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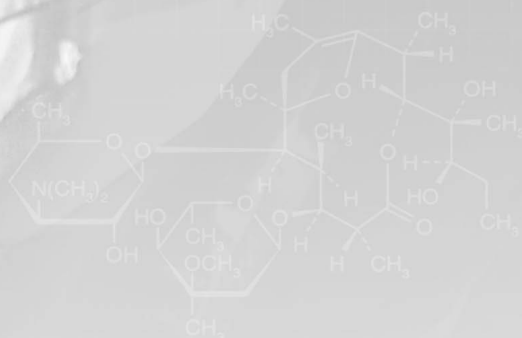


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New Impurities Control: Setting Specifications for Antibiotics and Synthetic Peptides

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PROCEEDINGS



NEW IMPURITIES CONTROL: SETTING SPECIFICATIONS FOR ANTIBIOTICS AND SYNTHETIC PEPTIDES

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**New impurities control: setting specifications
for antibiotics and synthetic peptides**

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Opening Remarks

Dr Michael Morris, Chair of the European Pharmacopoeia Commission

I am delighted to let you know that we have 135 participants registered from 30 countries, including China and India. It is my particular pleasure this morning to be here in the Palais de l'Europe, the headquarters of the Council of Europe. Many of you will be aware that the European Pharmacopoeia is established under the auspices of the Council of Europe, by a convention signed over 40 years ago, in 1964. It is very important; to first of all remember this, that the Pharmacopoeia has a wider applicability than just the EU. Secondly, that the Council of Europe itself represents 46 different countries, and covers a total population in excess of 850 million people. When one takes into consideration that the European Pharmacopoeia itself, in addition to its 35 member states, has 18 observer countries from other parts of the world; North Africa, from North America, from Austral-Asia and from a large part of Asia, you will understand that the topic we have before us today is a very important one, which affects a large number of manufacturers and users of pharmacopoeias, including regulators worldwide.

The European Pharmacopoeia is now part of the European Directorate for the Quality of Medicines, which is an institute within the Council of Europe, and it is particularly good news at this time that the new purposed design building of the EDQM is being located very close to here in the area of the Council of Europe headquarters, so we are returning back here after a number of years away from this area, and it is an exciting time that the new building is due to be occupied and finalized in the next few months.

I would just like to take you back a little to the origin of this particular conference, which goes back to the mid-90's, when there was the first attempt to set some kind of specifications and acceptance criteria for impurities on a rational basis, through the ICH-process. The resultant guideline Q3A, which was established for new active substances, specifically new active substances from chemical synthesis, set out a rational approach to controlling related substances, impurities, based upon not only chemical controls, but also on their toxicological evaluation. And those principles were rapidly taken up by many regulatory authorities and applied more widely, and the European Pharmacopoeia also, I think, extended its approach to controlling impurities, as is reflected in the general chapter on substances for pharmaceutical use 2034, and more recently the general information chapter (5.10), which sets out the policy of the European Pharmacopoeia to impurities. But the important thing is, while general monograph 2034 covers all substances which are covered by specific monographs in the European Pharmacopoeia, it excludes a number of types of active substances from the impurities section, in particular in the context of today's discussion; peptides, oligonucleotides and fermentation products and their semi-synthetic derivatives are excluded from the provisions currently of those impurities controlled, which were established through the ICH-Q3A process. This does not mean that you can ignore those ICH guidelines and the other general information chapters at the Pharmacopoeia. On the contrary, it just means that the general acceptance criteria established, are not necessarily appropriate, but the principles established within the guideline are very much appropriate. For various reasons, it may be that the general acceptance criteria are not easily met for antibiotics products of fermentation origin and their synthetic analogues, and for peptides. It may be, for simple practical reasons that it is not always feasible to adhere to the general acceptance criteria. It may be toxicologically unwarranted, and this has to be of course established on a case-by-case basis. Alternatively, it may be that more rigorous acceptance

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criteria are necessary for molecules where the related substances may exhibit more toxic type reactions. So, clearly, a general trend has been very much on developing specifications on a case-by-case basis.

So, the reason for the symposium this morning is to reflect this, and try to come up with a more systematic policy in going forward for the European Pharmacopoeia to deal with these particular categories of active substances, which are of course very important in pharmaceuticals.

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Opening Session

Development of the European Pharmacopoeia policy on impurities control
Mr Peter Castle, Secretary to the European Pharmacopoeia Commission
(EDQM, Council of Europe)

Introduction

Thank you very much for that kind introduction Dr Michael Morris. It is very nice to see so many people at the symposium this morning. What I want to do is to give a very brief kick-off speech, really outlining how we got to the point where we are at the moment and what we still need to do, particularly for antibiotics and peptides. So, we are concentrating on impurities control, which is really the core of all of the monographs that we draft on active substances, and six or seven years ago, there was a strong stimulus for us to begin to review our policy. Actually for us, the real stimulus was globalization, and we had been in a situation where active substances, their sources, were very well known, they were mainly in Europe, and the manufacturers were well known, we had good contacts with the manufacturers. That was changing, and of course now we know that the majority of active substances are manufactured outside Europe, and this changes the way that we have to see these products. There are new sources, and we have less contact, we do not have that good contact with the manufacturers, there are new impurity profiles, and this meant that we had really again to have a look at how we approach impurities control. At the same time, quantitative methods were replacing qualitative tests at the Pharmacopoeia, TLC being replaced by Liquid Chromatography and other methods. This, of course, was an opportunity for the Pharmacopoeia to improve impurities control, and in fact to make a change over, and use this in the way that we approached this problem created by globalization. It is essential that monographs reflect regulatory policy, and regulatory policy was of course developing with the new methods of control of impurities. We have to make monographs that are usable. We have to make monographs that are useful, and in the end we have to make monographs that are used by the different clients of the European Pharmacopoeia. Those are the three essentials from my point of view, and if we make monographs which in the end are not used, then really we have not achieved the aim, and we have put in a lot of work to no purpose.

Again, from my point of view, we need to have a uniform approach in our different groups of experts to impurities control. We have at least five groups dealing with synthetic active substances, and active substances of natural origin, alkaloids etc. Then we have one group dealing with antibiotics. We have one group dealing with synthetic peptides. Within the five groups dealing with synthetic active substances, we absolutely have to have a uniform approach; there is no justification for having a different approach in those groups. That also applies to some extent, and we will be seeing today and tomorrow just what the distinct features of antibiotics and peptides are. Nevertheless, for group 6 (Biological Substances) and 7 (Antibiotics) that means that we need to define our policy very clearly, if these groups are to be able to in fact follow a uniform policy.

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Events

These are some of the events that have contributed to the way our policy has developed. There was of course the ICH-Q3A guideline “Impurities in new drug substances”. That guideline represents a very widely accepted consensus on impurities control, widely accepted by manufacturers, regulatory authorities. In December 2001, after our reflections on globalization, EDQM decided to organize a seminar, where we went into the whole question of impurities control, and tried to begin putting on paper the general policy that we needed. There was already in preparation at that time, the general monograph on Substances for Pharmaceutical Use. That seminar affected the content of the general monograph. The general monograph came into force, with the 4th edition, in January 2002. One of the recommendations for the seminar in December 2001 was that we should have a general chapter on control of impurities. In order to explain very, very clearly what the policy of the European Pharmacopoeia was, and how the monographs should be interpreted, because there was some latitude, there were some differences in the way the monographs were interpreted, so we wanted to put down in black and white just what the proper interpretations of those monographs should in fact be.

General monographs and chapters

If we look at the general monograph 2034, “Substances for Pharmaceutical Use”, the first thing you notice is that it applies to all substances covered by European Pharmacopoeia monograph. At the moment, it does not apply to substances that are not covered by a specific monograph, although the regulatory authority of course does have the liberty to apply it to those substances, if the regulatory authority wishes to do so. For active substances, the decision was that the ICH approach for impurities control should apply. Of course, we know that the ICH approach excludes antibiotics as fermentation products, and also synthetic peptides. We know that the ICH guideline formally, is limited to new active substances, and ICH does define what a new active substance is. The decision for the European Pharmacopoeia was that since the ICH guideline had wide regulatory consensus and was based on sound principles, there was no reason, we could not justify in the Pharmacopoeia, limiting monograph 2034 and the impurities control to new active substances, and therefore the general monograph applies to all substances, new or existing.

That is the table taken from the monograph on Substances for Pharmaceutical Use, and of course it reflects very exactly, I hope, the Q3A guideline. I am sure you are all very familiar with the contents of that table, and that is now applied systematically in all new monographs that are drafted for the European Pharmacopoeia. This is the template that our groups of experts use, when they are drafting the test for related substances in the monographs. So, we now have a very standard, and I think a very clear template for specifications derived, using the terminology of the Q3A guideline, we have limits for specified impurities, and we have limits for unspecified impurities. Now, in older monographs, unspecified impurities come under the denomination of “any other impurity”, and we have had some difficulty with monographs on antibiotics, because we continued to call unspecified impurities “any other impurities”. This was to avoid any confusion with the definition of unspecified impurities, according to monograph 2034 at the identification threshold. I do not know whether keeping “any impurities” terminology has removed more confusion than it has caused and in fact, I

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do not know whether I should express a personal opinion here, but I have the feeling that it has probably caused more confusion than it actually removed. For the moment, we will continue to use that wording for the classes excluded from the paragraph on related substances in the general monograph. And of course finally, we have limits for total impurities in all of the monographs now. Now the older monographs do not, from a formal point of view, present this template very, very clearly. Although the older monographs mean the same thing as the newer monographs, it is a question of editorial presentation, and for the 6th edition, which will be published the middle of next year, we will make editorial revision of the older monographs, so that in fact they will be represented in the newer and clearer style. I think that will make a big improvement for the presentation of these monographs.

General chapter 5.10 on control of impurities in substances for pharmaceutical use was drafted as a recommendation from the symposium in December 2001. It sets out the basis for monographs and impurities control. It defines our terminology, and it has a large section on the interpretation of related substances test, which is particularly useful for the monographs that are in the older style. Once we have made the editorial revision of the monographs, this part of general chapter 5.10 will in fact, I hope, be just about obsolete. It also goes into many other aspects of impurities control in the European Pharmacopoeia.

A revision of that general chapter was published recently, where we tried to make things even clearer. In the first version, we had a number of examples of monographs, trying to explain, in that way, how to interpret them. In the more recent revision, we put in a decision tree, which we felt was a clearer way of explaining how to interpret the monographs, and I think that it made things so clear, that it then caused trouble because people realized just what the monographs meant. In any case, it is of course essential reading for anybody who is using the monographs of the European Pharmacopoeia.

Template for specifications

That is the decision tree, I do not know whether you can read it or not; you start in the top left hand corner and you move down, either moving to the right or moving down to the next box, and in that way, for the older monographs, you should be able to work out exactly how you are to go about interpreting the monographs and using the monographs.

This symposium is devoted to antibiotics and peptides. You can see from the general monograph that the paragraph on related substances does not apply to these products, antibiotics, because they are fermentation products. The general chapter 5.10 will tell you that they are excluded from the thresholds. I think that it is important to note, excluded from the thresholds. We have the same template for specifications in monographs on antibiotics and peptides, particularly in the new monographs, but as I said, unspecified impurities are usually referred to as “any other impurities”, to avoid confusion, or to cause confusion. In fact, at the moment, I think we have to say and recognise that we do not have a general policy for unspecified impurities in antibiotics and peptides, and that is an uncomfortable situation for us, because when you have a general policy, then the monographs go through the drafting, the elaboration process much more easily. When you do not have a general policy, then of course you will always have different points of view on how we should be drafting the monographs.

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So, here we have the decision tree again, and of course for antibiotics and peptides, when you start in the top left hand corner, in almost all cases, you immediately move to the box on the right, and that means that the specifications for unspecified impurities apply to anything. Now, if we interpret that as meaning “anything under the sun”, obviously that is not going to fly, so that policy at the moment is in fact too vague, and we need to, in some way, fill out this box at the top right hand corner, in the way that we will achieve consensus amongst the different users of the Pharmacopoeia.

Moving forward

Are antibiotics and peptides really a special case? Well, the impurity profiles are often more complex than with other substances, so from that point of view they are often a special case. There is also a less well-defined borderline between the active component and impurities, particularly for antibiotics. However, the general approach for impurities control is just as valid for antibiotics and peptides; the concepts of reporting threshold, identification threshold and qualification threshold are just as valid for antibiotics and peptides. At the moment of course, the practical application of those general concepts is decided for each individual product, and as I said, for us, this is a rather uncomfortable situation.

Moving forward, what we hope to do in the next day and a half is to have some clearer ideas on how to deal with unspecified impurities for the classes excluded from thresholds in 2034. What is the basis that will ensure safety and regulatory acceptability for these products? The basis of course has to be practically applicable, and it has to be clearly stated. Can we in monographs, in monographs alone, provide satisfactory standards for antibiotics and peptides? I hope the answer is yes. How can we reflect regulatory policies properly in monographs, to insure that the monographs will be useful, usable and in fact used? So, I have ended the presentation with four questions, and I leave it up to everyone in the audience to provide the answers over the next day and a half, thank you.

Session I: Antibiotics

Monograph specifications (case studies)
Dr Jan W. H. Smeets, DSM Anti-infectives (NL)

Dr Jan W. H. Smeets' slides are available on page 8 of the Impurities Control Symposium, Antibiotics presentations:
http://www.pheur.org/site/page_601.php

Abstract

Antibiotics as covered by Expert group 7 of the Ph. Eur. can be either pure substances or mixtures of different components. They consist of "Products of Fermentation" as defined in Ph. Eur. Monograph 2002:1468 or of substances obtained by semi-synthesis. These antibiotics fall outside the scope of the ICH impurity guideline Q3A and consequently the corresponding thresholds for reporting, identification and qualification do not apply either.

In the presentation the differences between antibiotic substances and chemically produced substances will be discussed and reasons why ICH thresholds not always can be met by antibiotic substances will be explained.

How Expert Group 7 has set specifications for antibiotics in the past and how these have been expressed in the substances monographs will be highlighted. Also the consequences of the introduction of general chapter 5.10 (Control of impurities in substances for pharmaceutical use) on the interpretation of the monograph specs will be discussed.

Finally the view of Group 7 on how impurity specs should be set in antibiotic monographs in future will be covered.

The topic of this presentation will be illustrated by relevant examples.

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Regulatory assessment and expectations
Dr Cornelia Nopitsch May, BfArM (D)

Dr Cornelia Nopitsch May's slides are available on page 22 of the Impurities Control Symposium,

Antibiotics presentations:

http://www.pheur.org/site/page_601.php

Guidelines and monographs with refer to fermentation

Guideline and monographs which refer to fermentation products are the general monographs of the European Pharmacopoeia such as “Products with Risk of Transmitting Agents of Animal Spongiform Encephalopathies”, the general chapter 5.2.8 on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Medicinal Products, “Chapter 5.10 Control of Impurities in Substances for Pharmaceutical Use” as well as the guidelines “Certification of suitability of Monographs of the European Pharmacopoeia, Content of the Dossier for Chemical Purity and Microbial Quality” and the “Note for Guidedance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products”.

Products of fermentation and semi-synthetic products are out of the scope of the Monograph “Substances for Pharmaceutical Use”. Therefore, the limits for impurities outlined in this monograph are not applicable for these kind of active substances.

How to deal with fermentation and semi synthetic products

Semi-synthetic products are obtained from a fermented starting material by a process involving at least cleavage and formation of covalent bonds followed by extraction and purification steps.

The characterisation of the starting material including a discussion of the possible carry-over of impurities from the fermentation process to the final substance and the control of intermediates by the impurity profile is very important for the assessment of semi-synthetic products.

For the assessment of fermentation products aspects such as source of the history and characterisation of the micro-organism used , purity of master cell bank, stability of genotype, biochemical markers should be taken into consideration. In addition stages as media ingredients and their sterilisation, the fermentation process, release and collection of the desired products and the purification should be described in detail. In-process controls (e.g. optical density, pH, temperature, agitation rate) should be outlined.

Concerning impurities in fermentation products, the applicant should provide an overview of the potential impurities including the related substances in the final product indicating how particular impurities (e.g. cellular residues, substrates, precursors, media ingredients, toxins, pesticides) are removed by subsequent purification steps.

Although semi-synthetic products are out of the scope of the Guideline “Impurities in New Drug Substances” and the Monograph “Substances of Pharmaceutical Use” the principal of

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this guideline or monograph respectively should be applied if the semi-synthetic product is similar regarding purity and description of a chemical substance.

The limits outlined in the Monograph “Substances for Pharmaceutical Use” are not applicable for fermentation products but from the point of view of our toxicological experts there is no reason to set limits for impurities of fermentation products less stricter than for impurities of chemical products.

In the newly revised chapter 5.10 “Control of Impurities in Substances for Pharmaceutical Use” a decision tree has been implemented, in which the following statement is outlined:

If a substance is out of the scope of the Monograph “Substances for Pharmaceutical Use” the general acceptance criterion described in the monograph of the active substance applies to all unspecified impurities and specified impurities, except those that have their own specific acceptance criterion in the monograph.

For the assessment of related substances in fermentation products covered by a monograph of the European Pharmacopoeia or a Pharmacopoeia of an European Member State the BfArM has the following statement:

Impurities should be identified and qualified if they are found in levels more than the limit given for unspecified impurities. Impurities found in amounts less than the limit for unspecified impurities should be qualified by comparison of the impurity profile with substances which are still marketed for example by history of the product.

Qualification of not identified impurities can be demonstrated for example by history of the product or by comparison of the impurity profile of substances which are marketed.

Case studies (antibiotics)

Nine cases resulting from the assessment of documentations have been presented. These case studies should demonstrate the procedure of assessing impurities of fermentation products.

The first case refers to benzylpenicillin, benzathine which is a semi-synthetic product. The manufacturer of the active substance has presented an own HPLC method which differs from the HPLC method of the Ph. Eur. monograph. Five impurities have been presented. The limits set for these impurities are in line with the requirements of the monograph for benzylpenicillin, benzathine.

The requirements regarding this case were:

Any recurring unknown impurity found in the drug substance should be identified and listed in the specification.

Furthermore the means by which they have been qualified should be discussed as they exceed 0.1 %.

The second case refers to potassium clavulanate, which is a fermentation product. It is covered by an monograph of the Ph. Eur. Three impurities were limited according to the requirements of the monograph. No limit for unknown impurities has been established. The disregard limit mentioned in the monograph is 0.05 %.

The requirements regarding this case were:

The limit for unknown impurities (0.1 %) should be established in the specification for the stability studies.

The limit of 0.1 % for unknown impurities should be qualified.

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Justification of these requirements was:

Taking into account that there is a disregard limit of 0.05 % mentioned in the monograph and taking into account that a relationship is mentioned in the monograph which refers to the limits of the monograph "Substances for Pharmaceutical Use" the requirements setting a limit of 0.1 % for unknown impurities is justified.

The third case refers to amphotericin B which is a fermentation product covered by a monograph of the Ph. Eur.. Amphotericin A is limited to NMT 2.0 % which is stricter than the requirements of the monograph (NMT 5 %, if used as parenteral dosage form). A new tetraene has been specified with a limit of NMT 4.0 %. This tetraene is not mentioned in the transparency box of the monograph.

The requirements were:

Based on the batch results found provide qualified limits for:

- the new tetraene found with a limit of NMT 4.0 %
- unidentified, specified impurities
- unidentified, unspecified impurities
- total of impurities

The fourth case refers to erythromycin, which is a fermentation product. It is covered by an Ph. Eur. monograph. A disregard limit of 0.06 % is mentioned in the monograph. Two known impurities were specified according to the requirements of the monograph.

The requirements regarding this case were:

Any unknown peak present above the disregard limit (0.06 %) should be identified and suitable qualified limits proposed.

The fifth case refers to roxithromycin which is a semi-synthetic product covered by a Ph. Eur. monograph. The disregard limit described in the monograph is 0.05 %. Two known impurities were found in the active substance which were limited according to the requirements of the monograph. A new impurity has been determined in the substance which is not mentioned in the transparency box of the monograph.

The conclusion regarding this case was, that the limit of 0.5 % was acceptable which has been set for the new impurity, because this impurity was covered itself by a monograph of the Ph. Eur. In addition the applicant has demonstrated that no other impurity was found above the disregard limit.

The sixth case refers to tobramycin sulphate which is a fermentation product. Tobramycin sulphate is not covered by a Ph. Eur. monograph, but the starting material tobramycin is described by a monograph of the Ph. Eur. Two known impurities were specified according to the requirements of the monograph tobramycin. A limit for unknown impurities has been set to NMT 0.3%.

The requirements regarding this case were:

Unknown impurities should be specified with NMT 0.1 %. Impurities found with a level up to 0.1 % should be qualified (i.e. qualified by product history, qualified by use).

The disregard limit of 0.25 % which is mentioned in the monograph for the substance tobramycin should not be taken into account for tobramycin sulphate based on the ground that the salification of tobramycin includes several purification steps.

The seventh case refers to gentamycin sulphate which is a fermentation product covered by a Ph. Eur. monograph. In this case no limits have been mentioned for impurities of the active

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substance, but a limit has been established for impurity E (7 %) in the finished product. This limit is not in line with the requirements of the Ph. Eur. monograph (NMT 3.0 %). The sum of impurities has been set to NMT 15 % which is also not in line with the requirements of the monograph (NMT 10 %).

The requirements regarding this case were:

The applicant has to document that the substance comply with the requirements of the Ph. Eur. monograph. This means:

- The limit for total of impurities should be set to NMT 10 %.
- The limit for impurity E should be set to maximum 3.0 % or less. The limit of impurity E (3.0 % or less) should be qualified taking into account that impurity E is mentioned in the monograph as other detectable impurity.

A declaration should be given about recurring identified or not identified impurities which are not mentioned in the transparency box including the qualification of these impurities.

The impurities of the active substance should be specified as follows:

- each single specified impurity eluting before gentamycin C1a
- each single unspecified impurity eluting before gentamycin C1a
- sum of impurities eluting before gentamycin C1a

Details about qualification should be taken into consideration for impurity E specified with a limit of 7 % in the release specification of the finished product.

The eighth case refers to rifaximin which is a fermentation product. Rifaximin is not covered by a Ph. Eur. monograph. The limit of an identified impurity has been given to NMT 0.5 %.

The requirements regarding this case were:

The applicant should demonstrate that the requirements of the Guideline on the Chemistry of New Active Substances as well as the Guideline for Fermentation Products are taken into account.

The following documentations should be provided:

- Characterisation of master cell bank and working cell bank
- Detailed description of the way of synthesis as well as the purification of the substance
- Declaration of all potential chemical and biological impurities including the estimation of their possible occurrence in the final substance.

The following specifications should be given for impurities:

- each single specified impurity
- each single unspecified impurity
- sum of impurities

Details about qualification should be given for the new impurity which has been specified with a limit of 0.5 %.

The ninth case refers to amikacin sulphate which is a semi-synthetic product. Amikacin sulphate is covered by a Ph. Eur. monograph. The disregard limit mentioned in the monograph is 0.1 %. Two known impurities have been limited in the specification of the active substance. The limits set for these impurities are in line with the requirements of the monograph. A limit for unknown impurities has not been specified.

The requirement regarding this case was:

Impurities found about 0.1 % which are not mentioned in the transparency box should be qualified.

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It should be taken into consideration that the decisions have been made before the implementation of the decision tree of the chapter 5.10 “Control of Impurities in Substances for Pharmaceutical Use”.

Session I: Antibiotics

Manufacturer's approach to specifications
Dr Hanno Binder, Sandoz GmbH Quality Management (A)

Dr Hanno Binder's slides are available on page 49 of the Impurities Control Symposium, Antibiotics presentations:

http://www.pheur.org/site/page_601.php

The control of impurities is described in several documents of ICH and European Pharmacopoeia. Whereas the regulations are clear for new drug substances, a "grey zone" exists for products of fermentation, semi-synthetic active pharmaceutical ingredients and for already marketed drug substances.

In our presentation we would like to demonstrate the origin of impurities under special consideration of antibiotics, to summarize the regulatory framework and the Sandoz approach for qualification of impurities. Finally we would like to give two examples regarding our experiences with authorities.

Impurities

Impurities may arise during the reaction and the isolation of the API as well as during the storage of the drug substance. They may either derive from starting materials including their impurities, or from solvents, and may pass the reaction without change. These impurities may stay at the initial level or may be depleted during the synthesis. Additionally, side reactions between starting materials and their impurities may occur. Impurities from above described pathways are commonly named "by-products".

"Degradation products" can be formed already during the reaction and the isolation of the API as well as during the storage of the material. One possible way of degradation during the manufacture of the API are side reactions with solvents, e.g. in case of alcohols or amines. Degradation during storage is mainly influenced by external factors like temperature, humidity, time, light, packaging, etc. as well as by internal factors like water, residual solvents provoking e.g. re-esterification.

Considering products of fermentation and semi-synthetic APIs derived therefrom, it has to be mentioned that by the nature of the fermentation process itself, impurities with a close relationship in structure and with similar solubility are formed. Due to this fact, these impurities react in the same way as the main fermentation product.

This is demonstrated with the example of semi-synthetic Cephalosporins. All Cephalosporins are derived from Cephalosporin C which is a product of fermentation. From the metabolism of the strain *Cephalosporium Acremonium* 3 impurities having just minor differences in the 3-position (Desacetyl-Cephalosporin, Desacetoxy-Cephalosporin, Cephalosporin Lacton) are formed.

As the chromatograms in the example of the synthesis of sterile Cefotaxime Sodium show, these 3 impurities are fully undergoing the same reactions during the 3 synthesis steps. In the chromatograms of Cefotaxime Acid also the formation of the dimer (degradation product) and the bi-acylated Cefotaxime (by-product) can be seen.

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Regulatory framework

This example demonstrates that in the field of antibiotics we are dealing with a wider range of impurities compared to fully synthetic APIs. Consequently the setting of limits has to consider the origin of these by- and degradation products.

In the currently valid ICH guidelines as well as in the corresponding Ph. Eur. Chapters fermentation products and semi-synthetic products derived there from are excluded from the scope. Ph. Eur. indicates that the general concepts of reporting, identification (wherever possible) and qualification of impurities are equally valid for these classes of substances but the thresholds stated in the general monograph do not apply. Furthermore it is stated that it is the responsibility of the user of the API to check for relevant identification and qualification thresholds. Normally this is done in close cooperation with the manufacturer of the API.

All the decision trees given in the guidelines deal with new drug substances only. In difference to FDA, for the European market there exists no regulatory framework for products already being on the market. This leads to the situation that for the assessment of dossiers the rules of new drug substances are widely applied.

The FDA approach described in the guidance “ANDAs – impurities in drug substances” introduces the concept of a comparison with the innovator’s drug product. That means if the generic API complies with the USP limits or if the same impurities at a similar level are observed in the innovator’s product, these impurities are considered as qualified. QSAR (Quantitative Structure Activity Relationship)-studies are well accepted by FDA reviewers as an additional justification. This program identifies whether an impurity is related to others with known toxicity.

Qualification of impurities

The ultimate goal of Sandoz’ approach for setting specifications is to ensure acceptable quality for the customers, i.e. that the biological safety of the product is established. To achieve this goal, three scenarios have to be differentiated: “new generic” products, “old generic” products and “process changes”.

Furthermore in case of antibiotics the impurities should be identified (wherever possible) to demonstrate that it has the same structural backbone as the desired fermentation product to consider it as safe based on analogy conclusions and as qualified, unless otherwise known. In case that the impurities are not originating from the fermentation process but were formed during the synthesis as a side-reaction (e.g. double acylation) ICH Guideline is followed. Limits should be set according to batch data at release and at the end of shelf life.

- For “new generic” products, that means for the first product on the market after patent expiry, the impurity profile is compared to the originator’s product. If the impurity profile is the same as that of the originator and the impurities are at equal or lower levels, the impurities are considered as qualified. If the impurities are the same as the originator product - but at higher levels - or if different impurities are observed, the above-mentioned general concept is applied.

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- For “old generic” products, that means where the product has already been marketed for several years, qualification is provided by “retrospective” qualification of the impurity profile (qualified “by use”). This is achieved by assessment of historical data including stability samples.
- In case of process changes either new impurities may arise or already specified impurities above the acceptance criteria may be formed. In such cases identification (whenever possible) and qualification is needed. The same general concept as described above is applied. In case that the outcome is not conclusive, QSAR studies or toxicological studies are performed.

Experiences with authorities

Finally let us describe our experiences with authorities. During the registration of Phenoxymethylpenicillin Potassium being on the market since 50 years, a limit for individual unidentified impurities of NMT 0.10% was requested. In our opinion this request was not justified since all the impurities have to be considered as qualified by use and secondly this API is a product of fermentation.

The second case deals with the registration of Amoxicillin dispersible tablets. For this API we had a CEP with approved limits for the impurities. We got a request to tighten the limits for the individual unknown and total unknown impurities of the API as well as the resulting drug product. Furthermore impurities exceeding the Q3B thresholds should be qualified. In our opinion this request was not justified since it was going beyond the regulatory requirements for semi-synthetic products derived from products of fermentation and the API with the same impurity profile is on the market since many years. Additionally it was not considered as justified to tighten specifications granted in a CEP.

DISCUSSION

Dr Jean-Louis Robert: I am going to challenge you on the last two slides; I can accept your frustration. I will come back to slide number 21, and also 22 and 23. Like you know, in 21 you speak about structural backbone. If you could maybe give me some explanation on what you understand on the structural backbone, and then you spoke in slides 22 and 23, you spoke about the concept; how you address new generic and existing generic, it says how you address it in your companies when you set your specifications or the policy in your company.

Dr Hanno Binder: So, coming back to your first question; structural backbone. This is the example of Cephalosporin, what I have given to you, that you have from the fermentation process these four impurities, which are structurally really closely related, and which are undergoing the same reactions.

Dr Jean-Louis Robert: Is it different from what we in the Pharmacopoeia we call related substances?

Dr Hanno Binder: This is some specific in product fermentation, which normally do not have in classic, synthetic APIs. The second question was, if we are applying this concept;

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yes, we are applying this concept. And, normally, we are rather successful in filing our products with this, and I think it is scientific based, and it is logical, that more or less everything is based on the data of the innovator by comparison.

Dr Lakshmi Prasad Alaparthi: I have another question. You have nicely classified the entire impurity segment in a chart, so one thing which I found, the solvents and reagents which you are using for the process, if those are having some impurities; it is very likely that those impurities also will get into the product.

Dr Hanno Binder: Yes, you are right, but I did not want to overcomplicate this slide, so I just was going to the basic principle, because you can throw this slide until I do not know.

Thank you and I think we will continue with questions in the workshops.

Session I: Antibiotics

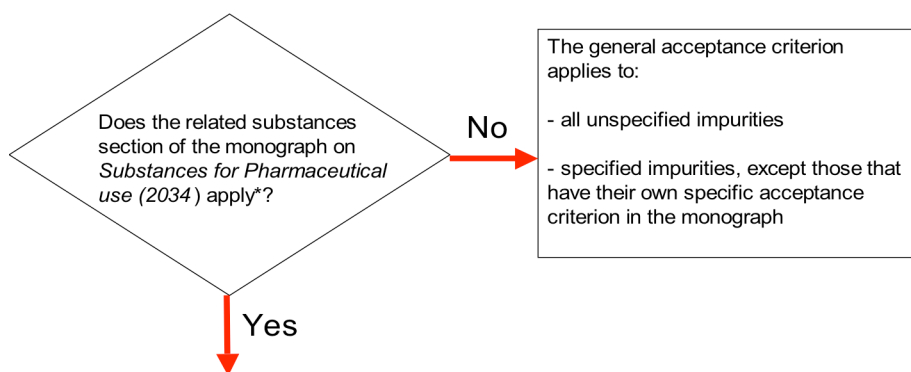
WORKSHOP

How to limit unknown impurities

Questions for discussion

1. What information should be considered by the expert group for setting impurity specifications for antibiotic substances of fermentation and semi-synthetic origin?
2. Can ICH impurity thresholds generally be applied to antibiotics?
3. Can one single acceptance limit for any other impurity (identification threshold) be applied to all antibiotics?

Relevant part of decision tree



* The requirements of this section apply to active substances, with the exception of: biological and biotechnological products; peptides; oligonucleotides; radiopharmaceuticals; products of fermentation and semi-synthetic products derived therefrom; crude products of animal or plant origin; herbal products

4. How are you applying the decision tree in General Chapter 5.10?
5. Is it justified to request stricter limits than specified in the Ph. Eur. Monograph during registration?

Case: Gentamicin sulphate

Ph.Eur. monograph:

- any impurity: max. 3.0 %
- total impurities: max. 10.0 %

- | | |
|--|---|
| <ul style="list-style-type: none">• <u>Regulators view</u>• fermentation product• no disregard limit• no limit for unknown impurities | <ul style="list-style-type: none">• <u>Expert group view</u>• fermentation product• disregard limit higher than ICH limit• limit for unknown impurities = limit for any impurity |
| <ul style="list-style-type: none">• Proposal:• Single specified impurities limit• Single unspecified impurities limit | <ul style="list-style-type: none">• Proposal:• unspecified impurities limit to be introduced (instead of "any other") |

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Workshop 1 Conclusion

Dr Bernhard Wolf, Merckle GmbH (D); **Mr Philippe Villatte**, Sanofi Aventis (F)

1. - Statement: the quality of marketed products is often better than that prescribed in monographs
 - Why?: specifications carried over from old, non-selective methods
 - How to modernise monographs?: Use selective, state-of-the-art methods. Collaboration with manufacturers needed: samples needed! If no information, stimulate comments via Pharmeuropa
 - How to move forward? What source of information?: The regulatory framework exists! (directive 2003/63). Be pro-active: EDQM website. Publication in Pharmeuropa should not be the start of collaboration. Authorities to disclose information to EDQM Expert Groups on an anonymous basis.
2. - In general No.
 - Some pure antibiotics can comply with ICH thresholds.
3. - No.
 - Case-by-case.
 - But: need for general guideline on how to apply the case-by-case approach.
4. - Authority 1: yes, however... "light" qualification procedure (eg history of safe use).
 - Authority 2: too much risk to apply a higher acceptance limit.
5. - No, Ph. Eur. Monographs are the compendial requirements.
 - Plea for harmonisation of approaches by licensing authorities (the safety is the same in all countries!).

Workshop 2 Conclusion

Workshop 2: : **Mrs Katjusa Kreft**, LEK Pharmaceuticals dd (SLO); **Prof. Dr Jos Hoogmartens**, Katholieke Universiteit Leuven (B)

1. - Establish close collaboration with manufacturers at early stages (a liaison person) in order to obtain the approved specifications and extensive batch data (release and end of shelf life)
 - Try to get also information from licensing authorities
 - Collaboration and communication is very important with Ph. Eur. Especially to improve out-dated monographs
2. - NO, as seen from the different presentations and discussions
3. NO
 - Looked at on a case by case basis / substance by substance; not manufacturer by manufacturer
 - This is due to the huge complexity of these compounds
4. Views of the authorities
 - MHRA - applies decision free as far as possible
 - Opinion of EDQM TAB Certification - in case of new or recently revised monographs and to substances already marketed in Europe it applies
 - Otherwise, with new manufacturer/new impurity profile, decision on a case by case basis asked to justify limits, qualification of new impurities required

Decision tree is not always clear: general acceptance criterion refers to the specific monograph
Add antibiotics to the special Ph. Eur. Revision programme

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5. NO, it is not
- As monographs were adopted by authorities, however, if new information is available on the genotoxicity of a particular impurity it could be justified
 - The limits apply until the end of shelf life
 - For the time being, each country has its own rules which is not an acceptable situation

ROUND TABLE DISCUSSION

Dr Michael Morris, Chair of the European Pharmacopoeia Commission, **Dr Jean-Louis Robert**, Chair QWP (EMA) and Chair of the Steering Committee of the Certification Procedure (EDQM, Council of Europe); **Mr Peter Castle**, Secretary to the European Pharmacopoeia Commission (EDQM, Council of Europe)

Dr Michael Morris: We have on the panel here for the round table, on my right, Dr Jean-Louis Robert, the Chair of the Quality Working Party of EMA, Peter Castle, Secretary to the European Pharmacopoeia, who spoke this morning. We do not have Professor Alain Nicolas, the Chair of group 7, unfortunately, but we do have Professor Jos Hoogmartens, who is also a long term member of group 7. We also have the speakers from this morning; Dr Jan W. H. Smeets, Dr Cornelia Nopitsch Mai and Dr Hanno Binder facing us. I do not have very much to say to start things off, but I think I will maybe defer to my colleagues on either side of me, and also of course look to the audience to see if anybody has any questions on what has just been heard, or on any of the topics that you have heard today, or indeed any comments or suggestions.

I think that we have heard from the presentations a set of points which were addressed by those, principally those five questions, and it seems as though some clarification is coming through, in terms of application of the principles of ICH, but without necessarily the acceptance criteria, which are built into Q3A, and the general monograph substances for pharmaceutical use, so from what I see, there is no fundamental issue with the way the work of the pharmacopoeia is being conducted. I see that maybe what we need to do, is to improve the transparency of the process. We also clearly need to move away from a situation where different regulatory authorities within Europe are applying different quality standards to the same pharmacopoeial materials, bearing in mind that pharmacopoeial monographs are binding on all the 24 member states. Of course, there are exceptional situations for given particular sources, but by and large, we should have a common approach within the member states regulatory authorities, and we also need to see more information flow, clearly we need to see more information coming in from manufacturers, to assist in the formation on monographs, and we need to see more information coming from the regulatory authorities into EDQM to highlight those areas, where perhaps there is an apparent deficiency in the current monograph text. So, with those comments stated, I would now perhaps maybe defer to, Jean-Louis, would you like to say something?

Dr Jean-Louis Robert: Thank you Dr Michael Morris. First I would like to welcome you all to this meeting, because I think it is a very important one, and then also to admit that we have tried to make a survey within the member states before I came here, and it appeared that we have, within Europe, or within the quality working party, not really a clear policy, or a harmonized policy at least, on how to deal with antibiotics and basically also with peptides, which we will talk about tomorrow. My first impression from what happened until

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now is that, at least I understood that there is a lot to do. I do not know exactly how we can do it, but I think we need really further discussion here, in order to come to a harmonized approach. I will try to make some clarification; it was very much discussed this morning about Q3A and ICH guidelines. First of all, it is clear that ICH applies only to new active substances and their corresponding products. However, there are some guidelines, for instance, for stability where we have a specific guideline on existing active substance products, where we took basically the whole philosophy from the ICH, and only, maybe the number of batches is less than for new products, but basically the same philosophy is applicable also, as for the ICH guideline has been applied. Residual solvents is another example, where it was decided that we apply the same limits for residual solvents that we apply then for new active substances, and in Europe, you know that we have a policy slightly different from the US. The quality parts of the dossier is a stand alone document, and it is assessed on its own merit, and basically from a quality point of view, we do not make any differences between new active substances and existing active substances, and basically this philosophy has also been taken on by the European Pharmacopoeia, that we applied ICH concepts and philosophies also for pharmacopoeial monographs, which makes sense, because as Peter has mentioned in his presentation this morning. 20 years or 25 years ago, we were mainly dealing with European sources, now we have much more sources, with the globalization, much more sources coming from different countries, which is good in itself, that is not the problem, but as we know less about these source materials, I think, we should also treat them as for new active substances. Q3A gives not only the threshold, but Q3A is a document which presents a general approach on how we should deal with impurities in an active substance.

We have to discuss about the manufacturing process, I think we have said not very much about the manufacturing process this morning, only slightly indicated, I think in the presentation from both Dr Jan W. H. Smeets and Dr Hanno Binder, is that the impurity profile depends very much from the manufacturing process, and that even for two manufacturers using the same strains, they can come up with different impurity profiles, so this stresses very much the importance of the manufacturing process. There should be a discussion about potential impurities; there should be discussions on which impurities really then appear afterwards in the products, its final active substance. We speak about method validation and so on. This present provided in this guideline is a general approach for dealing with impurities, and I think the philosophy of this approach is, it does not matter if we now speak about synthetic substances, or about substances coming from fermentation, I think the philosophy applies. Of course we have to deal differently, and in a different manner, that is fair enough, but the guideline should not, anyhow, tell you the how, but more tell you the what, what has to be done, and in fact the thresholds, if you look at the thresholds, there are a 1/3 of a page, so it is really not the most important part. And these thresholds are in fact the help also to say that if we recognise, if we accept these thresholds, which are commonly accepted, then the way of evaluating an application file will be easier for both the assessor and also the company, but threshold, that is also said in the guideline, that there might be cases when you have to have lower thresholds than those which are indicated in this table.

Now, coming to the antibiotics, and I think what I say here is in fact, we have not really discussed this in the Quality Working Parties, so it is a little bit my own opinion here, and

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what I got as information this morning, of course with antibiotics with the fermentation process, that is a much more complicated process than maybe a pure synthesis, we probably, I think that we probably will have to deal with it in a slightly different way, at least regarding the thresholds, than we do for synthetic substances, so basically I can say that the case by case approach is acceptable. Of course if we leave to the applicant or to the manufacturer to justify, and to show, based on a general approach, why it is more difficult to satisfy, or to comply with the identification threshold of 0.10%, or with the reporting threshold and so on, and Dr Cornelia Nopitsch May, this morning, said something in the initial seminar, that maybe the trend should go into trying to comply with the ICH thresholds. However, justifying why it could not be, why they should not be applicable, and I think for antibiotics, for fermentation products, it should not be too difficult.

Something which I said in the workshop which I attended, is that when we speak about case by case, it has been case by case per antibiotic, not case by case per company, so each company will be treated the same way, and I was not quite sure if it was clear to everybody. Maybe I misunderstood it, but I just wanted to make this clear. It was also said about information to the licensing authorities, I think that is fair enough, and it should be done. From the Quality Working Party Side, I think that we have a procedure in place, that each monograph which is published in Pharmeuropa is checked by the licensing authorities, basically to make work sharing, in order to identify if the limits which are proposed are acceptable, and also if the specified impurities are all covered, and I think Michael, this covers all the antibiotics. Is that correct? Yes, it covers also the antibiotics. Michael is that correct?

Comment from the floor: If I may add a remark still the flow of information can be improved.

Dr Jean-Louis Robert: Right, that was my next sentence. I agree with you, everything can be improved of course, but I am already happy that we have the mechanism. Now, as we have the mechanism, we work on it, but you are perfectly right, I think it could be improved.

The only thing which I am not quite sure, and we will now ask the question in our workshop is about the qualification threshold, and Dr Cornelia Nopitsch May said in her presentation that safety of the impurity should be regarded in the same manner, independent of whether it arises from a synthetic process, or from a fermentation process. I am not quite sure if Dr Hanno Binder in his presentation shares totally this view, this might be some kind of debate which we could have afterwards. It is something we probably need some more discussion with our safety expert, and with the Safety Working Party, in order to identify. Because basically, if we say that the same qualification threshold applies, that would mean that we have this qualification threshold, let us say for substances with a maximum daily dose 1 gram or less, that would be 0.15%, and for over 1 gram would be even lower, that would be 0.5%.

I can see some difficulties in the future, and, I think that we have here an open discussion, and not that the regulators have said that “full stop, OK”. This means that if we compare, and this is also shown by Dr Hanno Binder this morning; if we compare the impurity profile of an old generic with the originator, we compare basically an impurity profile, or a profile; let us put it this way, than rather discuss if all the related substances are also really impurities, or as for instance for proteins, quite amazing, for instance, the biotech people,

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when they deal with proteins, they do not speak about impurities, because the proteins may be part of the active substance, so sometimes we could wonder if for fermentation products, it could not be a similar situation, but that is another story. So, if we compare the profile of the originator, we see new active substances. In fact, we normally compare it by HPLC, or something like this. If we do not know if the identification threshold is rather high, because of technical reasons, fair enough, but the qualification threshold is below the identification threshold, basically, if 3.15% is applicable, but we have now an identification threshold for specific antibiotics of 0.2% for instance. I am not quite sure how it can be done in practice, or how we can deal with it, there must be a good justification done by the applicant; it is something on which maybe I would like to have feedback from some of my colleagues.

The last point which I would like to mention, I think the action which I will take, I will for sure make a report, and I know that some colleagues from the Quality Working Party are also attending this meeting to the Quality Working Party, and will try to identify the need for coming up with a general policy. Case by case is nice; however we should always have the same approach, same philosophy in the case by case, I think that is important.

Thank you chairman. I was a bit longer as I did not speak this morning, so I took the liberty to be a bit longer.

Dr Michael Morris: Thank you very much. You did raise some questions there, but maybe if the audience wants to jump in, and any of this mornings speakers who have been named, please if you hold and I'll ask my other two colleagues on my left, and then we can come back to any contentious points raised there by Jean-Louis or by others, so I move now and ask Peter Castle if he wants to make some remarks.

Mr Peter Castle: A few points, particularly coming from the workshop that I attended, I was in workshop number 2, and there are also some issues raised on the slides from workshop one; the question of upstream involvement of all the interested parties in monograph elaboration has been raised during the speeches: people tell me "sometimes we only see the monographs when they go into Pharmeuropa, and that is very, very late", and now I apologize to the people who were in workshop two, because I am going to say exactly the same thing that I said in that workshop; EDQM would like to have a liaison person with all of the manufacturers, so that we can feed information to the liaison person on the new monographs that we are going to draft, on the monographs that we are going to revise, and another function of a liaison person can be to feed to EDQM questions about the European Pharmacopoeia, and also, rather optimistically, we hope that the experience that liaison persons gather with dealing with the European Pharmacopoeia means that they can answer questions about the Pharmacopoeia within the company, and avoid the questions coming to us, maybe that is a bit too optimistic.

At this point I often ask the question; does anybody in this room know the name of their Pharmacopoeia liaison person, if you do, please raise your hands. I do not count these people here, because if you did not know...but there are one or two other hands, so that is slightly promising. For all of you who do not know who your Pharmacopoeia liaison person is, then on Monday morning, please go and find out, and if it is nobody, then try to convince your boss that you really need a Pharmacopoeia liaison person, in order to be aware of what

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is going on in the European Pharmacopoeia, and in order not to have any surprises. We will feed the information to you, and we hope that you will then follow up our requests to be involved in upstream elaboration and revision of monographs.

Of course, when you want to convince somebody else to do something, you either need a whip or a carrot, or both. The carrot in this case, is that you will be feeding information into the monograph, and you will be sure that the monograph fits your product. I am not so sure that we have a whip in this case, unfortunately. For chemical monographs, of course, if you do not participate upstream, for the synthetic chemical monographs, you may end up with a monograph which does not cover your product clearly. If we end up with a monograph where one of your specified impurities is not covered, then you are going to have to answer an awful lot of questions, and you are having to go through an awful lot of paperwork, so you have an interest, in fact, in making sure that you give the data to EDQM, to have the monograph covering your product adequately. I do not know whether that really applies to antibiotics, because there is this question of the unspecified impurities, and I am going to carry on calling them unspecified impurities, because I do not want to call them any other impurities. For unspecified impurities that are on a higher level than 0.1%, that may give you a let out for some of the impurities where you will not get whipped, because the monograph does not have these as specified impurities. But in any case, I think that there is a carrot for everybody to participate upstream in the development of monographs, and if you do not give any input early on, then I think EDQM is entitled, if you come late to say “hard luck”. Sooner or later, we will have to start saying more often “hard luck, we are going to adopt the monograph, we will revise it later of course, it will take time, but you have come too late”.

The question of whether the authorities apply the decision tree in the monographs, and the responses to this question remind me of the little song that they sing in France when they are picking the petals of a daisy, do you apply the decision tree “a little, a lot, passionately, not at all”. The question that they ask is not “are you applying the decision tree”, it is another question, I will tell you outside the room if you like, but of course, if the authorities are not applying the decision tree, that means that we have different decisions among the different authorities. That means that we have failed. We have failed because one of the basic aims of a common decision tree is that it applies the same policy for the same thing, what is good in one country will be good in all the countries. Who is we? It is all of us. It is not just the people in Strasbourg, it is not just group 7, it is not just the authorities, it is everybody. We need to move to a situation, where we do in fact have harmony, I think one good suggestion that we saw on a slide from the workshop one, is having a guideline to explain better the basis for the limits for unspecified impurities in the monographs on antibiotics. But until we have reached really a uniform approach for these monographs on antibiotics, as I say, we have really failed; we have not got to the winning post for these monographs.

One of the first slides from workshop 1 pointed out that the products on the market are often better than the monographs. I do not know what better than means. If it means that they are ten times better, I am worried. If it means that they are twice as good, I am not so worried, because I think that we can not set the monograph so tight, that everybody is living on the brink. You cannot afford to live on the brink, when you know what the consequences of an out of specification result are, so I would like to know what you mean by saying that the products are better than the monograph, twice as good, ten times as good?

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You have pointed out that moving over from TLC to quantitative methods, means that the limits often look too lax, because the limits have been taken over from TLC. When we have been running the special revision program, not for antibiotics, for the other products, that has not been the way that we have established the limits, well not always. We have preferred to, in fact set the limits in terms of current batch data. I do not know what proportion; I do not know whether you have some idea of this Dr Michael Morris? I think that probably for the majority of products, we have been able to set limits that are more in line with the batch data, than simply carrying over the TLC data, which was probably more based on detection limit, than anything that was really in the product, so there is an alternative to that. It does mean that you need to have batch data from all the interested parties. Then again, that is a carrot, if they do not give the data, then they may end up with a monograph which does not fit their product, which will give them trouble, of course.

Most of the antibiotics are not on the special revision programme, because the basis for the special revision programme was that the monographs were not in line with monograph 2034 and chapter 5.10, and we know that antibiotics are in fact excluded from that, so there was no reason to put them in the special revision programme. I think we have had some convincing arguments in fact now, for trying to get the antibiotics also on a fast track for modernization. So, those are the comments that I had Mike, thank you.

Dr Michael Morris: Thank you very much Mr Peter Castle. One of the things I really heard loud and clear there was a phrase that is usually used in Ireland in the context of the National Lottery;” “if you are not in, you cannot win”, and I would now like to turn over to Prof. Jos Hoogmartens, to see if you have got some words of wisdom for us, Jos, thank you.

Prof Dr Jos Hoogmartens: Thank you chairman, but I think that Mr Peter Castle has said the most important things, and I do not want to add anything at this moment, but I would like to participate in the discussion of course.

Dr Michael Morris: Ok, thank you. What about our three speakers from this morning, would you like to comment in the light of some of the comments raised, in particular, by Dr Jean-Louis Robert? Dr Hanno Binder, please.

Dr Hanno Binder: Yes, concerning qualification of impurities, this was one of your concerns. I think the concept what FDA is applying to qualify impurity profiles versus the innovator is legitimate. You were asking, HPLC alone is not enough. What I said in my presentation was that HPLC must be specific and stability indicating. That means, if a European Pharmacopoeia method does not fulfil this requirement, use an in-house method. For me, it is important that you run the innovator sample and your own product consecutively with the same method; otherwise you have no chance to compare. By this, you are getting a little bit more reliability and retention times, of course you can use older techniques what are possible, simplest technique diode array til mass spectroscopy. But this, I think is only valid if we are talking about pure antibiotics. If we are going in the direction of Nystatin and these compounds, which are rather heterogeneous than a chromatogram. First of all, you do not have such low disregard limits, especially if you are losing, we have now elaborated some monographs with PIT, where the detection limit is somewhere 0.2, 0.3, and by this, a qualification threshold of 0.15 per se is not applicable. That means, I think

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also in this, we should find a compromise, if it is a pure antibiotic, where we have seen examples that ICH can fully be applied, then there is no problem to go to the 0.15, but if you have complex mixtures, then it is getting difficult. And, for me, I do not know if the concept was clear. For me, the qualification limit in a generic product is not the matter if I can show that I have the same pattern in the innovator, because the innovator has qualified this particular in clinical studies. I think this is a major difference; a new substance compared to a generic substance. I do not know if I got you now the corresponding answers that you were asking, but I tried to do so.

Dr Jean-Louis Robert: The last comparison, the last point which you mentioned; I did not get it totally.

Dr Hanno Binder: Ok, if you compare your new generic product, let us say after patent expiry, to that of the innovator, and you have the same profile, no additional peaks, no higher level, then I think it is irrespective if this is 0.15 or more, because the innovator has proven by its clinical trials that the product is safe.

Dr Jean-Louis Robert: Correct. My question is just how you make the comparison.

Dr Hanno Binder: It is really complicated, it is absolutely complicated.

Dr Jean-Louis Robert: We will have to find one kind of justification for it, why you think that your source material is acceptable, from a toxicological point. Unless we have a source which we run on the market for X years, that is another story, but then we are again...

Dr Hanno Binder: I will try to give you an answer; you have a certain string producing a product, and even, if Dr Jan W. H. Smeets has said, even the same string can give different impurity profiles, but behind this is a biosynthesis. And biosynthesis is normally specific, product specific. That means that the fear that the same thing completely differently is produced by the micro organisms, I think the risk is not so high. In difference to our chemical synthesis, where really something can completely go wrong, but biosynthesis is normally very, very specific concerning stereo chemistry. That means you are getting what you want, even if the composition is a little bit different.

Dr Michael Morris: Would anybody else like to comment on that point? Please, Dr Bernhard Wolf.

Dr Bernhard Wolf: I think what we need is an additional identification test, that would be a fingerprint or anything like that, by HPLC, to compare it to anything which is the originators fingerprint. But you know what that means, we do not have something like IR in that case, we have an HPLC profile, and it is much more difficult to make it comparable than to do it with an IR spectrum. I have doubted if it is workable. You know, I remember what Dr Michael Morris said this morning; what we need; useful, usable and used monographs, and I think it does not work. Or was it Mr Peter Castle, I am sorry I do not remember, but I think it would not be usable.

Dr Lakshmi Prasad Alaparthi: Many times you cannot really do the real comparison, because in order to really be using a different quality excipient where the excipient peaks

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will be interfering, and you will always be confused whether they are really impurities or excipient peaks.

Dr Michael Morris: Any further comments?

Dr Jean-Louis Robert: Can somebody not address the questions which I raised, are there no problems? Can somebody raise this as a no problem. It would simplify a lot. But you agree that this is something which we have to deal with? Yes. I hope that somebody will stand up here, and say whether the question which I raised was in fact a no problem, that would have helped this, but I think this is something which we probably need to discuss further in the future.

Dr Hanno Binder: I am talking again about rather simple antibiotics. Each manufacturer has the impurities in house, and this is really helping, you can make spiking status, or at least you can fix most of the impurities you have in the impurity profile, but still, this does not help these complex antibiotics.

Dr Jean-Louis Robert: I understand the problem, that is why... Cornelia, do you want to add something, at least you raised the problem.

Dr Cornelia Nopitsch Mai: I think it is also necessary to discuss this point with toxicologists, isn't it?

Dr Michael Morris: Further comments? Yes, at the back there please.

Question from the floor: Yes, I have a comment to one of the statements from Mr Peter Castle, regarding the different interpretation from authorities, for example to application of the decision tree, so is it possible to have frequently asked questions, or something like that, where the industry or authorities communicate with the group of experts and then, to make some general questions, maybe when the monographs are not so clear, and then publish in an anonymous way, so that on the time, you know, we have more explanations regarding interpretation of monographs.

Mr Peter Castle: I am not sure that it is a problem that the monographs and that the decision tree in the general chapter are not clear. With a slight qualification of what we heard in workshop 2, I think that they are. I think that the problem is that they do not really represent a full consensus, on the part of the different interested parties. They are clear, but they do not represent a real consensus.

We have frequently asked questions on our website. We do not have one to cover this issue, because we think that the actual texts in the pharmacopoeia are clear. We hope that we are moving towards a better consensus on these issues. Dr Jean-Louis Robert asked a very simple question, and I think he wanted to have a simple answer, but I do not think the answer can be simple, really, and I think that one of the ways forward, is for us to develop better guidance on how we establish the thresholds for these substances, and as Dr Cornelia Nopitsch May said, the toxicologists should be involved in this kind of discussion. I think

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that if we can move forward in that way, then we will be getting towards consensus, and then we won't have any frequently asked questions.

Dr Jean-Louis Robert: Regarding the decision tree, the decision tree does not say anything about thresholds, or acceptance criteria, so I not quite understand why you have said that. It is obvious some member states do not follow the decision tree, what you mean by that, because basically somebody, one agency could say or competent authority could say that "ok, I think that for antibiotics, although rather for fermentation the ICH thresholds apply. " Full stop. This is our policy, right or wrong, I will not discuss this". This might be the policy from one member state, and basically from one member state, I got that answer from the survey. We do not discuss if it is right or wrong, but in fact you cannot say that you have not applied the decision tree, they have just applied their policy, and I think for antibiotics we should also apply ICH thresholds.

Mr Peter Castle: I think in that case, they really have fallen off one of the branches of the decision tree; they have plummeted to the floor, because for antibiotics, you start with the top left hand corner, you immediately move over to the right. That means that you may have an acceptance criterion, which applies to any other impurity of 0.3%. On the other hand, you are applying the ICH Q3A, then that will actually be meaningless, because at the same time, you want any other impurity to be qualified, if it is beyond the ICH threshold. So, it is quite right, they are not dealing with the same thing, but the two are contradictory.

Dr Jean-Louis Robert: I think that what we have to achieve here, and this was all part of the discussion in our workshop, is that, of course the European Pharmacopoeia deals with existing substances, substances which are already on the market, and for me the decision tree was very much for those substances who were in the pharmacopoeia, is that correct? And of course, one can argue, well if it is a new application, if it is a new antibiotic we do not have to apply, we can have a different policy for example, this is something which I would like to avoid in the future. I think that if there is a reason that we for antibiotics, we have an identification threshold of 0.2% or of 0.3%, because of the complexity of the production of the manufacturing process, and if we can define, maybe objective criteria, what will lead us to 0.2% or what will lead us to 0.3% whatever, then we should consist in two papers. What I would like is that we have, at the end of the day, at the end of the exercise, the same approach for new antibiotics, for new fermentation products, as well as how we deal with fermentation products in the European Pharmacopoeia. This is I think; we should aim at, because otherwise we might be more stringent eventually for new antibiotics, something which I would not like. I would not like to have two policies; one for new antibiotics, and one for existing ones.

Dr Michael Morris: Any further comments?

Dr Jan W. H. Smeets: Perhaps a short comment. Perhaps it looked from the slides in group one that we always just put the specifications from the TLC into the HPLC. I think, perhaps in certain cases it is, but I know for most of them that it is in fact not. We always try to look to batch results to set the specifications.

Comment from the floor: On the other hand, Dr Jan W. H. Smeets, we got the information from many authorities that the quality of the products which are on the market nowadays are

much better, maybe not a factor of 10, but maybe a factor of 3, 4 or 5, and that means, we have to fix new limits.

Dr Jan W. H. Smeets: OK, but that is a different story.

Dr Jean-Louis Robert: I have noticed that these products on the market are better than the monographs. I like it. I do not know exactly what it means. What is meant by that, probably this is coming from workshop one. I do not know, who raised the question, what does that mean?

Dr Jan W. H. Smeets: What is said in group one, is that some of the antibiotics on the market have a higher assay than is requested by the Pharmacopoeia. For instance antibiotics that have an assay of 98%, while it is allowed to have 95%, that kind of thing. And of course, I think that it all has to do indeed with the fact that monographs sometimes have been elaborated quite some years ago, when 95 perhaps was reflecting the quality at that time, and now processes have improved, the quality has been improved. Then you get a basic question of course; what should you do, and where should you base your specifications on? Should you base it on the safety, or should you base it on the quality of the current batches in the market?

Dr Jean-Louis Robert: But basically it is not a question from authorities, it just happened. The question is interesting, because we had the same debate in a workshop some time ago in the US; no, it was in a seminar in London about design space and this seminar had one workshop about specifications. Of course, it was not for antibiotics, but the question was similar, and I raise the question; what is better to have on the market, a source material with an impurity of 0.2% or one of 2%, even knowing that the 2% level is qualified. What is better?

Dr Lakshmi Prasad Alaparthi: There is a different dimension in this kind of cases. You have a product where 2% of impurities qualified under the limit, and in the same time the impurity which you are allowing is having no effect. So, eventually when you are making a 100% pure substance, you are enhancing the efficacy. I think that this is a very important aspect, especially when the impurities are having the higher limits, like 5%/10%, something like that. There is a difference.

Dr Jean-Louis Robert: Yes, but the question is should the specifications be according to the process capabilities, or should the specifications be according to qualification level. Is it allowed because some rats have been violated, and found that 2% of the impurities is safe, or at least for the rats, is it also then safe for the patients? Should we not avoid? In French we say “principe de précaution“. Should we not say we would like, if possible, to have as low impurities as possible. It is partly a philosophical discussion. Partly, when I speak with toxicologist people, they say that the safety studies are model; it is not the absolute truth. So maybe we are a little now switching out of the antibiotics, but only, just be reminded when you put products back on the market, actually the monographs, and the debate for this antibiotic how we can make the limit when 0.2% is possible, 2% is qualified, should we then say, “Ok let us put it at 1%” and everybody is happy, or, I do not know. This is not an easy discussion.

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Dr Jan W. H. Smeets: I think there is also always an economical factor on it, the price of the medicines, and we see all that the price of the generics is going down very fast, so if you on the other hand only raise the requirements, and the price is going down, we have a problem.

Dr Jean-Louis Robert: But then I will tell you the last story, that sometimes for reasons of marketing, the company will not have the impurities as low as possible, because he is able to do it, but his neighbour is not.

Dr Michael Morris: Any further comments?

Dr Hanno Binder: May I comment to this rather philosophical question. I think manufacturers and European authorities have one goal; this is the safety of the patients and to provide affordable medicines, or medicines at affordable prices, and I think we have to find a balance in this. If we are turning this group too much, the prices will rise, and I think this is not the goal of the European Union, that the people can not afford any more medicines

Dr Jean-Louis Robert: I agree.

Dr Michael Morris: Does anybody else in the audience have any points of view, at all, based on comments that have been made in the discussion so far, or, indeed, can I widen it out unless anybody has any questions or comments on any of the presentations that you have heard today, because if not, I think we will be thinking in terms of drawing the day's proceedings to the close.

Dr Jean-Louis Robert: I just wanted to add something. It is also important in all these discussions about impurities; we have very much discussed now, always the quality of the end product or of the active substance. What is also important is that we put very much effort in better manufacturing understanding of what is going on, although in fermentation process and so on, maybe by monitoring, this might also help us maybe in the future. I think what is important is that what we get really reproducible products on the market, and maybe by more product understanding, this might help us. I say this because now at ICH level, we have already drafted a guideline on pharmaceutical development, and of course until we see finished medicinal product, but we have also discussed that we should not do something similar for the active substance. This might although help us in the future, when we discuss this different threshold impurity levels and impurity profiles, at least we can insure that we have better process understanding, then at this moment we have at least always reproducible products on the market. I think that that is an important issue, which I would like to mention. I think it is not the best for debate, but we should always keep in mind that the impurity profile depends very much on what happens during the manufacturing process.

Dr Michael Morris: Anybody wish to respond to that point? .

Dr Lakshmi Prasad Alaparthi: I have a different question. See, we always talked a lot about the final products. I just wanted to understand if we can have some different guidelines for preparing the impurities for antibiotics. I mean, usually, our understanding in making difference analyse to have highly pure substances, but as we are allowing lot of impurities in the products itself, especially when I am making a difference in standards for

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impurity. What will be the acceptable purity levels, especially in pointing regulatory submissions, if I make 80%, will it be acceptable, in short?

Dr Michael Morris: Are you speaking about the purity of the active substance then?

Dr Lakshmi Prasad Alaparthi: Impurities.

Dr Michael Morris: Yes, you are speaking about the purity of the active substances then, not the product.

Mr Peter Castle: Purity of the impurities?

Dr Lakshmi Prasad Alaparthi: Yes.

Dr Jean-Louis Robert: Should I propose you a threshold?

Dr Lakshmi Prasad Alaparthi: Maybe some proposals would be useful for all participants, because everybody will have a different understanding for making reference standards, because we always insist of, the synthetic group, that until unless you get at least 95%, we will not be able to submit and always he will try to purify and purify and make a preparatory wonder, eventually, finally, end of the day, end of the month, he come and say that "I can not make it more than 92". In our company we have an understanding that since it is not possible to make it a further purification, we restrict ourselves at 90% or so on, because of that. Especially I wanted to understand some regulatory opinions, how European communities do in these kinds of situations?

Dr Jan W.H. Smeets: I think what you say is how pure should the impurities be when you are using them in the monographs. I think in the first place it is important here, where do you use the impurity for, only for identification, or is it really to quantify something? If you use it just for identification, very often we have a monograph with response factors, and then you do not need to have a certain purity, however if you use the impurity for quantification. I think that in the European Pharmacopoeia, we have a policy that if the content of the impurity is higher than 95%, you can regard it as pure, and if it is less than 95%, you have to determine the purity of that impurity, and you have to apply that in the monograph.

Dr Lakshmi Prasad Alaparthi: Surely, I mean for impurities for quantification, not for identification. I do understand that for identification, anything is adequate. Coming to quantifications, if there is a monograph which is officially in the European Pharmacopoeia, where you are allowing your final specification limiting from 80%, not less than 80%. In this situation, is it needed to have any what well to make 90% of impurity in this case, when you are making your final product purity at the level of 80%? In this case, I mean, my understanding is that I can make a little impure impurity something like that, that is what I was trying to understand.

Comment from the floor: I think that as long as we know how pure it is, it is Ok.

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Mr Peter Castle: See, what Dr Jan W. H. Smeets said about our general policy was perfectly correct. We do not insist on having very pure impurities for uses of external standards, although we usually prefer to have that, and we usually manage to obtain them, but what we do is that if it is 99% or more, then you can assume it is 100%, because we think that it makes no significant difference. If it is less than 95%, we give a figure, and you have to take account of that, when you are doing the calculation, but of course, an impure impurity might be just as good as an external standard, as long as you know what the content is.

Dr Lakshmi Prasad Alaparthi: I agree with that.

Mr Peter Castle: You will find our policy laid out in the general chapter on reference standards, which is chapter 5.10.

Dr Michael Morris: I was just going to add that also, thanks Mr Peter Castle. Ok, if there are no more questions or comments? Yes, please.

Question from the floor: I would really like to know what is going to happen next. We seem to have come up with some good ideas, but I do not see an action plan developing. Sure, the EDQM will do some of the things by the European Pharmacopoeia and Pharmeuropa, but are our regulatory authorities going to sort of nod their heads and say “Very interesting” and carry on as now. I would really like to see some sort of action plan, or some sort of response from the authorities present, just how they see the future.

Dr Michael Morris: Well I can tell you that tomorrow afternoon, when the formal conference finishes, there is a debriefing meeting for the national authority representatives, so according to my calculations, we have 18 European regulatory authorities represented in that group. These representatives may well be representatives of the pharmacopoeia authorities, and of the licensing parts of those authorities, so there will be a good opportunity for discussion, for listening, for taking forward the various comments that have been made, from the feedback, from the moderators from the two sessions, and coming up with a proposal that the pharmacopoeia can adopt. I am not sure, it depends on what the decision is tomorrow, I am not sure we will be in a position to make any recommendations to the next Commission session which is in November, but certainly it will be taken forward. We have a meeting scheduled of the chairs of the chemical groups maybe that will be something that can be discussed at that point also, because it may have implications for other groups, I am thinking aloud at this point. We also have of course, as I said, some members of the Quality Working Party present, including its Chairman, and as we have already heard, Jean-Louis has already committed to taking forward, back to the Quality Working Party, the views and the findings of this group, so that we will hopefully be able to get the buy-in from the licensing authorities, hopefully that maybe responds to your question.

Dr Lakshmi Prasad Alaparthi: I have a last question. In fact, this question, I have asked to Dr Hanno Binder in the morning, himself. That is regarding a deficiency posted by authority, where the European communities are having a relaxed limit, whereas other authorities have asked the company, corresponding company, to reduce the limit, because they have a trend analysis, and the level is low. Subsequently, they have responded that it is not justified to tighten that limit. In this focus, we have discussed with Dr Hanno Binder at the same time, in order to get quick clearance from the authority that eventually the

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manufacturer has to obey the authorities' deficiency. In many cases, in our observations, our experiences say that many companies get lot of deficiencies like that, even though it is justifiable, and finally another justification says that your explanation is good, but we would like to have this, so... Maybe, I just want to inform you how to address these kinds of situations to authorities.

Dr Michael Morris: I know what you say, but I am not sure that this is the correct forum to respond to that kind of issue. Ok, so if there are no more further questions or comments, then I think we should draw the meeting to its close. We are a little early, but we seem to have covered all the issues. I hope that we have come to some reasonably, harmonious conclusions and I did not hear anything in the summations around the tables from the moderators that conflicted with each other, so I think we have a fairly clear direction going forward. As I mentioned, we will have the debriefing meeting for the national authorities, and I hope that something definitive and concrete will come out of it, at the very least a clearer explanation to all users of the pharmacopoeia, as to exactly how the policy on related substances is applied to antibiotics, which are fermentation products, and their semi synthetic derivatives.

Thank you all for your contributions and your good questions today, and we look forward to continue the discussion tomorrow. Thank you very much.

Session II: Synthetic peptides
Monograph specifications (case studies)
Dr Peter Jongen, RIVM (NL)

Dr Peter Jongen's slides are available on page 1 of the Impurities Control Symposium, Peptides presentations:
http://www.pheur.org/site/page_601.php

Introduction

An overview is presented of peptide monographs currently described in the European Pharmacopoeia and their impurity sections.

Peptides can be defined by a family of short molecules formed from the linking, in a defined order, of various α -amino acids. Proteins are polypeptide molecules, the distinction is that proteins are long chains and peptides short chains. Sometimes peptides are defined by being short enough to be made synthetically. Other definitions take a size of 50 amino acids or of 5 kD as upper limit.

From an analytical and regulatory perspective peptides are interesting since they present a link between products derived from biotechnology and the small molecular chemical compounds.

Being analogues of compounds synthesised by means of RNA translation ribosomes, peptides do not need to be produced by biotechnological means. Several chemical routes are used to synthesise peptides out of amino acid source materials.

Peptides described in Ph. Eur.

In the European Pharmacopoeia 5th Edition, supplement 5.5 eleven monographs fulfil the definition of a peptide.

Six monographs cover analogues of pituitary anterior lobe (TSH, ACTH) or hypothalamus (gonadorelin RH) hormones. Three monographs describe hormones of the oxytocin and vasopressin family originating from the pituitary posterior lobe. (Slide p. 2: *Peptides in the Ph. Eur. 5th Edition, supplement 5.5*).

In size these peptides range from the tripeptide protirelin to the 32-aminoacid calcitonin. The salmon calcitonin monographs cover both the chemical as well as the biotechnological synthesis route. This again illustrates the borderline properties of these compounds. The Glucagon monograph only describes a biosynthetic compound.

Monograph tests addressing purity

The monographs address different aspects of the 'purity' of the peptide substances. All monographs describe a Reversed Phase HPLC test to detect related impurities, one older monograph also includes a TLC method for that purpose. Most of the monographs contain an optical rotation test which may provide some assurance on the enantiomeric purity. Bacterial endotoxins are specified in all monographs, reflecting the fact that these substances are administered parenterally. Typical tests for chemical compounds such as solvents, sulphated ash and appearance of solution are not commonly included in these monographs for well established compounds. The latter should not be considered as regulatory guidance for new peptides, since the deletion of these tests has only been performed after considerable batch analysis data for these substances had been gathered. Testing by amino acid analysis,

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infrared - and nuclear magnetic resonance spectroscopy are common in current monographs, but these test form part of the identification section and have not yet been specifically validated for purity assessment.

In the test for related impurities by RP-HPLC the monographs allow total impurity levels of 1.5 to 5 %. Many peptide monographs have recently been revised and contain a transparency chapter describing known impurities. Disregard limits in these RP-HPLC methods are generally 0.05 or 0.1 %. (Slide p. 3: *Limits related impurities in Ph. Eur. 5.5 monographs*).

Five monographs have not been updated. Three involve products which are hardly in use anymore. On the other hand the substances desmopressin and oxytocin are still in use. These monographs should be updated.

Related impurities in synthetic peptides

A synthetic peptide consists of a number of amino acids which are synthesised individually in consecutive steps into a peptide chain. Compared to synthetic processes for small chemical molecules the synthesis of a peptide consists of many steps. Therefore it is also likely to expect a greater diversity in impurities.

For a given peptide many structural variants are possible:

Amino acids have chiral centres and should be optically pure. Enantiomeric impurities therefore represent an important category. The best way to avoid this type of impurities is to strictly specify the enantiomeric purity of the source materials. In practice we still observe impurities in peptides chains due to a D-form of an amino acid where a L-form should have been. Other impurity types result from failure sequences: deletion, truncation and repetition. Common degradation routes for peptides which are also known for proteins are deamidation (asparagin, glutamin) and oxidation of f.i. methionine or disulphide bridges. Larger peptides may have the property to aggregate (e.g. fibrillation).

General Pharmacopoeial/ICH requirements on impurities

The European Pharmacopoeia monograph 'Substances for pharmaceutical use' and the ICH guideline 'Impurities in New Drug substances' (ICH Q3A) specify threshold limits for reporting, identification and qualification of organic impurities in drug substances. When a maximum daily dose is below 2 gram, impurities above 0.05 % should be reported, above 0.1 % be identified and above 0.15 % be qualified. However, both documents state that these general requirements are not applicable to peptides. Reasons for this exclusion are that these requirements would be too demanding for peptides. These compounds are typically dosed in small amounts, the amounts synthesised are low and the substance is expensive. It will be difficult to isolate and characterise all individual impurities which may originate from the multistep synthetic process. Therefore for peptides a case-by-case approach in the evaluation of related impurities is more appropriate than the approach prescribed by PhEur/ICH for conventional chemical drug substances.

This case-by-case approach rather concerns the specified threshold limits than the general principles thereof. The Ph. Eur. chapter 5.10 'Control of impurities in substances for pharmaceutical use' states that "although the threshold stated in the general monograph do not apply, the general concepts of reporting, identification (whenever possible) and qualification of impurities are equally valid for these classes".

Technical Guide for the elaboration of monographs for Synthetic Peptides and Recombinant DNA proteins

Expert group 6 of the European Pharmacopoeia has composed a technical guide for the elaboration of monographs for peptides and rDNA proteins which is now published on the internet. This document contains general guidance for a test on related peptides in a pharmacopoeial monograph:

- Typically, monographs for synthetic peptides contain a reverse-phase LC test for related peptides. Such tests are validated for specified impurities known to be potential contaminants, and where possible, are transparent with respect to these impurities.
- Where appropriate, specified impurities reference substances are provided.
- Where necessary, specified impurities may have to be separately quantified in independent methods.
- Where a monograph depends on a single purity test, then the capacity of the method to measure all relevant impurities should be demonstrated.

Related impurities of peptides raise concern from a theoretical point of view: the pharmacopoeial peptides have potent pharmacological properties and this may also apply to structural variants which could enhance, prolong or antagonise the activity of the main compound. When the peptides are becoming large in size also the potential immunogenicity raised by impurities may become a concern.

As is also a basic concept in the impurity assessment of biologicals: the quality of the product released for commercial scale is (at least) the same as the product evaluated in clinical studies. The analytical test methods must be able to establish product related purity in a thorough way. More than one method may be needed to establish this.

Specified impurities in Ph. Eur. monographs

The monograph of busserelin is presented as an example of an updated version. The structures of the common impurities are listed. For this nonapeptide Ph. Eur. limits for four different epimers A, B, D and E and a single truncated peptide "C" are given. In this overview the specified impurities are categorised for all current monographs. (Slide p. 6: *Specified impurities in Ph. Eur. Monographs*)

It is evident that epimers are the most frequently specified impurities in these monographs. Furthermore when comparing the impurities in the different nonapeptides the absence of any specified epimer in felypressin is striking. Maybe the current pharmacopoeial methods do not give us the full picture. An orthogonal analytical approach i.e. a detection method based on charge in addition to RP-HPLC is typically applied for rDNA proteins. Such an approach should be considered for new and revised peptide monographs as well. Occasionally new impurities are reported for well established substances when methods have slightly been changed.

Closing remarks

Ten Ph. Eur. monographs for synthetic peptides have been implanted in the Ph. Eur. 5th Edition. Most of them have recently been modernised. New peptides will be introduced in therapy and new monographs should be drafted and introduced. More specific guidance for related impurities is needed. The symposium provides a good forum to discuss this.

DISCUSSION

Question from the floor: I have a question. If I understood you correctly, then group 6 mainly treats the rather old, well known, peptides, so goserelin, calcitonin, glucagon; peptides like that; can you comment on your work on completely new peptides, so there are much more new peptides in clinical phases, and as a producer, I would be highly interested to know your thoughts about this group of new, completely new compounds or peptides.

Dr Peter Jongen: Basically we work on peptides when we are asked by the European Pharmacopoeia Commission, and I agree with you that to give guidance on new peptides, it would be useful to have more information than what we have right now, and therefore we should maybe elaborate some general guidance, but it is our experience from proteins, that it is often very difficult to start working on the monograph if a product is just appearing on the market. I agree with you that if you compare the situation now with 10 years ago, there are many promising new compounds coming, so we will have a new course. We need to work more on peptides, I agree with that, but we are dependent on the proposals from the European Commission, and I intentionally mentioned to you the example octreotide because I discovered that this is in use for more than 10 years, and it has a far higher use than the analogue, where we put effort in monographs, but it should be put on the agenda, so if you want to work on it, you should, I think, go to your national delegation, and ask them for proposal for monographs, and then the Commission can approve it, and the group can work on it, because I think it would be useful for us to see the new monographs, and also to see the new technology, I think at last meeting for instance, we had a discussion with an NMR expert group, what could be the added value of NMR for us in the analysis of peptides, so we realised that there are new things coming on, and we hope that we will get new activity on peptides.

Mr Peter Castle: With the drafting of monographs, one of the things is, you should not start too early, and you should not start too late, and it is a rule of thumb now that the European Pharmacopoeia Commission wants to have a monograph at least two years before patent expiry, so that when generic products are likely to be coming along, then we have a monograph in the Pharmacopoeia, is the basis that we try to work on. Of course, when we are looking at new control methods, then it is up to group 6 to be keeping step with analytical progress.

Dr Lakshmi Prasad Alaparthy: Yeah, I have an exchange of some ideas, especially single related compounds present in different peptides, from the list of European Pharmacopoeia, out of that 11 or 12 monographs which are listed, if we look at the total impurities allowed in all these peptides, varies from 1.5% to 5%, what I understand is that as the length of a peptide chain increases, there is a slightly relaxation in the total impurities. As the small peptides are there, then naturally, the total impurities are a lot less. Suppose, when we were working on some generic new peptides, can we take the stand that, as the chain length increases, we can allow more impurities, because right now there is no clear information to work on these kind of peptides. Of course, there are a few exceptions in these European Pharmacopoeia monographs, probably those are unpublished, maybe in the earlier times, but the latest peptides are having somewhat rigid limits for peptides like desmopressin, whereas

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if you take calcitonin, there is 5% of related compounds level. Can you share any experiences, that whatever we understand is correct? Is there any other differences.

Dr Peter Jongen: I do not like you to draw the wrong conclusion from my overview. This overview gives us the current status of these well established compounds. We have the information; these peptides have been manufactured first. Some time ago when they maybe even used other techniques, and I think that if you nowadays look at newer peptides, you will see that the number of impurities to be identified and characterized is a lot more than what you see in the monographs, so when we are defining monographs, we really need to, we are going to assess things on a case by case basis. It is over the mixture of what has been achieved technically, what you can do from an analytical perspective, but you should not think that “If I have a calcitonin and a peptide of the size of 30 amino acids, and I find 4 impurities, then I am ready, and the regulatory authorities will be satisfied”, I should not draw that conclusion, that is too dangerous.

Dr Lakshmi Prasad Alaparthi: Ok, there is another test in all the peptides, there is a busserelin identification test, by amino acid analysis. If you take different peptides, there are different limits for these amino acid analysis, like in the case of desmopressin the variance is about 5%, and other peptides, in some cases, some amino acids have relaxation of 2.10%. Basically this test is meant for an identification test, so I really do not understand this strict limit for peptides like desmopressin , and more relaxed limits for other peptides in an identification test by amino acid analysis.

Dr Peter Jongen: Yeah, this is more technical. I do not know, maybe one of my colleagues from group 6 knows what the background was about this differences. We are making the requirements also based on the comments from the manufacturer basically, and sometimes, maybe the experience of the manufacturer, that when they perform amino acid analysis, that the window is wider for one peptide compared to the other. But I must agree that it looks a bit strange.

Mr Peter Castle: I think that recently, some, or maybe all of the monographs have been revised, to move the amino acid analysis to the identification section. I do not know whether all the monographs were revised from that point of view, and since it is identification, then the tolerance was widened, because it is not regarded as a purity test. Now I am not sure just where those monographs are in the pipeline at the moment, maybe we could have a look at that and then come back to it at the round table, because I think we should move on now, and give the floor to Dr Diels J. van den Dobbelsteen who is a toxicologist with Organon, and of course toxicology must be central to our concerns with impurity control. Personally, I am looking forward very much to your talk, Dr Diels J. van den Dobbelsteen.

Session II: Synthetic peptides

Toxicological aspects of peptide - related impurities
Dr Diels J. van den Dobbelen, NV Organon (NL)

Dr Diels J. van den Dobbelen's slides are available on page 8 of the Impurities Control Symposium,
Peptides presentations:
http://www.pheur.org/site/page_601.php

Introduction

Peptides and their related impurities are explicitly exempted from the ICH Q3A and Q3B guidances on impurities in drug substance and drug product [1, 2]. From an development chemistry and analytical point of view this makes sense as the peptide-related impurity levels are generally higher due to the chemistry involved and in addition serious challenges are faced when developing the purification and analytical methods. Nevertheless, these guidelines could be used as a basis of an inventory of which biological and toxicological endpoints are of importance in the qualification of synthetic peptide-related impurities. In ICH Q3A and Q3B, in vitro genotoxicity, general toxicity and other relevant endpoints to be based on chemical structure and expected pharmacology are mentioned as the important aspects to consider in the qualification of impurities. In this paper it will be discussed which toxicological endpoints are most relevant for peptide-related impurities and whether similar thresholds as mentioned in in ICH Q3A and Q3B would be applicable.

Peptide drugs

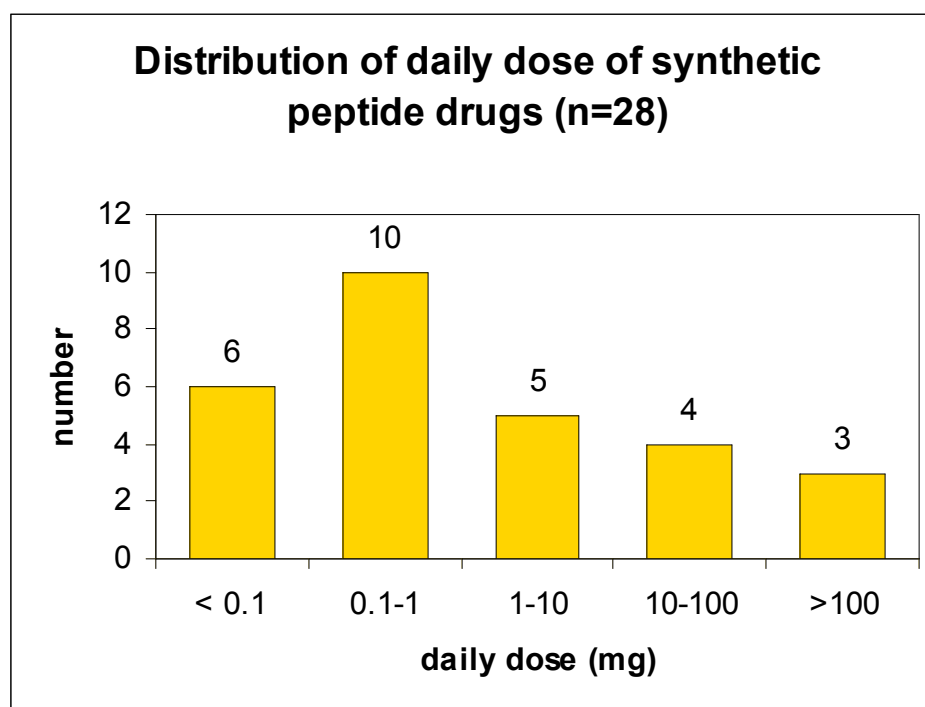
Peptide drugs generally have a high potency and high target specificity often being analogs of endogenous or natural products such as hormones and releasing factors. Their unique 3-dimensional structure is important for the potency and specificity and factors influencing these such as amino acid deletions, variations or modifications are likely to affect the biological effect. The clinical routes for peptides are mostly intravenous, subcutaneous or intramuscular and occasionally the inhalatory, intranasal or oral route are used. Peptide drugs have a limited distribution in vivo with little accumulation systemically or at the target site which is mostly extracellular. The size of the peptide and its related impurities precludes it from significant uptake into the cell unless peptides are substrates of specific transport systems. Proteases and peptidases in vivo limit the half-life of peptides, however capping with D-amino acids, acetylation or other chemical modifications are applied to increase the biological stability and decrease clearance. Likewise, for most peptide-related impurities a rapid clearance by proteases and peptidases is expected. Peptide drugs are cited to exhibit only low toxicity which basically should be interpreted as having a low probability of exerting chemical toxicity due to their inactive nature and their low tendency of being bioactivated to reactive species. Finally, as compared to (monoclonal) antibodies and other New Biological Entities (NBE), the immunogenicity of peptides and their related impurities is much less.

Toxicology view on threshold limit setting

Through the eyes of a toxicologist, it is not the percentage but the dose and duration of dosing of a related impurity which is important in the toxicological qualification of impurities. From a toxicological point of view a qualification threshold should be an absolute amount rather than a proportion. This explains the absolute limit of 1.0 and 3.0 mg per day as cited in ICH Q3A and Q3B for drugs dosed > 666 mg and 1500 mg per day, respectively. However, from an analytical point of view a percentage of the main peak would be more logical as a threshold for reporting, identification or qualification. Thus, an inventory of the recommended daily dose level of peptide drugs is required to estimate the absolute amount of related peptide at a particular relative qualification limit.

In *Figure 1* it can be observed that the vast majority of peptide drugs is dosed at a daily dose of ≤ 100 mg. The high potency of most peptide drugs results in a low daily dose, however exceptions exist for example for drugs that require (continuous) infusion to compensate for the rapid in vivo clearance. Furthermore, it should be considered that for many drugs different dose levels, various indications and different durations of dosing are applicable (e.g. calcitonins). Toxicologically, this would call for different threshold limits.

Figure 1: *Distribution of recommended daily doses of synthetic peptide drugs*



Types of peptide-related impurities

Several types of peptide-related impurities can be distinguished. In deletion sequences, insertion sequences and truncated sequences an amino acid has been omitted, inserted extra or the synthesis was discontinued at a certain stage. Diastereomers are generated by insertion of a D-amino acid or racemization during synthesis. Furthermore, functional

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groups of amino acids can be modified (oxidation, deamidation) or intra or inter peptide disulfide bridges could be altered. In drug products, with larger peptides in particular oligomers and aggregates can arise. Finally, genotoxic impurities should be mentioned as these are particularly relevant from a toxicological perspective.

Genotoxicity assessment

The subject of genotoxic impurities has received considerable interest from Regulatory Agencies and industry over the last years. In June 2006 EMEA has finalized the Guidance on the limits of genotoxic impurities and the FDA is about to publish a draft guidance on the same subject. A taskforce of the Pharmaceutical Research and Manufacturers of America (PhARMA) has also issued a White Paper on the establishment of allowable concentrations of genotoxic impurities in drug substance and product which proposes particular Thresholds of Toxicological Concern dependent on the phase of drug development and the duration of dosing. This paper recommends a 3-step approach starting with a classification of the identified and predicted impurities with regard to their genotoxic potential. The chance of genotoxic impurities being forwarded to the drug substance and drug product should be assessed based on the nature of synthesis conditions and type of chemistry applied, nature and quality of starting materials, solvents, resins, reagents, catalysts, protection and deprotection agents, the effect of purification steps and the results of in-process controls. Given the fact that hydrazide coupling of amino acids is historical, the above mentioned assessment for synthetic peptides manufactured at Organon – Diosynth has resulted in the conclusion that the chance of forwarding a genotoxic (peptide-related) impurity to levels above the threshold of 1.5 $\mu\text{g}/\text{day}$ is remote, if not non-existent.

General toxicity assessment

In the qualification with respect to general toxicological aspects, seldomly the impurity of interest is investigated due to the significant analytical and synthetic effort this would take. At best, new and reference drug substance batches containing different amounts of (peptide-related) impurity are being compared as suggested in the relevant guidances. However, this practice grossly overestimates the sensitivity of toxicity studies in revealing batch differences. The findings observed in the toxicity studies are related to the biological activity of the parent drug and effects of impurities even when present at %-level are mostly likely masked or overwhelmed by the effect of the parent drug. For example, for a peptide-related impurity at a 1% level the agonistic or antagonistic potency should be considerably higher as compared to the parent to be able to modify the biological response of a particular drug substance batch. Moreover, general toxicity studies are in general not specifically tailored towards detecting changes in pharmacological potency. Furthermore, given the low risk of chemical toxicity by peptide drugs themselves, in general, peptide-related impurities present a % level have an even lower risk of inducing chemical toxicity; this holds true even more considering that the parent compound, from which the impurity is derived, has been qualified and found safe.

Other endpoints important for qualification

Most likely other toxicological endpoints are more appropriate for peptide-related impurities. Altered pharmacological activity and immunogenicity are anticipated to be the most likely relevant in view of biological qualification. For some peptide products

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(quantitative) structure activity relationship data is (publically) available from lead optimization efforts to increase potency or prolong half-life. From this data it can be learned that in general peptide-related impurities with deleted or inserted amino acids or truncated sequences mostly result in decreased bioactivity. This is in line with the expectation that for natural peptides selection pressure during evolution has resulted in the optimal peptide – receptor combination in terms of specificity and potency. For diastereomers only positioning of a D-amino acid on either of the peptide ends will mostly result in increased metabolic stability, in most cases insertion of D-amino acids will result in decreased biological activity as a result of altered peptide folding. However, occasionally this mutation will result in increased biological activity. Oxidative modification or deamidation of lysine or arginine residues will most likely result in decreased activity. Modification of disulfide bridges will also result in changes in 3-dimensional structure of the peptide again most likely resulting in significant loss of biological activity. Thus, in summary the likelihood of adverse effects evolving from altered potency or specificity of a related-peptide impurity resulting in full antagonistic or super agonistic activity is estimated to be small.

Immunogenicity

Biological entities are associated with the risk for eliciting an immune response. Two markedly different immune reactions can be distinguished: classical immune reaction to neo-antigens and breakdown of immuno tolerance. The classical immune response to neo-antigens is observed with proteins of animal origin (complete or chimeric) and it tends to be a rapid reaction, sometimes already occurring after a single dose. The incidence of a classical immune response to neo-antigens has a rather high incidence, the antibodies are usually neutralizing and persist for a very long period. Breakdown of immuno tolerance is characterized by a response to antigens that should normally be regarded as non-foreign. The breakdown of tolerance is generally a slow process which can take years and the incidence is in most cases low. The antibodies also disappear if treatment is stopped or spontaneously reverse in long term treatment if continued. Breakdown of immuno tolerance is characteristic of human homologue products and often caused by the presence of oligomers or aggregates. Thus, the breakdown of immuno tolerance may be relevant for drug product in particular.

The chance of evoking a humoral or cellular immune response is proportional to the number of amino acid residues and dependent on the 3-dimensional structure. Peptides of < 8-10 amino acids as such are generally not immunogenic. Moreover linear epitopes are only weakly immunogenic as compared to discontinuous epitopes. As many peptides are analogs of the natural homologues or modified within the limits acceptable to not elicit an immune response, truncated peptide-related impurities can be expected to be at most equally immunogenic as compared to the peptide drug itself. However, related peptides impurities with amino acid deletions or insertions, diastereomers, disulfide exchange and functional group modifications may result in altered peptide folding, and thus in theory might result in an impurity directed immune response. Furthermore, the dosing frequency and route are of eminent importance to the chance of inducing an immune response. For most NBEs the subcutaneous and intramuscular route are most immunogenic followed by the inhalatory route. The intravenous and the incidentally applied oral route are typically associated with a very low chance of inducing an immune response. In addition, for single use peptide drugs

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used for example as imaging agents (e.g. Technetium-99 depreotide) the risk of a sensitization step being followed by a challenge in a second or following administration is very limited, if present. Finally, not only the intrinsic chemical properties of the peptide but also on extrinsic factors such as the host immunoglobulin repertoire, self-tolerance and various cellular and regulatory mechanisms definable only in the context of the host.

As indicated above unlike proteins, peptides by themselves are not very immunogenic. To elicit a humoral or cellular immune response conjugation or adhesion to a surface and/or co-administration of an adjuvant are required for peptides shorter than 15 residues. This provides the required T-cell help important for the initiation of an immune response. From the field of peptide vaccines a lot can be learned on the requirements of peptides to elicit an immune response. The low immunogenicity of peptides is probably best illustrated by Fromme *et al* [3] who synthesized a retro-inverso D-amino acid GnRH analogue to be able to induce a humoral immune response in rabbit and mice to this decapeptide. These drastic changes eventually succeeded in generating an immune response without the necessity to co-administer an adjuvant. In various publications on peptide vaccines the peptide dose reported in animals to elicit an immune response are in the range of 10 to 100 μg , however always in the context of an adjuvant and/or conjugation/adhesion of the peptide. This is a situation very different from human exposure to related-peptide impurities in synthetic peptides. Thus, even though some related peptide impurities could in theory be more immunogenic as compared to their parent peptide, the fact that they are only present at say a % -level makes it very unlikely that at these low levels in the absence of a context suitable for eliciting an immune response they act as immunogens.

An inherent problem in preclinical testing the immunogenic potential of proteins, peptides and peptide-related impurities is the fact that the preclinical models are incapable to predict immunogenic potential in man. A human protein sequence will in most cases elicit a classical immune response in any preclinical species. As a consequence, such preclinical studies on a peptide product are incapable of providing guidance to the size of a threshold dose below which an immune response to the peptide itself or its related-impurities will not occur in man.

Conclusion

In summary, if only toxicological considerations were taken into account when setting reporting, identification or qualification limits for peptide-related impurities a highly differentiated set of thresholds would evolve with amongst others daily dose, duration and route of dosing, related peptide length and structure as factors determining the value of the thresholds. However, this would be too complicated for regulatory purposes. Moreover, it would not give consideration to the practical limits of the synthesis, purification capability and analytical challenges often encountered.

Given the generally low toxicity of peptides and their related impurities, and the low dose in which synthetic peptides are mostly applied it appears to be justified to copy the absolute qualification threshold of 1.0 mg for peptide drugs with a high daily dose analogous to ICH Q3A. Taking into account the challenges in peptide process chemistry and analytics, a higher relative threshold (in %) is advocated in the lower daily dose range. A qualification threshold of 1% up to the daily dose of 100 mg for the peptide drug would result in a very straightforward definition of a threshold. The proposed qualification threshold for peptide-

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related impurities and its relation to the ICH Q3A and Q3B thresholds are depicted (Slide p. 14: *Relationship daily dose and qualification thresholds of ICH Q3A, Q3B and proposed for peptide related impurities*). Obviously, one should be aware that exceptions with more stringent qualification limits could apply for example in case of potentially genotoxic impurities (risk dependent on the synthesis, reagents etc.) or for products known to be very prone to result in an immune response such as peptides with non-human sequences.

References

- [1] ICH Q3A Impurities in New Drug Substance, Feb 2002
- [2] ICH Q3B (R2) Impurities in New Drug Product, June 2006
- [3] Fromme B., Eftekhari P., Van Regenmortel M., Hoebeke J., Katz A. and Millar R. A novel retro-inverso gonadotropin-releasing hormone (GnRH) immunogen elicits antibodies that neutralize the activity of native GnRH. *Endocrinology* **144**(7): 3262-3269 (2003).

DISCUSSION

Mr Peter Castle: We will take a couple of questions, if there are any right at the moment. Peter?

Dr Peter Jongen: You referred to the immunogenicity of calcitonin. I wondered: has there been also looked at whether this differs between the biosynthetic version and the synthetic version of calcitonin?

Dr D. J. van den Dobbelaars: I am not aware, the companies may have done. The only thing I know that eel-calcitonin and salmon-calcitonin show much higher incidence of immunogenicity because of their foreign nature. Maybe, I can try to look it up, but I am not sure that I will find the answer.

Dr Lakshmi Prasad Alaparthi: Is there a good relationship to this?

Dr D. J. van den Dobbelaars: Yeah, some of the data's are published, for example a lot of people have worked for a long time on GNRH analogues, and you pretty well know what kind of substitutions you are basically allowing to retain, pharmacological activity and antagonistic activity. Some of the data has been published, and some of the data I guess are still within the company, the innovator company, and they sort like to sit on it for obvious reasons.

Dr Lakshmi Prasad Alaparthi: Amongst these impurities, that maybe one immunized deletion, one immunized maybe will help us in predicting the correct toxicity.

Dr D. J. van den Dobbelaars: Yeah, from a toxicological perspective, I can only say that if you are able to predict the pharmacological activity, becoming less or more or antagonistic, you are already quite far in characterizing biological properties of a protein,

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because, as I said, the chemical toxicity of proteins, or polypeptides, is generally considered as very low.

Dr Lakshmi Prasad Alaparthi: Yes, what is the authorities perspective?

Dr D. J. van den Dobbelsteen: Sorry, can you repeat the question?

Mr Peter Castle: I think we should deal with that during the round-table, later on. I would just like to comment on your last slide; 1% or 1 milligram. 1 milligram seemed very, very generous for products where the dose maybe is just in the range of a few milligrams, so maybe it is dose related, I do not know, but again, that is probably something that we will come back to in the round-table.

So we are going to move on to the next speaker. On the programme we have two presentators for the next presentation; I do not whether both of them are going to be speaking. We have Dr Silvia Arrastia, who is, both of the presentators work for BCN Peptides, Dr Silvia Arrastia on Regulatory Affairs, and Dr Sergei Pavon on Quality Controls. Could I hand over to one or both of you please, so Dr Sergei Pavon is going to present.

Session II: Synthetic peptides

Manufacturer's approach to specifications

Towards the identification of related peptides impurities and interpretation of the monograph
Dr Silvia Arrastia and Dr Sergi Pavon, BCN Peptides SA (E)

Dr Silvia Arrastia and Dr Sergi Pavon's slides are available on page 25 of the Impurities Control Symposium,
Peptides presentations:
http://www.pheur.org/site/page_601.php

Introduction - General background

Peptides are small proteins (up to 50 residues, in monographs up to 32 with salmon calcitonin), but most of them show a complex structure with high molecular mass weight. There are many ways to get to a certain product. BCN Peptides produces synthetic peptides with the solid phase peptide synthesis technology (first introduced by Dr. Bruce R. Merrifield). Liquid phase synthesis or even recombinant methodology are alternative options. Each manufacturing procedure lead different impurity profiles.

At this slide typical impurities coming from the route of synthesis are listed. This is: deletion sequences (misincorporation of residues), truncation sequences, incompletely deprotected sequences, aminoacid overincorporations, sequences modified during cleavage, side reactions such as epimerization or aspartimide formation etc.

Furthermore, degradation processes after product manufacturing can also take place. Stability studies give us information on how important a particular degradation could be. Listed we have some examples of this degradation reactions: hydrolysis, deamidation (also at side chains), oxidation, epimerization, acetylation etc.

Identification of peptide impurities

The huge amount of potential impurities that could appear during the synthesis of a peptide, makes very difficult the identification of these peptide impurities. There could be too many potential impurities to individually synthesise all of them, especially when handling long peptides. And in case of having all the potential impurities synthesized it would be very difficult to find a method able to separate all of them. And therefore we have to focus on the identification of those which are present in the final product.

A good technique for this identification would be the HPLC-MS, but most of the analytical methods used for the related peptides tests in current monographs are analysed using buffers. It is known that salts contained in these buffers are not suitable when using a mass spectrometer.

Another problem is the small amounts of impurities present in the purified product (much below 1%), becoming often difficult the identification of the impurities from the pure product. We need samples that can be a valid source of impurities in order to isolate and identify them. The ways to get samples "rich" in impurities are: crude products (unpurified), and stressed samples where we force the increase of the impurity levels. With them we will be able to perform identification and decide whether a certain impurity has to be synthesized or not for further purposes.

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In this slide (Slide 6, p. 18) I explain how we usually proceed for the identification of related peptides:

First of all we take a sample rich in impurities and we run the HPLC analysis. We get a set of different peaks that are individually collected. These fractions will be desalted to obtain samples suitable to be analysed.

For that purpose we use commercial cartridges where the collected sample is loaded. The impurity is retained, but not the polar salts that we want to get rid of. The procedure follows washing with water, and finally eluting with a slightly acidified mixture of acetonitrile and water. A desalted sample is obtained (Slide 7, p. 19)

Then, the sample is analysed by Mass Spectrometry, which is a very useful identification technique, where a difference of a single unit in mass weight can be thoroughly determined. We want to point out that we check all our manufactured peptides using direct electrospray MS, and it appears to be more useful and quick in identification than NMR (complex spectra in peptides) or TLC, which are proposed in many monographs in the identification section. Next stage is checking the relative retention time of the isolated impurity. We inject it separately in the HPLC, and also a mixture of the main product and the adequate percentage of the impurity to determine its relative retention time.

Problems during the identification process (Slide 8, p. 19):

Some peaks elute very close to the main peak and we often find overlapped peaks, or even coelutions. Therefore, the chromatographic conditions need to be optimized in order to get a good separation to collect one peak per fraction. As an example that not all the impurities are always well resolved, we have the synthetic nonapeptide buserelin, where impurities D and E are limited in the specific monograph as the sum of them. Or salmon calcitonin, where the coelution of different specified impurities is accepted.

As we are working with an analytical HPLC, a very low amount of each impurity is obtained from each injection. To get higher amounts, the concentration of work can be increased in order to collect more amount of each impurity. But often we can't go to much increase because the more we inject, the worst is the separation.

So we have to find a balanced situation in order to have the best relationship between amount collected and chromatographic separation. And at the end this can mean that many injections need to be performed.

Degradation impurities

We have seen that apart from the impurities coming from the route of synthesis, there are other potential impurities coming from degradation (Slide 9, p. 20). Samples rich in these impurities can be obtained forcing the degradation of the pure product. For that purpose we have focused on these two ways:

- Temperature degradation (ex: accelerated stability studies).
- Sodium hydroxide degradation.

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We use the degradation of the pure product by temperature treatment mainly to identify the impurities that could be found along the stability studies (Slide 10, p. 20). Although these stability studies are usually performed at 5°C and at 25°C, a drastic treatment of the product up to 60 or even 80°C during some days can lead a sample “rich” in the impurities that could be observed along the stability studies. Not all the impurities found by this way would necessarily appear along the stability studies.

As an example, we have here the synthetic nonapeptide desmopressin. Applying a temperature of 80°C for several days, an increase of up to a 2% by normalisation in the content of the first impurity is obtained. With such percentage is much easier to work than with a poor 0.5% or less. These stressed samples have become very useful in our labs for identification purposes.

BCN Peptides has been always concerned about the epimer impurities, and it should be noted that the solid phase peptide synthesis is a well optimized technique which allows a high minimization of the epimerization side reactions. In some cases, all the epimers have been synthesised to know their relative retention time, but with long peptides too much work is needed to synthesize all the epimers.

As an alternative to the synthesis of all the possible epimers in long peptides, we introduce a stress treatment using sodium hydroxide (Slide 11, p. 21).

This treatment is similar to the method described to obtain the resolution solution in the Leuprorelin monograph, where impurity B (D-His-Leuprorelin) is generated.

The sodium hydroxide treatment has been developed to identify which are the most sensible epimerization sites in solution. By treating the pure product with sodium hydroxide and temperature (50°C is enough), the product is degraded and the degradation products show the same mass weight as the active substance, which allow to conclude that the degradation products are epimers. One to three hours are usually enough to complete the process, and neutralisation is applied afterwards.

It is true that this technique does not allow to know which is the residue where the epimerization takes place, we should need further analytical work for that, but we can be sure that the peak with that relative retention time corresponds to an epimer.

As an example we have here gonadorelin. We can see how dramatically new peaks arise. We compared the chromatogram with the current monograph method with a second HPLC method using no buffers but acidic water and acetonitrile. We can see how similar the obtained profiles between the two systems are; that permits us to work with collections in the non-buffered system, avoiding the desalting stage previous to MS. With this treatment we got as a result three epimerization peaks, unspecified at the monograph (there's no list of specified impurities in this case).

In this example, we can see again, that new impurities appear after the sodium hydroxide treatment (Slide 12, p. 21). But in this case, we had a disappointing surprise when comparing the two HPLC systems, buffered and unbuffered. The purity profile was very different between them: a peak of about a 33% by normalisation appearing just before the main peak in the unbuffered system. This peak coeluted with the active substance peak in the monograph method. Furthermore, poor separation was obtained among the peaks, having therefore to improve the separation conditions.

Fortunately, in our routine analysis for a batch release of any product, we run at least three chromatographic methods more apart from the monograph one, in order to ensure its quality and not to mismatch undesirable ghost peaks. The need for a second HPLC method and a

MS identification test for the pure product before the batch release appears to be here justified. It is good to point out how revealing a second method could be. Complementary methods to those of European Pharmacopoeia are often needed.

Monographs: Proposals

Having a look to the related substances section of the different peptide monographs, we consider that there are some aspects that need to be homogenized, and others that could be treated in a different way (Slide 13, p. 22).

1. First of all we would like to talk about the **Quantification** of the impurities. Looking at the peptide monographs, we can see that some of the monographs use the external standard method for the quantification of the impurities (such as Buserelin, Gonadorelin and Goserelin). This means that a diluted solution of the active substance is used as a standard to quantify the individual impurities, while in other monographs the quantification is performed by means of the normalization procedure (such as Salmon Calcitonin, Desmopressin, Leuprorelin,...).
At BCN Peptides Quality Control Laboratory it has been observed that the use of External Standard method lead to possible errors in the quantification. These errors are due to:
 - Handling, because an extra manipulation of the sample is needed, and to
 - sticking effects of the product on the glass vial. These effects are transformed into a lower response factor which leads higher values in the quantification of the impurities. Those effects have been also observed in the results of several collaborative studies.Then we propose homogenize the peptide monographs with the general use of the normalization Procedure
2. We would also like to talk about the **integration** methods. We think that a general statement regarding the integration method should be included in the monograph 2.2.46 *Chromatographic separation techniques* to avoid differences or discrepancies between laboratories. Sometimes the API manufacturer integrates the chromatogram in one way and the customer does it in another. We propose the Tangential Integration as the general procedure. In cases where the integration should be performed in another way it could be specified in the specific monograph.
3. Next we would like to propose a different way to describe the **stationary phase** of the columns that are used for each analysis.
We know that if a different column wants to be used it needs to be validated → But even if the method is validated with a different column, many customers want that the monograph is strictly followed. Therefore, we would like to propose a generic description of the stationary phase such as Reverse Phase.

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Stationary phase

It has been observed that different kind of reverse phase columns can lead identical results. In the example we can see that the chromatogram obtained when a sample of gonadorelin is analysed with an octylsilyl silica gel (C8) column is exactly the same as the one obtained when the same sample is analysed with an octadecylsilyl (C18) column (Slide 14, p. 22).

Usually each laboratory has its own preferences on certain columns due to different qualities, life time, and other reasons, so we would like to propose a change in the policy of the description of the stationary phase. As we said before, we would like to describe it as Reverse Phase. Then, if this general description is complemented with a system suitability and with the retention time range where the main product should elute it would be enough to establish a good description. As we said before many customers want that the monograph is strictly followed, and this measure would satisfy both, API manufacturers and Drug Products manufacturers.

Continuing with the stationary phase, it is known that the separation properties of the stationary phase do not depend only on the length of the aliphatic chain (such as octyl or octadecyl) (Slide 15, p. 23). It also depends on the interactions of the sample with the packing materials, and on other factors. This means that not all the columns that comply with the monograph description will lead to identical purity profiles, and may be a particular column could not be suitable for the purity analysis.

In the case of Buserelin, we analysed CRS standard with two different reverse phase columns. One analysis was performed with a C18 column and the other analysis was performed with a C8 column. It should be noted that in this case the C18 stationary phase is the one described in the monograph even the recommended brand in the knowledge data base.

In both cases the system suitability was met, but as it can be seen in the chromatogram only one impurity was detected when C18 column was used, while when a C8 column was used two impurities were detected.

It should be considered that since no chromatogram is supplied with the CRS there is no way to check which the profile that should be obtained is. So we would like **to propose that the CRS standard would be delivered with a purity chromatogram.**

Proposal of related peptide limits

After the comments on these general aspects, we would like to make a proposal for related peptide limits (Slide 16, p. 23).

As the requirements stated in the *Substance for Pharmaceutical use* monograph, do not apply for peptides, there is the need to establish some **general limits**:

We consider that for:

- *Reporting Threshold (Disregard limit)* $\geq 0.1\%$ (limit established in most of the peptide monographs)

For the

- *Identification Threshold* $> 0.5\%$
- *Qualification Threshold* $> 1\%$

(which are the thresholds recommended by the FDA in the last TIDES congress).

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As we said before, one of the main problems for the qualification and for the identification of the impurities is the obtention of enough amount of each impurity to be used in further studies.

In our case, the low amount of samples obtained is conditioning the use of analytical techniques where very low amount of samples is needed such as mass spectrometry. This technique allows us to identify the kind of impurities that are present in the sample. For example, for impurities with the same mass as the active substance we would identify the impurity as an epimer, but it would be impossible to say which is the residue that has suffered the epimerization. Sometimes, the mass obtained for an impurity does not match with any of the potential impurities and then we only could say that it is a related peptide. We think that this point should be taken into consideration in the identification requirements in the sense that not a full identification with sequence evidences such as NMR analysis and others are required.

Regarding the **specific monographs**,

It should be considered that the impurities present in a peptide product depend on the manufacturing process. Therefore, it is difficult that a monograph includes absolutely all the impurities. We know that if a product has different impurities than the ones described in a monograph, it should be notified to the European Pharmacopoeia, but it is a long process and takes a long time to be included in the monographs. Then, we consider a very important point the inclusion of a limit for *unspecified impurities* (impurities not described in the monograph).

Conclusion

As a conclusion we would like that the European Pharmacopoeia:

- Homogenize the peptide monographs.
- Consider a different way to describe the columns used.
- Tentative identification of the impurities should be accepted
- Consider the incorporation of a limit for Unspecified impurities

DISCUSSION

Mr Peter Castle: So we can take a couple of questions before we take the break. I think there are some very interesting suggestions in there, and I am sure we will in any case come back to them during the round table.

Dr Jan W. H. Smeets: The last slide you had, you say that you propose to include in the general monographs an identification threshold of 0.5%, and then you say also that you will include in the specific monograph an unspecified impurity limit. In fact it is not necessary, because you have it already in the general monograph; the identification threshold is exactly the same as the unspecified impurity limit, according to me.

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Dr Silvia Arrastia: Yes, but it was because in one of the monographs we saw there was no place for any other impurity.

Dr Jan W. H. Smeets: But I mean if you already included it in the general monograph, then there is no need anymore to put it in the specific monograph.

Dr Silvia Arrastia: No.

Mr Peter Castle: I am sure that what you say is correct, but we do not say that anywhere, black or white, we simply exclude the peptides from the identification threshold, because normally it should be, the limit for unspecified impurities should be at the, but we do not have to say that.

Session II: Synthetic peptides

Manufacturer's approach to specifications

Manufacturer's approach to control impurities in peptide manufacturing; a proposal to set specifications for peptides API's

Mr Bernard van Genugten and Dr Ivo Eggen, Diosynth BV (NL)

Mr Bernard van Genugten and Dr Ivo Eggen's slides are available on page 34 of the Impurities Control Symposium,

Peptides presentations:

http://www.pheur.org/site/page_601.php

Abstract

The production of synthetic peptides generally comprises the following steps:

- 1. the assembly of the protected peptide sequence, either by classical solution-phase synthesis (CSPS), solid-phase synthesis (SPPS) or Diosynth's patented DioRaSSP method,*
- 2. acidolysis to remove the protecting groups,*
- 3. other modifications like disulfide bond formation and fragment couplings,*
- 4. purification by preparative HPLC, and*
- 5. isolation by e.g. lyophilization.*

Impurities in the final product of a peptide synthesis may be roughly divided into six different categories.

- I. Deletion sequences lack one or more residues and originate from incomplete coupling and/or deprotection steps.*
- II. Insertion sequences contain one or more "duplicate" residues and originate from either the presence of free amino acid in the starting protected amino acid derivatives, or from incomplete removal of excess carboxylic compound after a coupling step.*
- III. Truncated sequences are formed when either capping or quenching is part of the synthesis protocol; the former is primarily applied in syntheses according to SPPS resulting in the formation of N-terminally truncated sequences, while the latter is applied in synthesis according to CSPS resulting in C-terminally capped sequences.*
- IV. Diastomeric sequences contain one or more residues in the undesired chiral form. The origin of such modifications may lie in the application of starting amino acid derivatives containing the undesired stereoisomer, as well as in specific synthesis steps which may be difficult to control.*
- V. Another group of impurities originates from modification of functional side chains and/or terminal groups, modifications of Asn residues being especially problematic.*
- VI. Finally, the pairing of disulfide bridges within the peptide may be modified to yield e.g. (anti)parallel dimers or polymers.*

The occurrence of impurities may often be controlled through optimization of reaction conditions and appropriate monitoring methods. The most suitable detection method for impurities in the final product is generally LC/MS, which is however not appropriate in case

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of diastereomeric impurities. For the identification of these impurities, isolation and or directed synthesis is the only conclusive method. With the number of residues in a synthesized peptide, the number of potential impurities increases. Moreover, since the influence of a specific modification on the overall structure of the peptide generally decreases with increasing peptide length, the difficulties of removing specific impurities also increases with increasing length.

There are currently no global or European guidelines on setting specifications for related substances in synthetic peptides. They are excluded from the ICH-guidelines Q3A and Q3B. Currently, specifications presented in Ph.Eur. monographs seem to be based on a case-by case approach; no generally applied identification and qualification limits are being applied.

Generally it is considered that peptides –and also their impurities- exhibit low toxicity. Based on the ICH Q3A(R1), it is acceptable that qualification of impurities is necessary if the daily intake of the impurity is more than 1.0 mg. Since the daily intake of peptides is generally not more than 100 mg per day, a qualification limit of 1.0% would therefore being considered acceptable.

Specifications for synthetic peptides should not only be based on safety aspects of impurities, also the technical limitations of synthesis and purification procedures are important. Synthesis of large peptides (typically >15 amino acids) leads to considerably larger number (and amount) of impurities than synthesis of small peptides. This should be expressed in different specifications for both types of peptides. Therefore, a specification for total impurities of 3.0% is proposed for peptides of 2-15 amino acids, while for larger peptides a specification for total impurities of 5.0% is considered appropriate.

Because related substances of peptides frequently elute close to the main peak, peaks often consist of multiple impurities, even when more than one chromatographic method is used. For this reason, for peptides, it is proposed to set specifications for peaks with a given RRT or RRT window, instead of individual components.

Identification of related peptide impurities may be a laborious task, especially if the impurity is a stereoisomer. Together with the information that the peaks often consist of more than one impurity, composite peaks it is acceptable to identify only those peaks that need a specification of > 0.5%.

The proposed thresholds for identification (0.5%) and qualification (1.0%) are values that are also employed by FDA/CDER.

DISCUSSION

Mr Peter Castle: I think that we will move straight on to the round table, and we can take questions on Mr Bernard van Genugten's presentation then, I think particularly on the proposals, which has come from several of the speakers this morning.

Session II: Synthetic peptides

ROUND TABLE DISCUSSION

Dr Michael Morris, Chair of the European Pharmacopoeia Commission, **Dr Jean-Louis Robert**, Chair QWP (EMA) and Chair of the Steering Committee of the Certification Procedure (EDQM, Council of Europe); **Dr Jan W. Dorpema**, President of the European Pharmacopoeia Group of Experts No. 6 Biological Substances; **Mr Peter Castle**, Secretary to the European Pharmacopoeia Commission (EDQM, Council of Europe)

Mr Peter Castle: I think that what we have all noticed at this morning session is that we have had some very concrete proposals on how we should be moving forward with the monographs in the European Pharmacopoeia. We have had an interesting presentation on toxicology, which I think must be really central to our considerations on impurities control. One of the experts around the table is Dr Jan W. Dorpema, who is the Chair of Group of Experts No.6 which drafts the monographs on peptides, and first of all, I would like to pass the word to you, Jan Willem, and maybe you could give us any comments and impressions, and some idea of what are the ideas you would like to take forward in Group 6.

Dr Jan W. Dorpema: Thank you very much, Peter. I would first like to thank all the speakers of this morning, and also of yesterday, but specifically this morning, for their excellent contributions. What we do in Group 6, like in the other groups, is that we try to make useful monographs, and this means, and it was also in the last contribution more or less mentioned, that we have a tremendous input from manufacturers on processes, and on products, and it all comes together. Now, to balance this and it is extremely valuable for us also to see what are the effects of this. Therefore the presentation on toxicology, I think is very helpful, although, I must say, it only gives us the data which we can use to monitor. The pharmacovigilance data have the same capabilities, we can not validate them. So what we still have to do is to find a way out with all this information and do the best. Nevertheless, we have got good information on the relevance of impurities, and I think it is extremely important, relevance, and I have discussed for instance with Dr Diels J. van den Dobbelen during the coffee break, that some of the impurities, finalized products, probably are also cleared by our body. We know that the DNA of our body is constantly changing. That means also that the products evolving from this will change, so also the peptides will show some of the, let us say the deviations, that we also see here when we do them synthetically, but apparently it seems that there is a mechanism for clearance for these products. But we can validate it, so at the moment what we have to do, we just have to take all these impurities very seriously. Up til now, Group 6 has dealt with impurities in, I would say, a relaxed way, and on a case by case basis, and I think the moment has come now to structurize it more, and therefore we are very thankful for the contribution which has been made this morning.

I seems that, if we looked, and it is very helpful that much of work has been done also in the direction of this structurization, and we have some proposals on the table, proposals that will bring us in line with FDA, which is helpful, but on the other hand, I must say, and just make one remark to the proposal given by Mr Bernard van Genugten, last proposal, is summarizing actually this, and it is in line with what we think in the group, although in the last meeting we had, there was one product with an impurity of 8%, so we have there some

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deviation which we should discuss, but as a whole, I think it is very helpful and it is a good base for discussion. Thank you.

Mr Peter Castle: Dr Michael Morris, do you have anything that you would like to contribute there?

Dr Michael Morris: Thank you Mr Peter Castle. What struck me first of all is that, like you, I am very encouraged to hear this morning some actual concrete numerical-type proposals, which we can take forward and debate this afternoon amongst the national authority closed meeting, and that is very helpful to have something specific to focus on, because we know some of the things that we have to do, primarily we have to try and remove some of the lack of clarity, to improve the transparency of the way in which monographs are designed, developed, written, and then used and interpreted by industry and regulators alike.

We have heard, effectively this morning, that the preparation of peptide monographs has similar difficulties to what we heard yesterday, the problems with antibiotics, and they have many of the same challenges in common, for example analytical difficulties, complex mixtures, particularly in peptides where you have the possibility of large numbers of very closely related species, which not only are difficult to separate analytically, but also may have similar therapeutic activity to the parent molecule. On the other hand, peptides represent the other end of a spectrum, if you like, in a sense that they are generally very physiologically active molecules. Unlike antibiotics they are presented and administered in very low doses, maybe below a milligram in many cases, as opposed to gram daily doses in the case of antibiotics. They are physiologically very potent, and of course that is the reason for the low dosing. Therefore, as we have heard again repeatedly, the likely actual levels of impurities, even if you have impurities of up to 10% being administered on a daily basis to patients, are likely to be very, very small, and we have heard the toxicological arguments, and like Peter, I strongly welcome this, in particular, the presentation on the toxicological elements in, to support this argument.

There is also the fact that in general terms, peptides are probably administered by different routes of administration, typically parenterally and not orally unlike antibiotics, which are often administered orally, so there is that element in the mix as well. Nevertheless, the same challenges beset us in moving forward into dealing with this, so I think that we have some good material now that we can work with, and hopefully come up with a greater clarity for the future in going forward, and working in particular for the national authorities, both the Pharmacopoeia authorities and the licensing authorities' perspective. I think that we have good work from the Pharmacopoeia already, by Dr Jan W. Dorpema's group, Group 6, and I certainly do not see the need to revise monographs that are within the kind of range we have been talking about, where they represent the existing good quality of products on the market, particularly for the older substances, but there may well be a need to review all monographs just and particularly with new developing monographs, to have a more coherent approach in the future.

Once again, we absolutely need input from manufacturers in dealing with this issue, and I have to say that it was, in response to a question I heard earlier this morning, about the work

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programme of Group 6, I think that it is true to say that some of the proposals, in the work programme for Group 6, have been seriously challenged by the Commission, because of the inability of the group to make progress, because of the lack of information from manufacturers. We have got to the situation where newly emerging molecules, included in the work programme, are actually having to be taken off the work programme, because of lack of progress, because of lack of information coming forward, so even more so in the case of newly developing peptide substances, getting near to patent expiry, or indeed recently off patent. These are the ones we critically need the input of manufacturers, of these kinds of substances, if we are able to make progress in the work of the Pharmacopoeia. So the same cry as yesterday; we need input from everybody into this overall process, and also from the regulatory authorities as well. So, that is really all I want to say. Thank you.

Mr Peter Castle: Thank you Dr Michael Morris. From the two manufacturers, we had some concrete proposals this morning. Quite by coincidence, they were exactly the same. More or less they were complementing the Q3A guideline, so I wondered whether Dr Jean-Louis Robert has something that he would like to say about that, or anything else that has happened this morning.

Dr Jean-Louis Robert: No, I would not say very much, because I would like to leave the floor to the audience. Basically I agree on what has been said by Dr Jan W. Dorpema and by Dr Michael Morris, and the proposal for having this, proposal for different thresholds to be included into chapter 5. I think it is important anyhow that we have here close collaboration between EDQM and the licensing authorities, especially also the safety working party, I think that they will need to consult them on different thresholds. Basically, I agree with Dr Diels J. van den Dobbelsteen; toxicologists are thinking in absolute value, and not in percentage, and what I have heard from my toxicology colleagues is that basically the one milligram level daily intake for an impurity which is the critical limit, and we might want, maybe, to build on this something. Thank you.

Mr Peter Castle: We did not take questions on Mr Bernard van Genugten's presentation. I would like to ask a question, Bernard. Dr Silvia Arrastia from BCN made a comment about the way that we go by quantification of impurities, and she reported that they find overestimation of impurities when you use an external standard. Does Diosynth have any experience which would confirm that?

Mr Peter Castle: Dr Silvia Arrastia; have you found it to be a general problem, or is it related to some particular peptides, the larger peptides, or something like that, where you might have more possibility of absorption?

Dr Silvia Arrastia: Well, indeed it is Dr Sergi Pavon who is working with this in the laboratory, so maybe he can answer.

Dr Sergi Pavon: We have observed it sometimes; even in collaborative studies, that difference in the response factor obtained was not in accordance with the normalization value that we obtained.

Mr Peter Castle: Dr Emmanuelle Charton?

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Dr Emmanuelle Charton: In the collaborative studies carried out by our laboratory at EDQM, we have asked the participants to carry out the calculations, using either normalization or external standard, and we came to the conclusion that this gives exactly the same results.

Dr Lakshmi Prasad Alaparathi: Yes, I do agree that both give the same results. In the normalization are external standard methods, so the only idea behind is that you can reduce some injections, because directly, you can get the results, that is one advantage, and specifically some products, example calcitonin, where the temperature is a factor, where it is affecting the stational phase and hardly the column glass ware 15-continuous injections, I am sure that many manufacturers who are making calcitonin are also having the same behaviour.

Mr Peter Castle: Thank you. I took my privilege to ask a question of Mr Bernard van Genugten. I wonder whether anybody else has any questions of Mr Bernard van Genugten's presentation. Peter?

Dr Peter Jongen: Yes, my question; I really liked the proposal of coming up with new figures, and apparently there has been some debate, but I just wondered whether the exclusion of the peptides and proteins from Q3A, was only related to the fact that the figures will not fit, or whether it was some additional concern, like the concern we have with the proteins, that reactions like immunogenicity will not really be predictable by low amount, so I am therefore concerned that if we start to defining figures, particularly for the larger peptides, that that was not the general idea of, then we automatically put these peptides in Q3A, while they should have become a philosophy more that they belong to Q6, so that is maybe also, because the whole Q3A is related to qualification of impurities, while for complex compounds, we often look at the qualification of material in clinical trials, rather than individual impurities.

Dr Jean-Louis Robert: Well, first of all, the Q3A is impurity testing, Q6 is specification guideline, and in fact, the one, the Q3, sets the thresholds for qualification reporting, identification and so on, and the Q6, we are intending to set specification. They are two different, complementary of course, but two different guidelines, we should not, Q6A or Q6B, whatever, they are really intended to set the specification, so we might have a threshold of 0.1, but we can have limits of acceptance criteria for 3%, 4%, I do not know. It depends also on the complexity of the process and so on.

Now, I must admit that I do not remember really, because it is a long time now ago, why peptides were not included into Q3A. I expect that it is more or less correct what Bernard has said, that it was felt at that time, it was new anyhow, it was already rather new for chemicals, and they did not want necessarily to make it more difficult, and they wanted really to come up with a guidelines, of course for chemicals it was much easier to come with a proposal, although in principal, enantiomeric impurities, also for synthetic substances are excluded from this guideline which is basically ridiculous, but that was also a deal, I think Japanese did not want to have enantiomeric impurities.

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Mr Peter Castle: The qualification threshold that has been proposed by the manufacturers is the same as in Q3A.

Comment from the floor: No.

Mr Peter Castle: No?

Comment from the floor: Why?

Dr Jean-Louis Robert: No, you take percentage, no. But if you take the concept that one milligram is a critical mass, if you like, then Ok, I agree.

Mr Peter Castle: Yes, that is true, I am sorry, and then, in fact the identification and reporting thresholds are different, and I take that to be an analytical problem. I suppose that that must have been a good reason for excluding them from Q3A, because simply, it is practically not possible, so if you had applied that, you would have had no peptides.

Dr Jean-Louis Robert: Then my question would be; could we step by step, have it at different levels, depending on the number of amino acids, that you might come up with, I do not know, 0.2% for identification instead of 0.5, something like this, or should we have a general, it is a question, it is not a statement, so it is a question. For smaller one, for instance 0.3, I do not know, but just to have it not uniformly 0.5, but to have it at different levels.

Comment from the floor: I think the problem I foresee is more related to the fact, that the larger a peptide becomes, the less acceptable it would be by qualification in animal studies, so people would rely on the clinical data, so we may be allow to come up with qualification figures for the smaller peptides, while we, it would not be acceptable for the larger peptides, because other problems, which are not covered by the common qualification procedure will emerge, I think that is the issue, and maybe also for this afternoon, maybe you also..

Comment from the floor: Yes, if you look in the literature, what is really sort of a cut off point is 9 amino acids, so as long as you are below that, you will be happy and safe with regards to immunogenicity, if you move above that, it becomes increasingly more complicated, because it is just a higher risk of the peptide carrying, basically these epitopes that could be involved in immunological recognition. Linear peptides also take a sort of special position. They are less immunogenic as compared to the more structurally complicated ones, carrying disulfide bridges *et cetera*.

Comment from the floor: Don't you think that this problem will vanish as the new peptides, especially peptide vaccines will be packed in special delivery systems, and containing adjuvant, and then there is the issue of immunogenicity all the time.

Comment from the floor: Right, yes, for peptide vaccines, because of the way that they are administered and the stuff that is added, they take a special position, yeah, so I would not really propose the limits above proposed for those kinds of drugs, vaccines.

Mr Peter Castle: I guess that peptide vaccines are not what we have been dealing with this morning.

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Dr Michael Morris: I just wanted to return to Dr Peter Jongen's initial question, comment, and to supplement what Dr Jean-Louis Robert said; I think that if we go back to the mid 90's, when the work was being commenced on the Q3 guideline, it was very much focused on new drugs produced chemically, and that was who the audience was, the audience being the people who had actually drafted the documents. I think from what I remember, we steered away from discussing more specialized molecules like peptides, but I participated in the Q6A discussion, which, as Jean-Louis have said, is a specification setting, and then we had in parallel the Q6B group who were looking at the biological, biotech, and there was a strong feeling that the peptides fell either into the Q6A group, or the Q6B, and it should be in one or the other, there was a strong feeling, but one of the difficulties we had with this very issue, is what is a small peptide and what is a large molecule, now we kind of toyed with the idea of 10 amino acids, so that is not too far away from your 9, so I am very heartened to think that we were on the right lines, but we could not actually agree on what the definition of a small peptide was. One of the earlier drafts of Q6A said that "large peptides should be excluded", and the question then was "what is a large peptide", and we said anything that "it is not a small one" and we said "a small one is below a certain point", but we could not agree on that cut off, so it is just really a simple question, but it illustrates the point that suddenly the regulators felt that the principles in the specification setting guidelines, and the impurities guidelines, were very much relevant to all the species, like peptides, it was only a question that we could not necessary speak with sufficient confidence to address all the issues, the kind of things that we had heard over the last two days in this meeting. So, that is just, you know, I think that we were on the right lines, but we did not have enough experience at the time to push them through.

Mr Peter Castle: Dr Jan W. H. Smeets, I think that you wanted to say something?

Dr Jan W. H. Smeets: I wanted to comment on the proposal of the thresholds. I see that the identification threshold is put on 0.5% and the qualification threshold on 1%, and I find the difference between the two very high. What does it mean in fact? It means that all the impurities of for instance 0.5%, 0.6%, 0.7% will be identified by what do we do with it? In fact, nothing at all, because we do not qualify them. So what is the use of doing a lot of work for detecting, identifying and specifying impurities between 0.5% and 1% if we do not qualify them, so my proposal would be to bring those two thresholds much closer together, then it is useful

Dr Jean-Louis Robert: I think that why we put the difference between identification and qualification threshold, was more that the applicant, or the manufacturer, he should identify an impurity, and I now speak about the chemical synthetic

Dr Jan W. H. Smeets: But they are quite small.

Dr Jean-Louis Robert: Yes, they are relatively small, I agree with you, but the idea was that you identify and you look at the structure, and you have this data base on structure alert system, and so on, and then you identify eventually that could potentially be a problem, a safety problem. And then, even if you are below the qualification threshold, you might then do some action, just to be aware about the structure and where, if potentially it could be something, that was the idea behind.

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Dr Jan W. H. Smeets: I understand, but then I would say; bring it something the 2 thresholds closer together, now the distances is rather big between 0.5% and 1%.

Dr Jean-Louis Robert: This is something which you could debate, I agree.

Mr Peter Castle: So, there were more hands raised earlier, when I asked whether there were questions at the back there.

Question from the floor: I have another question, and that is about, when I saw on page 72, and it was about the optical rotation, and in the old monographs, optical rotation is quite often used, and I see when we have peptides with, as we have been talking about now, 9 or 10 or more, it is really relevant to measure specific optical rotation, isn't that an old way, isn't reversed phase much better?

Mr Peter Castle: I think that is a question to the manufacturers. I guess that the question is; is it a sensitive way of detecting changes in the peptides?

Comment from the floor: I think by now, we have better methods, in fact to make sure that we have the proper chiral purity of a compound, and you could eventually also validate the reverse phase method that is capable of doing so, because we already saw that reverse phase method are apparently very well capable of identifying enantiomeric variants, so maybe we will have this method is suited, but it is a method which comes from the past, and it is indeed still present in most monographs, but there could be, there is analytical technology, or analytical validation strategy to overcome this.

Mr Peter Castle: And it is a very easy test to do. But I do not know whether Mr Bernard van Genugten and Dr Sergi Pavon have any experiences on the sensitivity of this method for detecting changes?

Mr Bernard van Genugten: I started telling that our company actually never made any validation of the optical rotation test; we did not test samples which had a high amount of one of the diastereomers or something like that, we never did anything to it, just we performed it and we knew actually Pharmacopoeia love optical tests, so that is really the reason why we did it. I must say it is bad for our company perhaps that we never commented on it, to remove it, but, yeah, it was our idea that something that they would not accept, but perhaps you can do it today.

Mr Peter Castle: Sergi, do you have any experience on that matter?

Dr Sergi Pavon: Not much, well, optical rotation is not so often used for other purposes than those of the final analytical control of the product, so not much.

Comment from the floor: What you have to realize is that, because we are often confronted with these kind of questions "can we just not replace it by modern technology", and basically we are in favour of it, but, let us say, the monographs are reflecting a harmonized opinion in Europe in a broad, and as long as there is one company on the market who uses this, unless we can not remove it, so this is, let's say, something which we have also to take into considerations.

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Mr Peter Castle: Ok, let's move on to another question.

Dr Rose-Marie Torstensson: Not that easy. Since I am working with Ferring pharmaceuticals and we are looking into new chemical entities, so we are looking into let's putting setting the standards for the next monographs, and we are doing these things mainly because we think that the authorities will ask for it, but I do not see any relevance of it, and I doubt really that you receive 1-2% of racemisation of a 10 amino acid peptide, so I don't think it is relevant to keep these tests, so then I am happy. Thank you very much.

Question from the floor: I have a question regarding the first presentation, started by saying that peptides are also produced by biosynthesis, but at the end of the presentation they were only talking about "impurities of synthetic peptides", so my question is if these suggestions for the set of impurities would be also applicable for small peptides produced by biosynthesis? Thank you.

Mr Peter Castle: What role does biosynthesis play for peptides i.e. up to 50 amino acids? Most of the ones we saw were in fact smaller, probably the biggest ones was about 24 or 25, or was it 30? 30. What role does biosynthesis play for those products? Is it commonly used?

Comment from the floor: I think, if you have a peptide the size of 30, it gets more and more common, and I think it is cost-effective to make it on a biosynthetic route, I am not so sure, and that is why you see how these products are getting manufactured biosynthetically, and they are also often, these are manufacturers experienced in rDNA technology, and as such, from a regulatory perspective, the biosynthetic peptides fully are under the scopes of the guidelines which we lay to the rDNA-products, and I think that they also fall under the scope of the general monograph of the rDNA-products, so that is also, because it was a symposium dedicated to synthetic peptides, I did not further elaborate on the impurities of those products.

Mr Peter Castle: So, this morning we have been dealing with chemical synthesis, and we should not think that you can extend this to the biosynthesis.

We are getting towards the close of the meeting, and I wonder whether we have some more questions, particularly from people who haven't had an opportunity yet. Michael?

Dr Michael Wierer: Thank you. Bernard, you will forgive me, but you proposed to identify the specified impurities by the relative retention window, or relative retention time. I can understand that, from a manufacturer's perspective, using always the same brand of column, this is feasible, but I am not sure that for multi source products, we can defend such an approach at our commission, when we think what is the requirement for the small molecules, because here we speak about levels of 0.6, 0.7%. Can that be reproduced on a different column? What does the audience think?

Mr Bernard van Genugten: My idea was that the relative retention times are given, but that together with that also a spiked reference standards should be sent in, perhaps I forgot to mention that, but that is the combination, there should be also a spiked sample together with

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the monograph actually, so that from that you have information about what your relative retention time be something, and then based on that, I think it is the best way to approach.

Mr Peter Castle: Ok. We are going to have one or two more questions, and I will then hand over the floor to Dr Michael Morris, to close the meeting.

Question from the floor: Ok, it is just that I wanted to come back on the presentation of Dr Sergei Pavon, it is on the first slide, he talks about the problem of buffered elements in the Pharmacopoeia methods. I think it is not specific to peptides, it is for any product in fact, and I always find curious that the new method proposed by the Pharmacopoeia are very rarely using not buffered, or differently buffered elements, mainly is phosphate buffer, so it is very difficult when you make developments to identify the impurities to transfer the method, so it is just a comment, and we heard yesterday that, from Prof. Jos Hoogmartens, that there were working on that in group 7, and it is a good news, I hope it is also true in the other groups, because it is just time to go there.

Mr Peter Castle: That is an analytical question; I do not know whether the analysts in the audience have something to add to that. Of course, identification of impurities, I get that is something you do once, and not in routine, and maybe the mobile phases that are used are the ones that are most convenient for routine use, I do not know. Bernard, do you have any comment about that? In the monographs, we are prescribing mobile phases that are not convenient for identification of impurities by MS, for instance. Could we think of moving over to mobile phases that would lend themselves better to that?

Mr Bernard van Genugten: I think, what we are talking now about, are more or less old monographs, which were developed almost before the time that LCMS, MS, or LCMS, were convenient method. I think from now on, at least within our company, we try to develop all methods which are indeed also applicable for LCMS, MS, so for the future monographs, I think this is being solved, but it is more for the current monographs that we have a problem, and for that, you have to choose for alternatives.

Mr Peter Castle: So we will have to decide what priorities should be given to revision of the monographs from that point of view, and the prioritization of our work programme is the privilege, and the duty of the European Pharmacopoeia Commission, and it is now time for me actually to hand over to the Chair of the European Pharmacopoeia Commission, not to answer your question about prioritization, but to in fact make the final address and close of the meeting.

Dr Michael Morris: Thank you very much Mr Peter Castle. I think that, I may be the Chair, but if I was to give you an opinion on prioritization of the work programme, I think I would be certainly over stepping my remit, that is the prerogative of the Commission as a whole.

It only remains to me really to thank everybody and to close the meeting. Thank you all very much for your good contribution during this round table session. I would like to thank Mr Peter Castle at my right here for moderating this morning session, very well. We have had some excellent presentations. I also want like to thank Dr Jan W. Dorpema, the Chair of Group 6 and Dr Jean-Louis Robert, the Chair of Quality Working Party, for participating in this round table, and all of you the speakers. We have had some excellent presentations this morning and of course yesterday. I did not specifically thank the speakers yesterday in

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regard to the antibiotics session, but I think that all of the presentations have been a very high standard, and it has been very thought provoking for us, and hopefully will help to direct the direction that the Pharmacopoeia takes in the next years moving forward. I do strongly feel kind of hopping back for a second to a comment. I think we can take in a lot of these things in moving forward, for example the solvents used, mobile phases. I think that is something for the future. It is for the new work, and we do not necessarily need to go back and revise existing monographs which are adequate in other ways, just in terms of a situation like that, but anyway that is just a personal view.

So, I also want to take this opportunity to thank the Chairs and the members of Group 6 and 7, and of course the staff of EDQM, for the, not only the good work, but for having the vision to bring this symposium together, to make it happen, because it was clearly something that was needed, and it has been a great opportunity for people to listen and to contribute, and to maybe go away with some clarifications, and of course for your energetic and enthusiastic and informative contributions, not only in the lead up, but during the two days of this symposium, they have been excellent. Thank you of course to all the participants for being the audience and for providing some useful questions, and I just wanted to mention that all the participants will receive an electronic evaluation sheet by e-mail, and it is very important that you provide us with your feed back, so it gives us the opportunity to dwell on what was good about the conference and to find areas for possible improvements for future development and future conference programs, and to make sure that they meet your needs. It should not be very taxing to fill out these questionnaires, but it is useful information.

Thank you very much.

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Report of the debriefing meeting held in presence of National Authorities

A meeting restricted to the participants of the symposium belonging to a National Authority took place after the symposium, in order to draw a roadmap for the respective substances.

Antibiotics: it was recognised that harmonisation of the approaches taken by the different member states was necessary. However most representatives stated that they would still, in most cases, request justifications from manufacturers when ICH thresholds were not applied. It was highlighted that a close cooperation between member states in solving the problem was needed. A possibility would be that they agree on a common decision tree that they would commonly apply. In the mean time the issues would continue to be treated on a case by case basis.

Synthetic peptides: there had been good agreement within authorities and manufacturers that common reporting, identification and qualification thresholds could be applied for all peptides. The monograph "Substances for pharmaceutical use" would be revised in order to take account of the proposal.

BIOGRAPHICAL NOTES

Dr Sílvia Arrastia, obtained his degree in chemistry from the 'Universitat de Barcelona' (UB) in Spain in 2002. She spent one year performing experimental research on peptides at the 'Universiteit Gent' (Belgium). She obtained a Master degree at the 'Universitat de Barcelona (UB)' in Spain, with the work on 'Efforts towards the orthogonal modification of peptides via Diels-Alder reaction', supervised by Prof. Dr. A Madder and Dr. J. Vilarrasa. In 2003, she started her work at BCN Peptides, S.A. (formerly Lipotec) at the Regulatory Affairs Department, where she is currently the manager.

Dr Hanno Binder obtained his degree in chemistry from the university of Innsbruck/Austria. He studied for his Ph.D. at the same university and obtained his Ph.D. in 1978. During the period 1978 – 1982 he was research assistant at the university of Innsbruck/Austria.

He joined Sandoz GmbH in 1982 and from 1982 – 1996 he was appointed as group leader and finally as head of the analytical R&D laboratories, being responsible for the development of β -lactam antibiotics.

He changed to Quality Assurance of Sandoz GmbH in 1996 and is now in the position as Head of Quality Management of Sandoz International.

Since 1998 he has been member of expert group 7 (antibiotics) of EDQM.

Mr Peter Castle graduated in biochemistry from Cambridge University, England in 1968. He worked on drug metabolism and determination of drugs in body fluids at the Pharmaceutical Society of Great Britain for three years before joining the animal health division of Smith Kline & French, UK. Since 1974 he has worked in the Technical Secretariat of the European Pharmacopoeia, now a division of the European Department for the Quality of Medicines (Council of Europe, Strasbourg). He is Secretary to the European Pharmacopoeia Commission and head of the division dealing with development of monographs and general chapters. Co-ordinates work on international harmonisation with the Japanese Pharmacopoeia and the United States Pharmacopoeia (Pharmacopoeial Discussion Group – PDG) and within VICH.

Dr Ivo Franci Eggen is Director of the Peptides Department at Diosynth BV, Oss, the Netherlands. Diosynth BV is part of Organon and produces chemical compounds (mainly steroids and synthetic peptides) and biological substances (using traditional biochemical and modern biotechnological processes).

Dr Ivo Eggen received his Ph.D. degree in Organic Chemistry at the University of Nijmegen in 1999. His Ph.D. investigation, which was supervised by renowned peptide chemists Zahn, Tesser and Brandenburg, was focussed on the synthesis of peptides by solid-phase peptide synthesis. In the following years at Diosynth, he gained much experience in solution-phase peptide synthesis and, in his function of Head of the Research Department for Peptide Synthesis, conducted the investigations towards the development of the patented DioRaSSP[®] method.

New impurities control: setting specifications for antibiotics and synthetic peptides

Prof. Dr Jos Hoogmartens (°1945) has been teaching Pharmaceutical Chemistry and Drug Analysis at the Université de Kinshasa (Congo) (1972-1976), at the Université Catholique de Louvain, Belgium (1988-1997) and at the Katholieke Universiteit Leuven (1977-) where he is a full professor since 1988. He is member (1985-1997, 2004-) of Ph. Eur. Group 7 of experts (antibiotics) and expert of the WHO (1996-), chairman of the Belgian Pharmacopoeia Commission (2000-) and member of the European Pharmacopoeia Commission (1992-).

Dr Peter Jongen obtained his degree in pharmacy from Utrecht University in the Netherlands in 1986. From 1986 until 1990 he was scientific officer at the National Institute for the Quality Control of Drugs. In 1991 he joined the National Institute for Public Health and the Environment as a senior scientific officer with special expertise in biological medicinal products. Mr Jongen is pharmaceutical assessor for biological medicinal products on the behalf of the Medicines Evaluation Board in the Netherlands. From 1993 until 2000 he was in charge of control authority batch release testing of vaccines and blood products. From 2000 he is projectmanager of the post marketing testing of biological medicines and of several projects with respect to research on new methods for the quality control of biological medicines. Since its establishment he took part in several advisory groups of the European OMCL Network. Mr Jongen is expert for the Netherlands in European Pharmacopoeia expert group 6: Biological substances and he is an alternate member of the Dutch delegation.

Dr Katjusa Kreft started her professional career in pharmaceutical company Lek as analyst in Quality Control where she has acquired knowledge and experiences in different analytical techniques after graduating on Faculty for Pharmacy in Slovenia in 1992. She has successfully introduced some new testing methods into laboratory, for example near-infrared spectrometry. In the meantime she obtained Master degree on Faculty of Pharmacy. At her present position she works as a Head of Quality Unit in Lek Pharmaceuticals, a member of Sandoz company, being responsible for quality of finished dosage products. Periodically she gives lectures on Faculty of Pharmacy on near-infrared spectrometry. She has been an expert in expert group for antibiotics at European Pharmacopoeia since 1997 and an expert in expert group for NIR since 2003. She is also a member of Slovenian Pharmacopoeia committee.

Dr Michael Morris graduated from the University of Manchester, UK, with a degree in Pharmacy and a PhD in Pharmaceutical Chemistry. Following some early work in R&D in industry, he moved to QC/QA in hospital based pharmaceutical manufacturing operations. In 1987 he joined the NDAB in Dublin, Ireland, as Senior Pharmacist and became Pharmaceutical Director of the newly formed Irish Medicines Board in 1996. As the IMB is the competent authority for animal and human medicines, Dr Michael Morris was in charge of pharmaceutical assessment activities, until a reorganisation took place in 2003. Currently he is Senior Scientific Advisor and a member of the Management Committee to the IMB. Dr Michael Morris was a member of the Quality Working Party, EMEA until 2003, and a former member of the Biotechnology Working Party. Since 1996, he has been a representative for Ireland of the European Pharmacopoeia Commission and was elected to the post of President in March 2004. He has also been active in ICH during this period and is currently EU topic leader for its Q4B group (pharmacopoeial harmonisation).

New impurities control: setting specifications for antibiotics and synthetic peptides

Dr Cornelia Nopitsch-Mai obtained her degree in pharmacy from Free University Berlin in Germany. She obtained her Ph.D in 1990. During the period of six years she was research assistant at the Institute for Pharmaceutical Biology, Free University Berlin of Germany. Field of activities: elimination and structural characterization of proanthocyanidines in *Cassia auriculata*.

Since 1991 she has been scientist at the Federal Institute for Drugs and Medical Devices in the assessment of the quality part of the dossier. Since 2000 she has been assessor for the Certification Procedure (EDQM). Since 2003 she is TAB-member and she has become chairperson of the TAB since July 2005.

Dr Sergi Pavón obtained his degree in chemistry from the 'Universitat Autònoma de Barcelona' (UAB) in Spain in 1995. He studied his Master on Biotechnology at the 'Universitat de Barcelona' (UB, Spain), working at the Peptide Synthesis group of the Faculty of Chemistry with professor Dr. David Andreu. After developing there a collaborative project for the pharmaceutical company Lacer, in 1998 started his work with BCN Peptides, S.A. (formerly Lipotec) at the R&D Department.

Since 2004 he is the Quality Control Manager of BCN Peptides, S.A., concerning both the final products and raw materials.

Dr Jean-Louis Robert studied chemistry at the University of Basle (CH) and obtained his Ph.D. from there in 1976. He had a post-doctoral training at the Pharmaceutical Institute of the "Eidgenössische Technische Hochschule" (ETH) in Zurich (CH). He spent one year with a pharmaceutical company before joining the National Health Laboratory (LNS) in Luxembourg. In his current position he is, Head of the Department of Control of Medicines, an official medicine control laboratory at the LNS.

He is a member (co-opted) of the Committee for Human Medicinal Products (CHMP) at the European Medicines Agency (EMA) in London and chairman of the CHMP/CVMP Quality Working Party.

Within the International Conference on Harmonization (ICH), he is rapporteur for the Guideline Validation of Analytical Procedures, EU-topic leader for the Common Technical Document-Quality (rapporteur for step 4 and the implementation working group), rapporteur for the revision of the guidelines on impurities Q3A and Q3B and member of the EU Q8 team (pharmaceutical development). Currently he is member of the ICH EWG of Q10 (pharmaceutical Quality System). At the European Pharmacopoeia, he is a member of the Commission and of the group of experts 10 B (synthetic products). Currently he chairs the Steering Committee of the CEP and serves as a pharmaceutical expert at WHO.

Dr Jan W. H. Smeets obtained his Ph. D. in chemistry at the University of Utrecht, The Netherlands, in 1988. Since 17 years with DSM with different positions in R&D and Regulatory affairs for APIs and intermediates.

Currently Senior International Regulatory Affairs Manager within DSM Anti-Infectives.

Responsible for worldwide submissions and approvals of registration dossiers within DSM Anti-Infectives.

Since 2000 Dutch representative in Expert Group 7 (antibiotics) of the European pharmacopoeia.

Dr Diels J. van den Dobbelaars studied Biomedical Health Sciences at the University of Nijmegen, the Netherlands (graduation: 1989). He obtained a Ph.D. in biology at the University of Utrecht, the Netherlands (1995) on a biochemical mechanistical toxicology

New impurities control: setting specifications for antibiotics and synthetic peptides

subject. During the period 1995-1997 he was a post-doctoral fellow at the Karolinska Institute, Division of Toxicology, Stockholm, Sweden.

Mid 1997 he joined Organon in the Department of Toxicology and Drug Disposition as a Study Director in general, genetic and reproductive toxicology, later on head of Study Directors. In his current position as Expert Toxicologist he was the toxicologist in a multidisciplinary company Working Party defining the strategy of toxicological qualification of organic impurities and setting of acceptance criteria for drug substance and drug product throughout drug development (2004-2006).

Mr Bernard van Genugten is Project Coordinator at the Quality and Regulatory Unit of Diosynth. He has over 25 years of experience in the pharmaceutical bulk industry. Areas of experience include analytical development and quality control. He supports new products launch teams from the Quality perspective. He is also responsible for the implementation of pharmacopoeial techniques and Quality related guidelines. He is a member of one of EDQM's Expert groups (Organic Chemistry-Synthetic Products).

Mr Philippe Villatte obtained his degree in chemistry from ESCOM and Paris VI University, France. In 1980, he was appointed as head of analytical development laboratory by Roussel-Uclaf. He developed his career as responsible for Strategic Quality Control, API Quality Operations, HMR France (1996). He is currently worldwide support for Industrial Regulatory Compliance, Chemistry, at Sanofi-Aventis.

He has been member of EDQM Expert Group 7/Antibiotics since 2001.

Dr Bernhard Wolf studied chemistry at the Technical University of Aachen where he received PhD in Biochemistry. After 4 years in clinical chemistry he started his career in the pharmaceutical industry in 1986 at Grünenthal GmbH in Aachen. After 3 years as protein chemist in the biotechnological field he was responsible over 10 years for the analytical development in R&D. Major goals here were the development of analytical methods for new drug substances and the analytical part of galenic development of new drug products. Later he started as the head of quality control of Grünenthal GmbH. Since 3 years he is the head of quality control at ratiopharm, Ulm a leading company of the generic pharmaceutical industry.

Participants list

Title	Name	Employer	Ctry
Ms	AGAPOVA KOLEVA Neli	Bulgarian Drug Agency	BG
Dr	AGASØSTER Tone	Statens Legemiddelverk	N
Ms	AKTAN Ferhan	Mustafa Nevzat Pharmaceuticals	TR
Dr	ALAPARTHI Lakshmi Prasad	Sun Pharmaceuticals Industries Limited	IND
Mr	ALMELING Stefan	EDQM Division III - Laboratory	COE
Mme	ANDLAUER Béatrice	EDQM	COE
Mme	ARMEL Sylvie	AFSSAPS Unité Pharmacopée	F
Ms	ARRASTIA I CASADO Silvia	BCN Peptides SA	E
Dr	ARTIGES Agnes	Director EDQM	COE
Ms	BAUMGARTHEN Francine	EDQM	COE
Dr.	BEN MOUSSA Ines	National Drug Control Laboratory	TU
Dr.	BERARDO Benedicte	Biogaran	F
Dr	BINDER Hanno	Sandoz GmbH Quality Management	A
Mrs	BOUIN Anne Sophie	EDQM	COE
Dr	BREIPOHL Gerhard	Coley Pharmaceutical GmbH	D
Mrs	BRØNNUM HANSEN Kirsten	Leo Pharma DK	DK
Mrs	BRUGUERA Hélène	COE-DEQM	COE
Dr	BURGENER Roger	Swissmedic	CH
Mme	CAIZERGUES Lama	AFSSAPS DEMEB	F
Ms	CARAMELLA Carla	Università di Pavia	I
Dr	CASTELLANOS Aida	Andrés Pinaluba, S.A.	E
Mr	CASTLE Peter	EDQM	COE
Dr	CHARTON Emmanuelle	EDQM	COE
Ms	CHOHBANE Katia	COE-DEQM	COE
Dr	COUNE Claude	EDQM	COE
Mr	CRESPO Nicolas	IDD	F
Mrs	CSEH PALOS Andrea	National Institute of Pharmacy	H
Dr	DE BEER Jacques	Scientific Institute of Public Health	B
Dr.	DICK Fritz	Bachem AG	CH
Ms	DONG Suying	Beijing Municipal Institute for Drug Control	RC
Dr	DORPEMA Jan Willem	HAL Allergy	NL
Mme	DUCLOS Laurence	AFSSAPS	F
Dr.	EGGEN Ivo	Diosynth BV	NL
Dr	EIDELMAN Chaim	Novetide Ltd.	IL
Dr	EK Marianne	Medical Products Agency Laboratory	S
Dr.	EL BLIDI Karima	Laboratoire National de Contrôle des Médicaments	MA
Dr	ENGEL Sabine	BfArM	D
Mrs	FORSTER Christiane	Federal Office of Consumer Protection and Food Safety	D
Mr	GAO Wei	Beijing Municipal Institute for Drug Control	RC
Ms	GARNIER-POIDEVIN Anne	EDQM	COE
Dr	GASSMANN Peter	Swissmedic	CH
Dr.	GIERLICH Uwe	Boehringer Ingelheim Vetmedica GmbH	D
Ms	GILCHRIST Fiona	EDQM	COE
Dr.	GOEDEMOED Jaap	RIVM-KCF	NL
Mrs	GONZALEZ KOHRS Citali	Boehringer Ingelheim Vetmedica GmbH	D
Mme	GRABY Nicole	AFSSAPS Unité Physico Chimie	F
Dr.	GUMZ Thorsten	BfArM	D
Dr.	HAAS Gerhard	Bachem AG	CH
Dr.	HAEBERLI Adrian	Swissmedic	CH

Participants list

Title	Name	Employer	Ctry
Mr	HILBERT Christophe	Lilly France SAS	F
Ms	HØJELSE Christina	Danish Medicines Agency	DK
Prof Dr	HOOGMARTENS Jos	Katholieke Universiteit Leuven	B
Mrs	HURLE KIENTZ Carine	COE-DEQM	COE
Dr	HUSAGER Lars J	Danish Medicines Agency	DK
Mrs	JANSSEN Marjo	Janssen Pharmaceutica NV	B
Dr.	JIRICEK Rolf	Bachem AG	CH
Miss	JONCKERS Nettie	DSM Anti Infectives	NL
Mr	JONES Sean	MHRA	GB
Mr	JONGEN Peter M J M	RIVM BMT	NL
Mr	JUNKER Christian	Swissmedic	CH
Dr.	JURISIC Blazenka	Croatian Institute for Medicines Control	CRO
Ms	KANKKUNEN Tarja	National Agency for Medicines	FIN
Dr	KNIE Ulrich	Dr August Wolff GmbH and Co Arzneimittel	D
Ms	KREFT Katjusa	LEK Pharmaceuticals dd	SLO
Dr	KUBBINGA Marlies	Nat. Inst. for Public Health and the Environment	NL
Ms	LAAKSO Teija	National Agency for Medicines	FIN
Mrs	LARSEN LE TARNEC Caroline	EDQM	COE
Dr.	LECLERC Robert		F
Ms	LI Mengmeng	State Pharmacopoeia Commission of China	RC
Dr	LIPKE Uwe	Federal Institute for Drugs and Medical Devices	D
Mr	LIU Yu	Servier _Tianjin_ Pharmaceuticals Co. Ltd	RC
Dr	LIVINGSTONE Alan	GlaxoSmithKline	GB
Dr	LODI Andrea	EDQM	COE
Dr	MANN Warren C		GB
Ms	MAUKONEN Liisa	National Agency for Medicines	FIN
Ms	McLEOD Fiona	COE-DEQM	COE
Mr	McMATH Andrew	COE-DEQM	COE
Mme	MEHMANDOUST Maryam	World Health Organisation	CH
Dr	MORRIS Michael	Irish Medicines Board	IRL
Dr	NADAL Eva Maria	Agencia Espanola del Medicamento	E
Dr.	NIEDAN Wolfgang Volker	Lonza Ltd	CH
Dr	NOPITSCH MAI Cornelia	Bundesinstitut für Arzneimittel und Medizinprodukte Bf	D
Dr.	OETTL Martin	Bachem AG	CH
Ms	ORTEGA DIEGO Isabel	Spanish Medicines Agency	E
Ms	OSTERBERG Malin	PolyPeptide Laboratories AB	S
Mr	OVERBALLE PETERSEN Carsten	Alpharma	DK
Dr	OVERBEEKE Pla Toine	Diosynth BV	NL
Ms	PAVLIDOU Eleni	Demo SA Pharmaceutical Industry	GR
Mr	PAVÓN Sergi	BCN Peptides SA	E
Prof	PLAT Michel		F
Dr.	PLATZER Peter	AGES PharmMed	A
Dr	POTUCKOVA Laura	State Institute for Drug Control	RSL
Dr.	RAIJMAKERS Huub	Diosynth BV	NL
Dr	RAUTMANN Guy	EDQM Division III - Laboratory	COE
Mr	RAUX Jean Christophe	GlaxoSmithKline	B
Mr	RENE Hanselaer	Federal Public Service of Public Health	B
Dr	ROBERT Jean Louis	Laboratoire National de la Santé	L
Mrs	SAAREMAEL Laivi	State Agency of Medicines	ZES

Participants list

Title	Name	Employer	Ctry
Ms	SAUVAGE Yvonne	Lilly France SAS	F
Dr	SCHLUMBOHM Wilhelm	B. Verbraucherschutz und Lebensmittelsicherheit	D
Dr	SCHMID Beate	Dermapharm	D
Miss	SCHULTZ Birgit	Welding GmbH Co	D
Mrs	SCHUTZ Theresa	InfectoPharm Arzneimittel und Consilium GmbH	D
Mrs	SEJKOTOVA Blanka	State Institute For Drug Control	RTC
Dr	SMEETS Jan W H	DSM Anti Infectives B V	NL
Mrs	SORINAS JIMENO Monica	EDQM	COE
Dr	STEINER Samuel	Health Department Canton of Bern	CH
Dr.	SUCHY Josef	Inst. for State Control of Vet. Bio. and Med.	RTC
Dr	TACONET Laure	EDQM	COE
Mrs	TORSTENSSON Rose-Marie	Ferring Pharmaceuticals A_S	DK
Dr.	VAN DEN DOBBELSTEEN D. J	NV Organon, Dept. of Toxicology and Drug Disposition	NL
Mr	VAN GENUGTEN Bernard	N V Organon API_Biotech	NL
Mr	VAN HOVE Ben	Janssen Pharmaceutica NV	B
Mr	VAN ZOMEREN Paul	DADA Consultancy BV	NL
Dr	VATOVEC Maruja	Institute of Pharmacy and Drug Research	SLO
Mr	VILLATTE Philippe	Sanofi Aventis	F
Mr	VIOLAKIS Vassilis	National Organisation for Medicines	GR
Dr	WALHAGEN Karin	Ferring Pharmaceuticals A_S	DK
Mr	WALSH Patrick	Irish Medicines Board	IRL
Mr	WHITE Brian	GlaxoSmithKline	GB
Dr	WIERER Michael	EDQM	COE
Mr	WILLGRESS Stephen	Athlone Laboratories	IRL
Dr.	WIPPO Ursula	BfArM	D
Dr	WOLF Bernhard	Merckle GmbH	D
Dr	WOOD Virginia	Elanco Animal Health	GB
Mr	WYLIE David	Veterinary Medicines Directorate	GB
Mrs	WYSZECKA KASZUBA Ewa	The Office for Registration	PL
Mrs	XENOU KOKOLETSI Magdalene	Demo SA Pharmaceutical Industry	GR
Mrs	ZAHARIEVA Svetlana	Bulgarian Drug Agency	BG
Ms	ZHANG Huiyan	State Pharmacopoeia Commission of China	RC
Ms	ZHANG Peipei	State Pharmacopoeia Commission of China	RC

