SESSION 1: Current regulations on alternatives and review of progress

European Pharmacopoeia activities - an overview

Dr Emmanuelle Charton, Deputy Head of the European Pharmacopoeia Dept,
EDQM, Council of Europe

Dr Charton's slides are available on the website under the download section http://www.edqm.eu/site/Download-527.html#966, click on Events organised by the EDQM - Follow-up previous events - 2008: Alternatives to animal testing, 23-24 April 2008
Presentations Session 1: Current regulations & review of progress

I would like to dedicate this article to the memory of Peter Castle, who left us a few days after the symposium was held.

The European Convention on the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes was opened for signature on 18 March 1986. This marked the beginning of an intensification of the activities of the European Pharmacopoeia Commission to review all animal tests in monographs with a view to applying the precepts of the Convention for replacement, reduction and refinement of the use of animals for test purposes. After 22 years, the amount of progress is considerable. This was acknowledged by Peter Castle on the occasion of the 20th anniversary of the Convention [1] and on earlier occasions [2].

Reference to the Convention is made in several places in the European Pharmacopoeia, not only in the general introduction to the European Pharmacopoeia, but also in the general monographs on *Vaccines for Human Use (0153)*, *Vaccines for Veterinary Use (0062)* and *Immunosera for Veterinary Use (0030)*. Since the monographs cover all vaccines for human and veterinary use and all immunosera for veterinary use, whether or not there is a specific monograph for the product, animal welfare continues to be a concern for assessing the quality of biological substances and preparations. This is reflected in the daily work of the Groups of Experts responsible for the elaboration of the corresponding Ph. Eur. monographs.

Biological and biotechnological products

Replacement of the rabbit pyrogen test by the bacterial endotoxin test
In the field of biological and biotechnological products, the rabbit pyrogen test (2.6.8) has been replaced by the bacterial endotoxin test (2.6.14). This has necessitated the collection of data showing the equivalence between the tests and justifying an appropriate limit for bacterial endotoxins, as recommended in Chapter 2.6.14. In all cases, monograph revisions were undertaken by the appropriate Groups of experts, with the support of the former Group 1L (Bacterial endotoxins) for the review of the files submitted by manufacturers.

Replacement of batch safety tests (abnormal toxicity, histamine, depressor substances) by upstream validation requirements)
Upon review of data which demonstrated the absence of positive batches over a significant period of time, the test for abnormal toxicity (2.6.9), the test of histamine (2.6.8) and the test for depressor substances (2.6.11) have been moved into the production section of the

© EDQM/Council of Europe

appropriate monographs. This prevents the tests from having to be carried out on every batch. The corresponding chapters have been left in the Ph. Eur. in order to allow manufacturers to use these tests during development. In the context of the recent heparin event it has been shown that the test for depressor substances is no longer of value and it has been replaced by modern physico-chemical analysis methods aimed at screening for specific chemical entities.

Replacements of hormone bioassays by HPLC assays
During the last 2 decades considerable effort has been made to replace bioassays by HPLC assays for hormones, whether they are produced by chemical synthesis or by rDNA technology [3, 4]. This has been achieved after having organised collaborative studies, not only to establish the replacement methods but also to provide an equivalence between International Units (I.U.) of activity and mg. Indeed, for some of these hormones, I.U. are still being used by practitioners although the substances are analysed exclusively by physicochemical methods.
The replacement of erythropoietin bioassay by an *in vitro* assay or a physico-chemical assay remains a challenge for the future. The complexity of the molecule makes it difficult for a single test to be used. The introduction of capillary zone electrophoresis for the characterisation of the glycoforms is a first step forward [5]. Ph. Eur. experts are presently working on the introduction of a glycan mapping test in the monograph as a replacement for the test for sialic acids. These tests, complemented by an *in vitro* assay, might be the solution for a monograph free of animal tests.

Antibiotics

Replacement of the rabbit pyrogen test by the bacterial endotoxin test
The same exercise was carried out as for the Biological and biotechnological products.

Replacement of batch safety tests (abnormal toxicity, histamine) by upstream validation requirements)
The same exercise was carried out as for the Biological and biotechnological products.

Radiopharmaceuticals

Replacement of the rabbit pyrogen test by the bacterial endotoxin test
The same exercise was carried out as for the Biological and biotechnological products.
Physiological distribution
Ten monographs on Technetium (99mTc) injections include a test for physiological distribution. This is based on the fact that the distribution pattern of radioactivity observed in specified organs, tissues or other body compartments of an appropriate animal species (usually rats or mice) can be a reliable indication of the expected distribution in humans and thus of the suitability for the intended purpose. Replacement of this test by a physico-chemical test remains a challenge.

Blood products

Deletion of abnormal toxicity based on historical review
The test for abnormal toxicity was removed from 21 monographs after demonstration that the test was not necessary as a batch release test.

© EDQM/Council of Europe

Introduction of serological assays as replacements of in vivo challenge assays
The Ph. Eur. monograph on tetanus immunoglobulin (0398) refers to the possible use of an *in vitro* assay but does not describe a detailed protocol. It is considered that such a statement is a first step towards the replacement of the assay in animals. Current work is underway to develop and validate an appropriate method and it is expected that the monograph will include a standardised serological method in the not too distant future.

Replacement of the rabbit pyrogen test by the bacterial endotoxin test
The Group of experts responsible for monographs on blood products is currently working on the replacement of the pyrogen test by the bacterial endotoxin test. This has been facilitated by interested parties that have provided data to base limits on. Revision proposals have been published for four monographs in Pharmeuropa 20.1 [6] and an enquiry for the possibility to expand the revision to all other blood products is under way.
The EMEA is currently revising the Note for guidance on plasma-derived medicinal products for the introduction of an Addendum on the replacement of rabbit pyrogen testing by an alternative test for plasma-derived medicinal products [7]. The revised monographs together with the EMEA note for guidance will be a powerful combination of tools that will help users to implement a replacement of the rabbit pyrogen test.

Alternative to the rabbit pyrogen test

As mentioned previously, the bacterial endotoxin test is the most common replacement for the rabbit pyrogen test. However, the test can also advantageously be replaced by *in vitro* methods that will detect pyrogens other than Gram-negative bacteria. As a result of a workshop organised by ECVAM [8] and subsequent funding by EU [9], a set of *in vitro* tests have been validated. The work was the basis for the writing of chapter 2.6.30, *Monocyte activation test,* which has recently been published for enquiry [10].

Vaccines for human use, vaccines for veterinary use

A considerable number of achievements have been made in the last few decades and studies are ongoing which will be reported by other speakers during the conference. A few highlights are summarised below.

Deletion of safety tests
In the case of vaccines for human use, the test for abnormal toxicity was removed from 50 monographs based on historical review. With regard to veterinary vaccines, a waiver for safety test after testing of initial batches has been included in the general monograph, allowing manufacturers to omit the test after demonstrating that it is no longer necessary in routine analysis.

Humane endpoints: a way to move forward
A section on *Animal tests,* which recommends that animal welfare be taken into account during testing, has been added in the general monographs *Vaccines for human use (0153)* and *Vaccines for veterinary use (0062)*. This statement cannot be expected to have far-reaching practical effects but it does have some merits: it can be used by a manufacturer to justify the use of, for example, humane end-points when submitting an application for marketing authorisation; it can be used by an assessor who wishes to convince a manufacturer to apply,

© EDQM/Council of Europe

for example, a humane end-point. The paragraph was added following the Zeist meeting as a result of discussion in a workshop [11].

At its 127th Session (March 2007) the European Pharmacopoeia Commission adopted revisions of the monographs *Rabies vaccine for human use prepared in cell cultures (0216)* and *Rabies vaccine (inactivated) for veterinary use (0451).* The revised monographs include a section on alternative end-points describing typical clinical signs to be noted and a typical score-chart. This approach is the most likely to lead to use of alternative end-points for other vaccines. The analyst is expected to "validate" the end-point for a sufficient number of batches by scoring the test in the usual way and also using the alternative end-point. Since the test is carried out routinely for release of batches of vaccine, manufacturers have the opportunity to do the alternative scoring without having to do separate tests for validation.

The situation would be improved if the approach given for rabies vaccine could be extended to other monographs. This means developing disease by disease a definition of typical signs and a score chart. As with future challenges for improving animal welfare in the field of vaccines, it is best to take an opportunist approach, choosing to work on those topics that will yield results most readily. The choice is a matter of judgement on the part of specialists in the various diseases. Priority should also be given to major products and to those tests where most distress is caused. Combining these factors should lead to a programme of work for the next decades.

The contributors to the introduction of the 3Rs in the European Pharmacopoeia

The achievements of the last years could not have taken place without the dedication of all the players in this exercise and the excellence of the relationship between EDQM and all European or non-European partners, who are gratefully acknowledged.

Groups of Experts of the European Pharmacopoeia
Animal welfare is a permanent concern in the work of the Ph. Eur. Groups of Experts. There is no meeting organised in Strasbourg without the subject being discussed at some point on the agenda. There is no Commission session without a text being revised in the direction of the 3Rs. It is the addition of all these efforts which drives the Pharmacopoeia towards a set of specifications where the use of animals is reduced, refined and replaced.

The EDQM Biological Standardisation Programme
The 3Rs cannot be introduced in the Ph. Eur. without first having been validated. The validation phase can be a lengthy process and involves a lot of testing. Thanks to the EDQM Biological Standardisation Programme, scientifically sound projects are regularly submitted to the Ph. Eur. Groups of Experts for inclusion in the Pharmacopoeia.

The EDQM Laboratory
The EDQM laboratory performs no tests on animals. However, it actively participates in the implementation of *in vitro* or physico-chemical methods used in the replacement of animal tests. The EDQM laboratory not only systematically participates in the collaborative studies organised by EDQM or external partners, but contributes to the initial preparatory phases before the start of collaborative studies.

© EDQM/Council of Europe

Our external partners

The WHO, which has observer status with the European Pharmacopoeia, is an active contributor to the 3Rs. Participation of WHO representatives in Ph. Eur. Groups of Experts meetings extends the international dimension of the European work. This is also demonstrated by the results of collaborative studies, jointly organised by EDQM and NIBSC or the participation of one partner in collaborative studies organised by the other.

The FDA which also has observer status with the European Pharmacopoeia and is an active contributor to the 3Rs, in particular in the field of blood products.

The validation studies carried out by ECVAM are often the basis for revision of Ph. Eur. monographs and its contribution is significant.

The EU Commission, through the allocation of funding for the various projects, is a significant contributor to progress in animal welfare. On a technical basis, collaboration with EMEA assures that a consistent approach is applied within Europe.

Finally, the work could not be done without the contributions from the manufacturers of the substances or preparations concerned. Their generosity, patience and good will have been essential to the progress made.

There are probably many more years to go before no animal test is cited at all in the European Pharmacopoeia, but the future looks promising, thanks to all the efforts of the interested parties.

References

[1] P. Castle. Replacement, Reduction, Refinement (3Rs), Animal welfare progress in European Pharmacopoeia monographs. *Pharmeuropa* 2007;**19.3**;430-441.

[2] E. Charton and P. Castle. Reduction, Replacement and Refinement of animal tests in the European Pharmacopoeia: recent developments for monographs on biological substances and preparations. *Pharmeuropa* 2001;**13.1**;94-96.

[3] E. Charton. Hormones: the role of the European Pharmacopoeia. In: Alternatives to animals in the development and control of biological products for human and veterinary use. F. Brown, C. Hendriksen and T. Sesardic (eds). Published by *Dev Biol Stand*. Basel, Karger, 1999;**101**;159-167.

[4] A. Bristow and E. Charton. Progress and Problems in the replacement of *in vivo* pharmacopoeial bioassays for hormones. In: Advancing science and elimination of the use of laboratory animals for development and control of vaccines and hormones. F. Brown, C. Hendriksen, D. Sesardic and K. Cussler (eds). Published by *Dev Biol*. Basel, Karger, 2002;**111**,79-88.

[5] A. Bristow and E. Charton. Assessment of the suitability of a capillary zone electrophoresis method for determining isoform distribution of erythropoietin. *Pharmeuropa* 1999;**11.2**;290-300.

[6] Human albumin solution (0255), Human coagulation factor VIII (0275), Human normal immunoglobulin (0338), Human normal immunoglobulin for intravenous administration (0918). *Pharmeuropa* 2008;**20.1**;90-98.

© EDQM/Council of Europe

[7] Note for guidance on plasma derived medicinal products (CPMP/BWP/269/05 rev. 3) Addendum on the replacement of rabbit pyrogen testing by an alternative test for plasma derived medicinal products (EMEA/CHMP/BWP/452081/2007) 11

[8] T. Hartung, I. Aaberge, S. Berthold, G. Carlin, E. Charton, S. Coecke, S. Fennrich, M. Fisher, M. Gommer, M. Halder, K. Haslov, M. Jahnke, T. Montag-Lessing, S. Poole, L. Schechtmann, A. Wendel and G. Werner-Felmayer. Novel pyrogen tests based on the human fever reaction. The report and recommendations of ECVAM Workshop 43. *ATLA* 2001;**29**;99-123.

[9] 5th EU Framework Program Key Action: 3.1.3 – Cell Factory, QLRT-1999-00811.

[10] Monocyte Activation Test (2.6.30), *Pharmeuropa* 2008**;20.3**;505-511.

[11] P. Castle (1998). The European Pharmacopoeia and humane endpoints. In: Humane Endpoints in Animal Experiments for Biomedical Research. *Proceedings of the International Conference*, 22-25 November 1998, Zeist, the Netherlands. C. Hendriksen and D. Morton Eds. (Published by the Royal Society of Medicine Press), 15-19.

© EDQM/Council of Europe