impurities including heavy metals. The guideline applies to new finished products as defined in ICH Q6A *[G43]* and ICH Q6B *[G9]* and to new products whose API already existed previously. The scope of ICH Q3D includes recombinant allergens while excluding allergenic extracts and herbal products. The guideline advises on the risk assessment/setting of permitted daily exposure limits, the elemental classification which is based on the toxicity and the likelihood

of appearance, and the risk assessment of the manufacturing process (assessment of sources of elemental impurities and their identification). Special considerations for biotechnology-derived products are provided. For biotechnological active substances including recombinant allergens, the risk of elemental contamination is considered to be low. The manufacture of the finished product is more prone to elemental contamination as the impurities may be introduced via excipients, the equipment or the primary packaging (leachables, extractables). The allergen guideline EMEA/CHMP/BWP/304831/2007 *[G1]* does not mention elemental impurities and heavy metals as a concern for recombinant allergens.

Allergen extracts:

The guideline EMEA/CHMP/BWP/304831/2007 *[G1]* mentions heavy metals as a concern for allergen extracts of plant origin. Risk of heavy metal contamination may be due to environmental pollution (e.g. fossil fuel or coal combustion). Both the allergen Ph. Eur. general monograph *Allergen Products (1063) [EP1]* and the pollen-specific Ph. Eur. draft monograph *[EP7]* advise on the testing of heavy metals for pollen source material without referring to the according Ph. Eur. chapter. The herbals guidelines EMA/CPMP/QWP/2820/00 *[G36]* refers to the Ph. Eur. chapter *2.4.27. Heavy metals in herbal drugs and herbal drug preparations [EP11]* for heavy metal testing. The monograph itself refers to a variety of further monographs for specific test methods to analyze heavy metals. It is not clearly stated, how the acceptance criteria may be set. The general Ph. Eur. chapter *5.20. Metal catalysts or metal reagent residues [EP12]* which is based on EMEA/CHMP/SWP/4446/2000 *Guideline on the specification limits for residues of metal catalysts or metal reagents* provides acceptance criteria which however, refer to metal catalysts and metal reagents which served a purpose during the manufacturing process. The monograph points out, that extraneous metal contaminants should be “addressed by GMP, GDP or any other relevant quality provision”. Good agricultural and collection practice as described in the guideline EMEA/HMPC/246816/2005 *[G30]* may help to avoid heavy metal contamination. The herbals guideline EMA/CPMP/QWP/2820/00 *[G36]* states that “acceptance criteria … depend on safety considerations”.

Impurities – pesticides

Contaminations which are specific for allergen extracts of plant origin are contamination of the source material with residues of pesticides (please see definition of the term “pesticide” as defined in Dir. 2009/128/EC article 3(10) (a) (b) *Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 establishing a framework for Community action to achieve the sustainable use of pesticide [RE4]*. In order to avoid pesticide contamination GACP should be practiced *[G30]*. The draft allergen monograph for pollen as source material refers to the Ph. Eur. chapter *2.8.13. Pesticides residues for pesticide testing [EP13]* for testing. The monograph provides limits for different pesticides and information on their analysis.

Viral contamination/TSE contamination

Viral contamination - Recombinant allergens:

The guideline ICH Q5A(R1): *Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin [G44]* [is applicable to all cell bank based products including recombinant DNA-derived products and hybridomas. Any biotechnological product bears the risk of viral contamination because the MCB may carry endogenous viruses, was obtained from infected animals as source or was infected during upstream processing. Therefore, the MCB needs to be carefully selected and the manufacturing process should be capable of reducing or inactivating viruses. The ICH Q5A(R1) advises on the virus analysis to be performed at the MCB, the WCB and the cell line at the end of its use for production, on analytical procedures and presents a scheme to decide on the necessity of viral clearance studies. For recombinant allergens manufactured using a eucaryotic cell line, the guideline will apply.

The Ph. Eur. chapter *5.2.8. Minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products [EP8]* also applies to recombinant allergens and refers to ICH Q5A(R1) and guideline CPMP/BWP/268/95 *[G21]* for further information. Guideline CPMP/BWP/268/95 applies to any biological medicinal product including recombinant allergens. Guideline CPMP/BWP/269/95 *[G22]* may be considered a supportive guideline for recombinant allergens.

The guideline EMA/CHMP/BWP/457920/2012 *[G23]* informs about testing for adventitious agents (bacteria, fungi, mycoplasma, and particularly for viruses) as well as about viral inactivation and is applicable to recombinant allergens as was discussed in chapter 4.2.1.2 of this thesis. The Ph. Eur. monograph *Bovine serum (2262) [EP15]* advises on quality specifications for bovine serum and should be considered when selecting bovine serum batches for medicinal product manufacturing.

The note for guidance EMEA/410/01 *[G24]* and the corresponding Ph. Eur. chapter *5.2.8 Minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products [EP8]* also apply as already discussed above. Compliance with the guideline/monograph might be certified by the TSE Certificate of Suitability (EDQM). This certificate may be used as a basis to assess the risk of using such materials for the manufacture of a medicinal product.

Viral contamination - Allergen extracts:

As already discussed in chapter 4.2.1.2 the guidelines CPMP/BWP/268/95 *[G21]* , CPMP/BWP/269/95 *[G22]* both of which are referred to in the guideline EMEA/CHMP/BWP/304831/2007 *[G1]*, as well as EMA/CHMP/BWP/457920/2012 apply to allergen extracts. These guidelines are concerned with viral and further adventitious agents. Ph. Eur. chapter *5.1.7. Viral safety [EP14]* also applies to allergen extracts.

With respect to potential BST/TSE contamination guidance EMA/410/01 *[G24]* and the corresponding Ph. Eur. chapter *5.2.8. Minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products [EP8]* will apply as already discussed above.

Little information is provided for good collection practice of animal source material. Some information is available in the annex 1 of the *WHO Technical Report Series* 822 [24] and in the Ph. Eur. chapter *5.2.8. Minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products.*

Further microbial contamination

The allergen Ph. Eur. general monograph *Allergen Products (1063) [EP1]* states that "Allergen products presented as parenteral preparations, eye preparations, preparations for

inhalation or preparations for skin testing comply with the test for sterility." The same is requested by the allergen guideline EMEA/CHMP/BWP/304831/2007 *[G1]*. Although not mentioned directly this will imply testing according to Ph. Eur. chapter *2.6.1. Sterility [EP16]*. For other dosage forms (e.g. for oral administration) the Ph. Eur. chapter *5.1.4. Microbiological Quality of Pharmaceutical Preparations [EP17]* is requested. In case of e.g. multi-use containers the allergen guideline requests efficacy testing of the preservative according to Ph. Eur. chapter *5.1.3. Efficacy of antimicrobial preservation [EP18]*.

Achieving the requirements of the Ph. Eur. monograph on preservatives may be challenging, as not only the efficacy of preservation but also the compatibility with the active substance and other excipients as well as safety issues need to be considered and may limit the amount of preservative to be used.

Sterile allergen products with respect to preservatives need to fulfill the "A criteria" for parenteralia of the Ph. Eur. chapter *5.1.3. Efficacy of antimicrobial preservation*. Only if justified, the "B criteria" may suffice. The Japanese Pharmacopoeia (JP) in its monograph *Preservative Effectiveness Test* [25] assigns the term "Category 1A" to these products. In the United States Pharmacopeia (USP) monograph *<51> antimicrobial effectiveness testing*, [26] the products mentioned above are called "category 1 products". Both the JP and the USP provide less stringent requirements than the Ph. Eur. monograph (see table 3).

Table 3 JP, USP, and EP requirements for antimicrobial effectiveness test

Time	JP	USP	EP criteria A	EP criteria B
Requirements for bacterial log reduction				
6 h	Not required	Not required	2	Not required
24 h	Not required	Not required	3	1
7 d	Not required	1	No recovery	3
14 d	3	3	No recovery	Not increase
28 d	No increase compared to day 14 count	No increase compared to day 14 count	No recovery	Not increase

Time	JP	USP	EP criteria A	EP criteria B
Requirements for fungal log reduction				
7 d	Not required	No increase	2	Not required
14 d	No increase	No increase	No increase	1
28 d	No increase	No increase	No increase	No increase

Elder *et al.* question in their publication [27] the necessity of such stringent requirements as provided for in the Ph. Eur. chapter *5.1.3. Efficacy of Antimicrobial Preservation* and emphasize that "there is no clear evidence that the USP performance criteria have led to poorly preserved products within the US" and refer to Sutton *et al.* [28] for evidence and consequently request the harmonization of the according monographs of Japan, USA and EU.

4.3.4 Excipients

The guideline EMEA/CHMP/QWP/396951/2006 *Guideline on excipients in the dossier for application for marketing authorisation of a medicinal product [G45]* provides general information on excipients such as fillers, lubricants, solubilizers, flavouring, antioxidants, preservatives, adjuvants etc. All medicinal products for which a MAA or a variation is filed are in the scope of this guideline. This includes products from allergen extracts and recombinant allergen products. The guideline informs about the required information to be provided with the dossier and refers to guidelines, monographs etc.. In its annex 1 the guideline informs about different types of excipients (e.g. single chemical entities, ready to use mixtures, physically or chemically modified excipients), in annex 2 it informs about antioxidants and antimicrobial preservatives and in annex 3 it informs about solubilisers and permeation enhancers.

Excipients which are of particular concern for allergens are preservatives and adjuvants. Therefore, these two topics will be discussed further.

4.3.5 Preservatives

Many allergen products require to be sterile. In case of multi-use containers preservatives are used to avoid microbial growth during the period of use. The efficacy of the preservatives

need to adhere to the Ph. Eur. chapter *5.1.4. Microbiological Quality of Pharmaceutical Preparations [EP17]*. The review article by Meyer *et al.* [29] provides an overview of preservatives used in parenteral medicinal products and a list of the most common preservatives and their concentrations used in peptide and protein biological medicinal products. The part one of a three-part review by Elder *et al.* also provide information on the preservatives available, their mode of action etc. [27].

For choosing a preservative, different aspects such as their antimicrobial efficacy in the environment of the dug product (e.g. pH), compatibility with the active substance and other constituents of the product including their influence on the interaction of the biological active substance (e.g. the allergen) with an adjuvant as well as toxicological aspects need to be considered. In chapter 4.3.3.3 of this review the requirements of the Ph. Eur. were discussed.

4.3.6 Adjuvants

The guideline EMEA/CHMP/VEG/134716/2004 *Guideline on adjuvants in vaccines for human use [G46]* addresses the use of adjuvants in new or established vaccines. The guideline applies to adjuvants which are "... a component that potentiates the immune responses to an antigen and/or modulates it towards the desired immune responses". The principles of this guideline as far as quality and non-clinical aspects are concerned are also applicable to "therapeutic vaccines" which, according to this guideline includes *inter alia* "allergen-specific immunotherapy" irrespective of the allergens being of recombinant origin or obtained by extraction. Diagnostic allergen products have no therapeutic purpose and therefore, are not within the scope of the guideline. However, diagnostic allergens are usually applied in an unmodified state (no adsorption to an adjuvant) anyway as is explained in the Ph. Eur. general monograph *Allergen Products (1063) [EP1]*. The guideline EMEA/CHMP/VEG/134716/2004 *[G46]* informs about quality issues of the adjuvants, the combination of adjuvants/antigens and the finished product (e.g. development, manufacture, control, stability). The clinical aspects of the guideline only refer to vaccines. Vaccines as opposed to "therapeutic vaccines" are defined as "those that provide immunity against infectious disease" thereby excluding allergens.

The guideline provides examples of adjuvants some of which may be used for recombinant allergens such as a CpG motif which would be one functional sub-unit of the recombinant

molecule. Other adjuvants are not an integral part of an either recombinant allergen or an allergen extract such as human immunomodulators (e.g. cytokines). The Ph. Eur. general monograph *Vaccines for human use (0135) [EP19]* explains that adjuvants "may be included in the formulation of the final vaccine or presented separately". The information contradicts the adjuvants in the vaccines guideline CHMP/VEG/134716/2004 *[G46]* which informs: "The incorporation of adjuvants into vaccine formulations is aimed at enhancing, accelerating and prolonging the specific immune response towards the desired response to vaccine antigens." This sentence is also quoted in Dir. 2001/83/EC *[RE1]* as is pointed out in the explanatory note CHMP/VWP/244894/2006 *Explanatory note on immunomodulators for the guideline on adjuvants in vaccines for human use [G61]* which concludes that an adjuvant "should be part of the (reconstituted) formulation".

Allergen extracts are commonly adsorbed to aluminum hydroxide. However, aluminum is questioned as adverse health effects are suspected [30] [31] whereas e.g. the GACVS does not see a health concern [32] and refers to studies performed by the US-FDA for support. The US-FDA used an assessment model of aluminum vaccines and came to the conclusion, that after vaccination (injections) the body burden even in low-weight infants never exceeded the acceptable thresholds. The FDA data *per se* were not presented.

Information on the use of aluminum in allergens can be drawn from the CHMP Safety Working Party´s response to the PDCO *CHMP Safety Working Party's response to the PDCO regarding aluminium hydroxide contained in allergen products [G47]*. The question and answer document discusses the applicability of the guideline on adjuvants EMEA/CHMP/VEG/134716/2004 *[G46]* as allergens are used in higher frequency and for a longer duration than vaccines which may lead to an accumulation of aluminum hydroxide in the body. In its response the CHMP confirms the applicability of the guideline also to allergens products. Furthermore, in the response document plasma levels of aluminum are calculated from food intake and allergy treatment. From these calculations it is concluded, that only for small children with a body weight of about 20 kg a theoretical health concern may arise during 6-weeks of maintenance therapy with an allergen product including > 0.5 mg Al/dose. From surveillance of adverse effects in pharmacovigilance and clinical studies no reason for concern was observed as only local side effects (e.g. granuloma) were detected.

The absorption rate of aluminum is not clear and may cause short term elevated aluminum levels in blood plasma. Therefore, the response document recommends to measure Aluminum plasma and urine levels e.g. at the beginning, during and after one year of maintenance therapy. Until such pharmacokinetic studies are available allergen products with an aluminum content of up to 0.5 mg/dose in children are considered acceptable.

With respect to acute toxicity triggered by AIT (neurotoxicity, osteomalacia, anemia) conducted with an aluminum containing product it is concluded that the aluminum exposure levels are comparable to other safe exposure levels (e.g. food). The theoretical concern of acute toxicity can be addressed with pharmacokinetic studies.

In a recent publication Jensen-Jarolim [33] the author discusses effects of aluminum due to environmental and occupational exposure and medicinal application. The author in conclusion suggests refining the tolerable weekly intake, to harmonize occupational exposure, to reduce the level of aluminum in parenteralia (especially for infants) and appeals to put products with alternative immune-modulators or allergen delivery system on “fast track” for approval.

In a recent safety evaluation of the Paul-Ehrlich-Institut (PEI) on aluminum in therapeutic allergen preparations [34] the authors, based on a model conclude that a three year lasting immune-therapy would result in an accumulation of 12 mg aluminum provided that eight applications per year with a single application every six weeks of an allergen finished product including 0.5 mg of aluminum/dose would be administered. 12 mg of accumulated aluminum is considered to be low as compared to other sources of exposure. A pharmcovigilance survey over a period from 1986 to 2013 was performed by the PEI. During this period some local effects were reported none of which were of serious nature. Furthermore, none of the neurologic effects reported could be connected to the use of aluminum as adjuvant.

Although the PEI considers the benefit-risk balance for the use of aluminum containing allergen therapeutics to be positive, the PEI suggests observing blood plasma levels during dose finding studies with adults in order to draw conclusions for the use in children, as children are the most vulnerable population with respect to exposure to aluminum.

Alternatives to aluminum as adjuvant for immunotherapy are discussed. MPL (3-O-desacyl-4′-monophosphoryl lipid A) which modulates the immune response is one candidate. The

combination of MPL (as immunostimulating agent) and the amino acid tyrosine (carrier of the allergen) also is under investigation [35]. CpG (non-methylated cytosine-guanine dinucleotiedes), an immune-modulators binding to Toll-like receptors of APCs is another candidate for allergens. MPL in combination with aluminum is already marketed as anti-virus vaccine (AS04). Information of Adjuants for use in AITis provided in a review by Pfaar et al. [36]. General information on adjuvants can be found in Davies G. (eds): Methods in Molecular Biology Vol 626/Vaccine Adjuvants. 2010, Humana Press Inc. [37].

4.4 Comparability/Biosimilarity

4.4.1 Comparability

The note for guidance ICH Q5E *Comparability of biotechnological/biological products [G48]* and the guideline EMEA/CHMP/BMWP/101695/2006 *Guideline on comparability of biotechnology-derived medicinal products after a change in the manufacturing process. Non-clinical and clinical issues [G49]* are concerned with "Proteins and polypeptides, their derivatives, and products of which they are components, e.g., conjugates" (quoted from ICH Q5E *[G48]*) irrespective of them being recombinant or non-recombinant or isolated from tissues and body fluids. Therefore, the principles of these guidelines may apply not only to recombinant allergens but also to allergens extracted from plant and animal tissues (pollen, epithelia etc.). However, the guidelines suggest to seek advice on their applicability from the regulatory authorities.

Both guidelines introduce the "comparability exercise" which is intended to evaluate if changes to the manufacturing process, be they during drug development or post MA, may influence the quality, safety and efficacy of a medicinal product. The more advanced the development process at the time of process modification, the more comprehensive the comparability exercise needs to be.

ICH Q5E *[G48]* is focused on the quality aspects of the comparability exercise whereas the guideline CHMP/BMWP/101695/2006 *[G49]* focuses on the pre-clinical and clinical aspects.

During the comparability exercise aspects which may impact the safety and efficacy such as the CQAs, critical manufacturing steps, the relationship between both of them and the reproducibility of the product quality need to be considered. Based on the knowledge about

the impact of the manufacturing process on the quality of the product and the influence of the quality attributes on the safety and efficacy, the criteria for a “highly similar” product can be defined. ICH Q5E *[G48]* refers to ICH Q6B *[G9]* for the characterization of the molecule before and after the manufacturing process change. If the quality attributes of the product after change of the manufacturing process deviate from the previous quality attributes and if these differences are suspected to impact the safety and/or the efficacy of the product pre-clinical and clinical testing should be considered. The guideline CHMP/BMWP/101695/2006 *[G49]* provides according advice for the testing.

A comparability exercise is “passed” by the modified manufacturing process if the quality parameters do not change or only change to such a small extent that the safety and efficacy of the product remains unchanged.

Comparability exercise – recombinant allergens

The ICH Q5E *[G48]*, the guideline CHMP/BMWP/101695/2006 *[G49]* as well as ICH Q6B *[G9]* to which ICH Q5E refer for quality issues encompass the same type of product including recombinant proteins, and polypeptides. Therefore, these guidelines apply to recombinant allergens without any limitations. These guidelines also apply to recombinant allergens conjugated to some other functional units without limiting the nature of such functional units to synthetic or biological units.

Comparability exercise – allergen extracts

Both, ICH Q5E *[G48]* and the guideline CHMP/BMWP/101695/2006 *[G49]* refer *inter alia* to proteins and polypeptides which are isolated from tissues and body fluids and may be of recombinant or non-recombinant manufacture. The guidelines also state that the proteins and polypeptides “can be highly purified and characterized”. However, high purification and characterization is not a prerequisite for the applicability of the guidelines. Therefore, their principles are applicable to allergen extracts.

Allergen extract molecules are not considered to be well characterized molecules. Despite improved manufacturing processes and analytical procedure, a protein cocktail is isolated by allergen extraction which cannot be characterized entirely. Therefore, CQAs of allergen extracts are defined by parameters, such as the major allergen content and total allergenic

activity, allergen and protein profile without characterizing the co-extracted proteins. As for recombinant allergens it may be required to apply additional test methods to allergen extracts in order to evaluate the impact of the manufacturing change on the CQAs and further attributes not analyzed on a routine basis. It is concluded that a comparability exercise at least for well-known allergen-extracts is possible.

4.4.2 Biosimilarity

The guideline CHMP/437/04 *Guideline on similar biological medicinal products, [G50]* lies down the principles for biosimilar medicinal products. The guideline states that "A biosimilar is a biological medicinal product that contains a version of the active substance of an already authorised original biological medicinal product (reference medicinal product) in the EEA." This definition refers to quality characteristics, the biological activity, the safety and efficacy of the "biosimilar". The guideline clarifies, that bioequivalence is not sufficient for MAA but that instead a comprehensive comparability exercise is required. The guideline provides some information on the biosimilarity approach and refers to EMA/CHMP/BWP/247713/2012 *Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: quality issues [G51]* and to ICH Q5E *[G48]* for more details.

The question and answer document EMA/837805/2011 *Questions and answers on biosimilar medicines (similar biological medicinal products) [G52]* clarifies that a biosimilar medicinal product may show minor differences of the molecule due to the complexity and natural variability of biologicals. However, the status of "biosimilarity" can only be granted if it is proven that any differences do not affect the safety or efficacy of the biosimilar product.

The guideline on similar biological medicinal products EMA/CHMP/BWP/247713/2012 *[G51]* also informs about the setting of QTTPs and on the performance of the biosimilarity evaluation which e.g. requires parallel extensive analysis of the "biosimilar" product and its reference product. The biosimilar approach according to this guideline is applicable to recombinant molecules which includes recombinant allergens. For other biological products (e.g. allergen extracts) at least the principles may be applied.

Currently no recombinant allergen products are on the market to which a biosimilar product might be developed. For allergen extracts considering their complexity the biosimilarity

approach is very likely not feasible. Therefore, the topic of biosimilarity is not discussed any further.

4.5 Stability

The guideline ICH Q1A(R2) *Stability Testing of New Drug Substances and Products [G35]* provides information about the "core" stability data for new active substances and finished products to be submitted with the MAA. The guideline introduces basic concepts for the testing of the stability of new chemical-synthetic active substances and finished products and refers to further ICH guidelines for additional information. The principles of ICH Q1A(R2) can be applied to biological/biotechnological products. The guideline introduces the basics of the stability design as there are e.g.: batch selection, storage conditions (long-term, intermediate, and accelerated conditions), testing frequencies, specifications etc.. It should be noted that ICH Q1A(R2) *[G35]* introduces the concept of "re-test" period and "shelf-life" for the active substance and finished product, respectively. The guideline at the same time points out, that many biological/biotechnological substances are labile and therefore only should be assigned a "shelf-life". This is confirmed by the guideline EMEA/CHMP/BWP/304831/2007 *[G1]* which only refers to "shelf-life" with respect to the stability of the active substance and finished product.

CPMP/QWP/ 122/02 *Guideline on stability testing: Stability testing of existing active substances and related finished products [G53]* is an extension to ICH Q1A(R2) (dealing with the same topics) for already existing active substances and finished products. As opposed to ICH Q1A(R2), this guideline only refers to chemical active substances and the related finished products and to herbal drugs/preparations/products. Biological and biotechnological active substances and their finished products are explicitly excluded from this guideline thereby also excluding allergens.

ICH Q5C *[G34]* is an annex to ICH Q1A(R2) *[G35]* and provides the basis for stability testing of biological active substances and finished products. The guideline only refers to "well-characterised proteins and polypeptides, their derivatives and products of which they are components, and which are isolated from tissues, body fluids, cell cultures, or produced

using rDNA technology." This includes recombinant allergens. It is stated that the guideline may be applied to conventional vaccines after consultation with the authorities. However, allergenic extracts are excluded from the scope of the guideline. The allergen guideline EMEA/CHMP/BWP/304831/2007 *[G1]* concurs with ICH Q5C *[G34]* by describing recombinant allergens as "predefined allergenic polypeptides", consisting e.g. of a specific major allergen or a specific mix of polypeptides. Allergen extracts instead consist of a variety of major and minor allergens and non-allergenic substances/proteins. Therefore, the ICH Q5C does not apply to allergen extracts. However, in chapter 4.2.5 of guideline EMEA/CHMP/BWP/304831/2007 *[G1]* it is stated that "the general principles defined in the ICH Q5C guideline for biological/biotechnological products should also be considered for allergen extracts." This reference to the ICH Q5C may be due to the fact that the manufacturing procedures for allergen-extracts have improved since the time when ICH Q5C came into force.

ICH Q5C *[G34]* provides some important information to be considered for allergen active substances and finished products (extracts and recombinant allergens). E.g only a shelf-life and no re-test period is foreseen for the active substance thereby confirming the according statement of guideline EMEA/CHMP/BWP/304831/2007 (see above). Moreover, the stability data submitted for the MAA need to be obtained from long-term, real-time, real condition (e.g. light, temperature, humidity etc.) stability studies as also is stated in the allergen guideline EMEA/CHMP/BWP/304831/2007 *[G1]*. As allergens may be conjugated to another component (in case of recombinant allergens) or adsorbed to an adjuvant which influences the potency of the allergen product, it should be verified during stability testing that no dissociation or degradation occurs by evaluating the biological activity of the allergen. The ICH Q5C also "strongly" suggests accelerated stability testing and stress testing. Accelerated testing may help to determine the shelf-life of the active substance or the finished product. Stress testing may help to detect weaknesses (e.g. strong susceptibility to pH changes) in the stability of the active substance or the product and according surveillance (e.g. testing and trend analysis) may be applied during routine storage. Stress testing conditions will very likely differ from those proposed in Q1A(R2) *[G35]* as the products are of biological nature. Therefore, proper test conditions must be established on a case-by-case basis.

Another guideline concerned with stability testing is the ICH Q1B: *Photostability testing of new active substances and medicinal products [G54]* for photostability testing. The guideline, which is an annex to ICH Q1A(R2) *[G35]*, covers the same active substances and products as its parent guideline. Therefore, its principles are applicable to biological and biotechnological active substances and finished product. With respect to photostability testing the ICH Q5C *[G34]* suggests to consult with the authority on a case-by-case basis.

The ICH Q1C *Stability testing: Requirements for new dosage forms [G55]* which also is an annex to ICH Q1A(R2) *[G35]* is not applicable to biological products including any type of allergen. The ICH Q1C states that in "certain justified cases" a reduced stability program may be acceptable when submitting a MAA for a new dosage form (different route of administration, different functionality/delivery system, different dosage forms). A new dosage form usually requires changes to the composition and the manufacturing process. However, any change to the composition and manufacturing process of a biological/biotechnological product is considered critical with respect to quality, safety, and efficacy as can be seen in the *Guidelines of 16.05.2013 on the details of the various categories of variations [RE2]*. Therefore, very likely a full set of stability data within the scope as described in the EMEA/CHMP/BWP/304831/2007 *[G1]* will be required.

Although the guideline EMA/CHMP/CVMP/QWP/441071/2011 *Guideline on stability testing for applications for variations to a marketing authorisation [G56]* states that biologicals, immunologicals and biotechnologicals are outside its scope the guideline provides valuable information on the requirements of stability testing in connection with variations for these types of products.

The allergen guideline EMEA/CHMP/BWP/304831/2007 *[G1]* permits reduced stability testing in the scope of the homologous group concept. According to this concept a full set of stability data of the representative allergen of a homologous group is required whereas for the non-representative allergens data may be extrapolated from the respective representative allergen. While real-time stability data of the representative allergens are required at the time of MAA, data for the non-representative allergens may be obtained during ongoing stability testing. However, the choice of test parameters which are extrapolated need to be justified, as

some characteristics which differ between the representative and non-representative allergens of a homologous group may influence the stability of the product.

The guideline ICH Q1D: *Bracketing and matrixing designs for stability testing of drug substances and drug products [G57]* may be applied to stability testing as is indicated in ICH Q1A(R2) *[G35]* and ICH Q5D *[G19]*. Although EMEA/CHMP/BWP/304831/2007 *[G1]* does not refer to ICH Q1D, it refers to the parent guideline ICH Q1A(R2) and to ICH Q5D both of which consider bracketing and matrixing. Therefore, bracketing and matrixing may be applied to allergens in addition to reduced testing in the scope of the homologous group concept. Based on justification it may be possible to add allergens to an already existing homologous group or to establish a new homologous groups based on the requirements for the establishment of homologous groups as presented in guideline CHMP/BWP/304831/2007 *[G1]*. However, in any case scientific advice should be sought.

Details on the conditions for in-use stability are provided in CPMP/QWP/2934/99 *Note for guidance on in-use stability testing of human medicinal products [G58]*. This note for guidance specifies in more detail the conditions for in-use stability testing which is only briefly described in ICH Q1A(R2) *[G35]* and may be applied to all types of products including biological/biotechnological products.

The ICH Q1F *Stability data package for registration in climatic zones III and IV [G59a]* on climatic zones which as opposed to ICH Q1A(R2) *[G35]* considered climatic conditions in countries outside the ICH region was withdrawn on 08. June 2006 (notification and explanation see CPMP/ICH/421/02, June 2006 *[G59b]*. Please note that the EMA reference number of the explanatory note is identical to the ICH Q1F guideline reference number itself. The information of this guideline is still of value for products which are marketed in countries of climatic zones III and IV. The parent guideline ICH Q1A(R2) still refers to this guideline. Biological and biotechnological products in the most cases require storage in the refrigerator or freezer. In case storage is possible at ambient temperatures, information on stability testing for products delivered to the mentioned climatic zones can be found in ICH Q1F *[G59]* and in the *WHO Technical Report Series*, No. 953, 2009, annex 2, appendix 1, table 1[38].

The statistical evaluation of stability data can be performed according to ICH Q1E *Evaluation of stability data [G60]* which applies to all types of products (chemical-synthetic, biological/biotechnological) including allergens.

The note of guidance CPMP/QWP/072/96 *[G33]* informs about the calculation of the finished product shelf-life. Typically, the shelf-life should be calculated from the day of batch release unless the release is later than 30 days after the production date (production date: date at which the API is combined with another ingredient the first time). In such a case the shelf-life needs to be calculated from the day of production. The guideline however, excludes biological/biotechnological medicinal products including allergens. The allergen guideline EMEA/CHMP/BWP/304831/2007 *[G1]* expects real-time stability data. In order to evaluate the shelf-life of the allergen finished product (extract or recombinant) the allergen guideline suggests to perform stability testing of the finished product using active substance at the end of its shelf-life. Kaul et al. [39] present a figure depicting scenarios of shelf-life determination for allergen products.

4.6 Primary packaging

The guidelines CPMP/QWP/4359/03 *Guideline on plastic immediate packaging materials [G62]* advises on the information needed for plastic immediate packaging material which will be introduced for an already approved product in the scope of a variation application and for new medicinal products in the scope of a MAA. The immediate packaging may be for packaging active substance and finished product irrespective of them being of chemical-synthetic or biological nature thereby including any type of allergen product. The guideline provides decision trees to decide on the topics (e.g. specifications, extraction studies, interaction studies (compatibility studies), toxicological information) which need to be addressed in the documentation. Furthermore, the guideline offers explanations concerning the studies which may be required to assure the suitability of the primary packaging. Further information on plastic containers and closures are provided in EP. Eur. chapter *3.2.2 Plastic containers and closures for pharmaceutical use [EP20]* which lists the most common polymers and informs about their testing.

The EP. Eur. chapter *3.2.1 Glass containers for pharmaceutical use [EP21]* which refers to glass containers such as vials, bottles and ampoules introduces the different types and classes of glass containers (Type I, II, and III) and surface treatments of glass. It discusses the risk of delamination and recommends compatibility testing (compatibility with the pharmaceutical preparation) by the pharmaceutical manufacturer. Furthermore, the monographs informs about testing of glass containers in order to assure their quality.

5 Conclusion

There is a wide range of regulatory documents which provide advice on the development, the manufacturing, the control and the stability of allergen extracts and recombinant allergens.

Allergen extracts:

Extensive information regarding allergen extracts can be found in the European Pharmacopoeia. The Ph. Eur. general monograph on *Allergen Products (1063)* which specifically addresses allergen extracts is the core monograph for allergen extracts. This monograph is joined by a variety of further general monographs which may be applicable under particular conditions (e.g. the general monograph on *Products with risk of transmitting agents of animal spongiform encephalopathies (1483)* and a wide range of general chapters which advise on quality testing.

Several ICH/EMA guidelines are also applicable to allergen extracts. The core documents for allergens are the guideline CHMP/EWP/18504/2006 which concerns the clinical development and was not further discussed in the scope of this thesis as well as guideline EMEA/CHMP/BWP/304831/2007 on the production and quality issues. Further guidelines, in particular the guidelines addressing biological/biotechnological products may apply partially considering certain topics or may be applied based on the “principles” provided in these guidelines.

Recombinant allergens

As for allergen extracts, a variety of general monographs apply to these allergens. The general monograph *Recombinant DNA technology, products of (0784)* may be considered the core monograph for recombinant allergens. A variety of general chapters provide further advice.

Further core guidelines on allergens are the EMA guidelines mentioned above which are applicable not only to allergen extracts but also to recombinant allergens. In addition, all ICH/EMA guidelines concerning recombinant biological products are applicable to recombinant allergens joined by ICH/EMA guidelines with general applicability.

The review presented here has the intent to provide an overview over the most important regulatory documents concerning allergen extracts and recombinant allergens. It is the hope, that the reader will find this review helpful in navigating the regulatory environment of allergen extracts and recombinant allergens.

6 Literature

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Appendix: Overview applicable regulatory documents

x applicable
x * applicable upon coming into force
(x) partially applicable or applicable according to the principles of the guideline
n.a. not applicable

Sequence of appearance	Document	Title Document	Chemical products	Recombinant allergens	Allergen extracts
		Regulations/Directives			
RE1	Dir. 2001/83/EC	Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the	x	x	x
RE2	C (2013) 2804	Guidelines of 16.05.2013 on the details of the various categories of variations, on the operation of the procedures laid down in Chapters II, IIa, III and IV of Commission Regulation (EC) No 1234/2008 of 24 November 2008 concerning the examination of variations to the terms of marketing authorisations for medicinal products for human use and veterinary medicinal products and on the documentation to be submitted pursuant to those procedures	x	x	x
RE3	Reg. (EC) No 726/2004	Regulation (EC) No 726/2004 of the European Parliament and of the Council of 31 March 2004 laying down Community procedures for the authorisation and supervision of medicinal products for human and veterinary use and establishing a European Medicines Agency	x	x	x
RE4	Dir. 2009/128/EC	Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 establishing a framework for Community action to achieve the sustainable use of pesticides	n.a.	n.a.	n.a.
	EMA Reference Number	**Guidelines and supportive Information**			
G1	EMEA/CHMP/BWP/304831/2007	Guideline on allergen products: production and quality issues	n.a.	x	x
G2	ICH Q8(R2) CHMP/167068/2004	Pharmaceutical development – Step 5	x	x	x
G3	ICH Q11 CHMP/ICH/425213/2011	Development and manufacture of drug substances (chemical entities and biotechnological / biological entities) – Step 5	x	x	x
G4	ICH Q9 CHMP/ICH/24235/2006	Quality risk management - Step 5	x	x	x
G5	ICH Q10 CHMP/ICH/214732/2007	Pharmaceutical quality system - Step 5	x	x	x
G6	ICH Points to Consider ICH Q8/Q9/Q10 (R2)	ICH Quality Implementation Working Group Points to Consider (R2), ICH-endorsed guide for the impler	x	x	x
G7	CHMP/ICH/265145/2009	ICH guideline Q8, Q9 and Q10 - questions and answers, volume 4 – Step 5	x	x	x
G8	ICH Q8, Q9, and Q10 training material	ICH Q8, Q9, and Q10 training materialhttp://www.ich.org/products/guidelines/quality/training-programme	x	x	x
G9	ICH Q6B CPMP/ICH/365/96	Test procedures and acceptance criteria for biotechnological/biological products - Step 5	n.a.	x	(x)
G10	ICH Q7 CPMP/ICH/4106/00	Good manufacturing practice for active pharmaceutical ingredients - Step 5	x	x	x
G11	EMA/CHMP/CVMP/QWP/BWP/70278/2012	Guideline on process validation for finished products - information and data to be provided in regulatory submissions	x	(x)	(x)
G12	CPMP/QWP/155/96	Note for guidance on development pharmaceutics	x	x	x

G13	CPMP/QWP/054/98	Decision trees for the selection of sterilisation methods (CPMP/QWP/054/98) Annex to note for guidance on development pharmaceutics (CPMP/QWP/155/96)	x	x	x
G14	CPMP/BWP/328/99	Development pharmaceutics for biotechnological and biological products (CPMP/BWP/328/99) Annex to note for guidance on development pharmaceutics (CPMP/QWP/155/96)	n.a.	x	x
G15	EMA/CHMP/QWP/96664/2015	Draft guideline on the chemistry of active substances	x*	n.a.	n.a.
G16	3AQ5A	Chemistry of active substances	x	n.a.	n.a.
G17	CPMP/QWP/130/96	Guideline on the chemistry of new active substances (CPMP)	x	n.a.	n.a.
G18	EMA/448443/2014	Reflection paper on the requirements for selection and justification of starting materials for the manufacture of chemical active substances	x	n.a.	n.a.
G19	ICH Q5D CPMP/ICH/294/95	Derivation and characterisation of cell substrates used for production of biotechnological/biological products - Step 5	n.a.	x	n.a.
G20	EMA/CHMP/BWP/187338/2014	Draft guideline on process validation for the manufacture of biotechnology-derived active substances and data to be provided in the regulatory submission	n.a.	x	(x)
G21	CPMP/BWP/268/95	Note for guidance on virus validation studies: the design, contribution and interpretation of studies validating the inactivation and removal of viruses	n.a.	x	x
G22	CPMP/BWP/269/95	Note for guidance on plasma-derived medicinal products	n.a.	x	x
G23	EMA/CHMP/BWP/457920/2012	Guideline on the use of bovine serum in the manufacture of human biological medicinal products	n.a.	x	x
G24	EMEA/410/01 (2011/C 73/01)	Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products	x	x	x
G25	CPMP/BWP/CPMP/5136/03	Guideline on the investigation of manufacturing process for plasma- derived medicinal products with regard to vCJD risk	n.a.	n.a.	n.a.
G26	EMEA/CPMP/BWP/498/01	Joint Committee on Proprietary Medicinal Products / CVMP note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products: explanatory note for medicinal products for human use on the scope of the guideline	x	x	x
G27	Eudralex Vol 4, annex 2	Manufacture of Biological active substances and Medicinal Products of Human Use	n.a.	x	x
G28	Eudralex Vol 4, Part II	Basic Requirements for Active Substances used as Starting Materials	x	x	x
G29	ICH Q5B CPMP/ICH/139/95	Analysis of the expression construct in cell lines used for production of r-DNA derived protein products - Step 5	n.a.	x	n.a.
G30	EMEA/HMPC/246816/2005	Guideline on good agricultural and collection practice (GACP) for starting materials of herbal origin	n.a.	n.a.	x
G31	CPMP/QWP/486/95 3AQ2a	Note for guidance on manufacture of the finished dosage form	x	n.a.	n.a.
G32	EMA/CHMP/QWP/245074/2015	Draft guideline on manufacture of the finished dosage form	x*	(x)*	(x)*
G33	CPMP/QWP/072/96	Note for guidance on start of shelf-life of the finished dosage form (annex to note for guidance on the manufacture of the finished dosage form)	x	n.a.	n.a.
G34	ICH Q5C CPMP/ICH/138/95	Stability testing of biotechnological/biological products - Step 5	n.a.	x	(x)
G35	ICH Q1A(R2) CPMP/ICH/2736/99	Stability testing of new drug substances and products - Step 5	x	x	x
G36	EMA/CPMP/QWP/2820/00	Guideline on specifications: test procedures and acceptance criteria for herbal substances[1], herbal preparations[2] and herbal medicinal products[3]/traditional herbal medicinal products	n.a.	n.a.	x
G37	EMA/CPMP/QWP/2819/00	Guideline on quality of herbal medicinal products[1/] traditional herbal medicinal products	n.a.	n.a.	x
G38	ICH Q3A(R2) CPMP/ICH/2737/99	Impurities in new drug substances - Step 5	x	n.a.	n.a.

G39	ICH Q3B(R2) CPMP/ICH/2738/99	Impurities in new drug products - Step 5	x	n.a.	n.a.
G40	CPMP/BWP/382/97	Position statement on DNA and host cell proteins (HCP) impurities, routine testing versus validation studies	n.a.	x	(x)
G41	ICH Q3C(R5) CHMP/ICH/82260/2006	Impurities: Guideline for residual solvents - Step 5	x	x	x
G42	ICH Q3D CHMP/ICH/353369/2013	Guideline on elemental impurities - Step 4	x	x	n.a.
G43	ICH Q6A CPMP/ICH/367/96	Test procedures and acceptance criteria for new drug substances and new drug products: chemical substances – Step 5	x	n.a.	n.a.
G44	ICH Q5A(R1) CPMP/ICH/295/95	Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin - Step 5	n.a.	x	n.a.
G45	EMEA/CHMP/QWP/396951/2006	Guideline on excipients in the dossier for application for marketing authorisation of a medicinal product	x	x	x
G46	EMEA/CHMP/VEG/134716/2004	Guideline on adjuvants in vaccines for human use	n.a.	x	x
G47	EMA/CHMP/381064/2010	CHMP Safety Working Party's response to the PDCO regarding aluminium hydroxide contained in allergen products	n.a.	x	x
G48	ICH Q5E CPMP/ICH/5721/03	Comparability of biotechnological/biological products - Step 5	n.a.	x	(x)
G49	EMEA/CHMP/BMWP/101695/2006	Guideline on comparability of biotechnology-derived medicinal products after a change in the manufacturing process. Non-clinical and clinical issues	n.a.	x	(x)
G50	CHMP/437/04	Guideline on similar biological medicinal products	n.a.	x	x
G51	EMA/CHMP/BWP/247713/2012	Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: quality issues (revision 1)	n.a.	x	(x)
G52	EMA/837805/2011	Questions and answers on biosimilar medicines (similar biological medicinal products) (EMA document library)	n.a.	x	x
G53	CPMP/QWP/ 122/02	Guideline on stability testing: Stability testing of existing active substances and related finished products	x	n.a.	n.a.
G54	ICH Q1B CPMP/ICH/279/95	Photostability testing of new active substances and medicinal products - Step 5	x	(x)	(x)
G55	ICH Q1C CPMP/ICH/280/95	Stability testing: Requirements for new dosage forms - Step 5	x	n.a.	n.a.
G56	EMA/CHMP/CVMP/QWP/441071/2011	Guideline on stability testing for applications for variations to a marketing authorisation	x	(x)	(x)
G57	ICH Q1D CPMP/ICH/4104/00	Bracketing and matrixing designs for stability testing of drug substances and drug products - Step 5	x	(x)	(x)
G58	CPMP/QWP/2934/99	Note for guidance on in-use stability testing of human medicinal products	x	x	x
G59a	(ICH Q1F) (CPMP/ICH/421/02)	Stability data package for registration in climatic zones III and IV	n.a.	n.a.	n.a.
G59b	ICH Q1F CPMP/ICH/421/02	Stability data package for registration in climatic zones III and IV, explanatory note on the withdrawal of ICH Q1F from the ICH Website	x	x	x
G60	ICH Q1E CPMP/ICH/420/02	Evaluation of stability data - Step 5	x	x	x
G61	CHMP/VWP/244894/2006	Explanatory note on immunomodulators for the guideline on adjuvants in vaccines for human use	n.a.	(x)	(x)
G62	CPMP/QWP/4359/03	Guideline on plastic immediate packaging materials	x	x	x

		European Pharmacopoeia Monographs and Chapters			
EP1	07/2014:1063	Allergen products (1063)	n.a.	n.a.	x
EP2	PA/PH/Exp. ALG/T (14) 1 ANP	Allergen products (1063) – draft	n.a.	n.a.	x*
EP3	PA/PH/Exp. ALG/T (14) 2 ANP	Animal epithelia and outgrowths for allergen products (2621) – draft	n.a.	n.a.	x*
EP4	PA/PH/Exp. ALG/T (14) 3 ANP	Hymenoptera venoms for allergen products (2623) – draft	n.a.	n.a.	x*
EP5	PA/PH/Exp. ALG/T (14) 4 ANP	Mites for allergen products (2625) – draft	n.a.	n.a.	x*
EP6	PA/PH/Exp. ALG/T (14) 5 ANP	Moulds for allergen products (2626 – draft)	n.a.	n.a.	x*
EP7	PA/PH/Exp. ALG/T (14) 6 ANP	Pollen for allergen products (2627) – draft	n.a.	n.a.	x*
EP8	07/2011:50208	5.2.8. Minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products	x	x	x
EP9	01/2008.50400	5.4. Residual solvents	x	x	x
EP10	01/2008:20424 corrected 7.2	2.4.24. Identification and control of residual solvents	x	x	x
EP11	07/2014:20427	2.4.27. Heavy metals in herbal drugs and herbal drug preparations	n.a.	n.a.	x
EP12	04/2013.52000	5.20. Metal catalysts or metal reagent residues	x	n.a.	n.a.
EP13	07/2014:20813	2.8.13. Pesticides residues	n.a.	n.a.	x
EP14	01/2008:50107	5.1.7. Viral safety	n.a.	x	x
EP15	01/2008:2262	Bovine serum (2262)	n.a.	x	x
EP16	04/2011:20601 corrected 7.7	2.6.1. Sterility	x	x	x
EP17	01/2014:50104	5.1.4. Microbiological quality of pharmaceutical preparations	x	x	x
EP18	01/2011:50103	5.1.3. Efficacy of antimicrobial preservation	x	x	x
EP19	01/2013:0153	Vaccines for human use (0135)	n.a.	(x)	(x)
EP20	04/2015:30202	3.2.2. Plastic containers and closures for pharmaceutical use	x	x	x
EP21	04/2015:30201	3.2.1 Glass containers for pharmaceutical use	x	x	x
		Data Bases			
DB1	EMBL-EBI Protein family database	http://pfam.sanger.ac.uk/	n.a.	x	x
DB2	WHO/IUIS Allergen data base	http://www.allergen.org/	n.a.	x	x
DB3	EDQM Reference standards	https://crs.edqm.eu/ https://crs.edqm.eu/db/4DCGI/web_catalog_CRS	n.a.	x	x
DB4	USP Reference standards	http://www.usp.org/reference-standards/find-reference-standard	n.a.	x	x
DB5	WHO Reference standards	http://www.who.int/bloodproducts/catalogue/en/	n.a.	x	x

Hiermit erkläre ich an Eides statt, die Arbeit selbstständig verfasst und keine anderen als die angegebenen Hilfsmittel verwendet zu haben.

Dr. Christa Lamping

Unterschrift