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Guideline on the principles of regulatory acceptance of 3Rs (replacement, reduction, refinement) testing approaches

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This guideline replaces the Position on Replacement of Animal Studies by in vitro Models (CPMP/SWP/728/95).

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Guideline on the Principles of Regulatory Acceptance of 3Rs (Replacement, Reduction, Refinement) Testing Approaches

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Executive summary

In accordance with Directive 2010/63/EU, the principle of the 3Rs (Replacement, Reduction and Refinement) needs to be considered when selecting testing approaches to be used for regulatory testing of human and veterinary medicinal products. A general overview is provided on implementation of 3Rs principles in this context.

Further information on current or future implementation of specific 3Rs testing approaches for human and veterinary medicinal products can be found in separate reflection papers providing an overview of the current regulatory testing requirements for medicinal products for human and veterinary use and opportunities for implementation of the 3Rs [1, 2]. Regulatory acceptance is defined and guidance is given on the scientific and technical criteria for regulatory acceptance of 3Rs testing approaches. Pathways for regulatory acceptance of 3Rs testing approaches are described and procedures for submission and evaluation of a proposal for regulatory acceptance of 3Rs testing approaches are described.

This guideline aims to encourage stakeholders and authorities to initiate, support and accept development and use of 3Rs testing approaches.

1. Introduction

Non-clinical regulatory testing of medicinal products for human use is carried out to support clinical trials and marketing authorisation (MA) applications. For veterinary medicinal products, regulatory testing is carried out in the target species to demonstrate the safety and efficacy of the products, with additional safety testing performed in non-target species. For both human and veterinary medicinal products regulatory testing is also conducted to control quality during (in-process) and/or at the end (final product batch testing) of the production of the product.

To comply with Directives 2001/83/EC [3] and 2001/82/EC [4] and their associated guidelines, quality, non-clinical[a] and target animal safety and efficacy[b] testing can require the use of animals for the development of human and veterinary medicinal products. Ethical and animal welfare considerations demand that animal use is limited, and preferably avoided, as much as possible. In this respect, Directive 2010/63/EU [5] on the protection of animals used for scientific purposes, which is fully applicable to regulatory testing of human and veterinary medicinal products[c], unambiguously fosters the application of the principle of the 3Rs (Replacement, Reduction and Refinement) when considering the choice of methods to be used.

The application of the 3Rs is currently embedded in the drafting process of scientific guidance both at the European and at international (V)ICH (International Cooperation on Harmonisation of Technical Requirements for registration of Pharmaceuticals for Human Use [and Veterinary Medicinal products]) level. New 3Rs testing approaches have been accepted for regulatory use via multiple and flexible approaches, either as pivotal, supportive or as exploratory mechanistic studies, wherever applicable [1, 2].

[a] In marketing authorisation applications for veterinary pharmaceutical medicinal products this is referred to as “safety testing”. From this point forward the term non-clinical is used for testing of both human and veterinary medicinal products.
[b] Safety and efficacy studies in the target species are conducted for pharmaceutical and immunological products.
[c] With the exception of field clinical trials for veterinary medicinal products, which are specifically excluded from the scope of the Directive

With respect to quality testing, the European Directorate for the Quality of Medicines and Health Care (EDQM) upholds the principles of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" of the Council of Europe (No 123, Strasbourg, 18/03/1986) and also Directive 2010/63/EU in the development of European Pharmacopoeia monographs and through its Biological Standardisation Programme, which aims to validate novel 3Rs testing methods for inclusion in the European Pharmacopoeia (Ph. Eur.). As such, for quality testing of human and veterinary medicinal products, where applicable, animal tests are either deleted or replaced by 3Rs testing approaches [1, 2].

Whilst replacement of animal studies remains the ultimate goal, approaches aiming at reducing or refining animal studies are routinely implemented in regulatory guidelines, where applicable.

The European Pharmacopoeia also encourages animal-free approaches to be used by manufacturers including, for example, through the proof of consistency to avoid unnecessary tests in animals on intermediate stages of production or on the final product. The consistency approach for release testing of authorised vaccines promotes the use of in vitro, analytical systems for monitoring of quality parameters during the whole production process. The consistency approach has the potential to improve both the quality of testing but also the implementation of the principles of the 3Rs for quality testing of human and veterinary biologicals and vaccines [6, 7].

2. Scope

This guideline applies to requirements to support regulatory applications for:

- Non-clinical testing during development of human and veterinary medicinal products.
- Target animal safety and efficacy studies during development of veterinary medicinal products.
- Residues testing in the target species during development of veterinary medicinal products for food producing species.
- Quality batch control as part of the manufacturing process of human and veterinary medicinal products (in-process and/or final product batch testing).

3. Legal basis

This guideline has to be read in conjunction with:

- Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use (Consolidated version: 05/10/2009) [3].
- Directive 2001/82/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to veterinary medicinal products (consolidated version: 18/7/2009) [4].
- Directive 2010/63/EU on the protection of animals used for scientific purposes on 22 September 2010 [5].

4. Replacement, reduction and refinement of animal studies and legal obligations

The principles of 3Rs were first defined by Russell and Burch (1959). Meanwhile, these principles have been further expanded and now encompass:

- **Replacement**: testing approaches that avoid or replace the use of live animals in an experiment where they would have otherwise been used. Replacement could include the use of established animal and human cell lines, or cells and tissues or mathematical and computer models or physicochemical methods.
- **Reduction**: approaches that minimise the number of animals used per experiment or study, either by enabling researchers to obtain comparable levels of information from fewer animals, or to obtain more information from the same number of animals, thereby avoiding further animal use. Examples include improved experimental design and statistical analysis, combination of studies, international harmonisation of testing requirements (e.g. (V)ICH) to avoid duplicate testing and the use of technologies, such as imaging, to enable longitudinal studies in the same animals.
- **Refinement**: approaches that minimise the pain, suffering, distress or lasting harm that may be experienced by the animals. Refinement applies to all aspects of animal use, from the housing and husbandry used to the scientific procedures performed on them. An example of refinement is the use of appropriate anaesthetics and analgesics.

Directive 2010/63/EU on the protection of animals used for scientific purposes of 3 June 2010 [5] fully endorses the principle of replacement, reduction and refinement by stating in article 4 that:

1. Member States shall ensure that, wherever possible, a scientifically satisfactory method or testing strategy, not entailing the use of live animals, shall be used instead of a procedure[d].
2. Member States shall ensure that the number of animals used in projects is reduced to a minimum without compromising the objectives of the project.
3. Member States shall ensure refinement of breeding, accommodation and care, and of methods used in procedures, eliminating or reducing to the minimum any possible pain, suffering, distress or lasting harm to the animals.

The choice of methods is to be implemented according to article 13 which states that:

1. Without prejudice to national legislation prohibiting certain types of methods, Member States shall ensure that a procedure is not carried out if another method or testing strategy for obtaining the result sought, not entailing the use of a live animal, is recognised under the legislation of the Union.
2. In choosing between procedures, those which to the greatest extent meet the following requirements shall be selected:

   (a) use the minimum number of animals;

   (b) involve animals with the lowest capacity to experience pain, suffering, distress or lasting harm;

   (c) cause the least pain, suffering, distress or lasting harm;

[d] A 'procedure' means any use, invasive or non-invasive, of an animal for experimental or other scientific purposes, with known or unknown outcome, or educational purposes, which may cause the animal a level of pain, suffering, distress or lasting harm equivalent to, or higher than, that caused by the introduction of a needle in accordance with the good veterinary practice [5].

and are most likely to provide satisfactory results.

3. Death as the end-point of a procedure shall be avoided as far as possible and replaced by early and humane end-points. Where death as the end-point is unavoidable, the procedure shall be designed so as to:

   (a) result in the deaths of as few animals as possible; and

   (b) reduce the duration and intensity of suffering to the animal to the minimum possible and, as far as possible, ensure a painless death.

5. Regulatory acceptance of 3Rs testing approaches

5.1. Definition of regulatory acceptance

In the scope of this guideline regulatory acceptance of a new 3Rs testing approach can be ultimately defined by its incorporation into a regulatory testing guideline and/or the Ph. Eur. It may also include on a case-by-case basis the acceptance by regulatory authorities of new approaches not (yet) incorporated in testing guidelines but used for regulatory decision making (e.g. an in vitro test accepted through a submission of a MA application or variation or qualification of novel methodologies for human medicines [8, 9]).

The process and decision of acceptance for incorporation in a regulatory guideline is usually carried out by a working group of experts involved in drafting guidance documents and/or Ph. Eur. monographs before being adopted by the Committee for Human and/or Veterinary Medicinal Products (CHMP and/or CVMP) or the European Pharmacopoeia Commission.

Regulatory guidelines concerned are those related to the quality or non-clinical (safety) requirements for human or veterinary medicinal products, residues requirements for veterinary medicinal products and safety and efficacy target species test requirements for veterinary medicinal products.

5.2. 3Rs testing approaches

3Rs testing approaches include tests specifically tailored to a single product (especially for biological products and vaccines), individual endpoint-specific testing methods, testing batteries and integrated testing strategies. These could be introduced through modification of existing approaches or introduction of entirely new approaches.

The modification of existing testing approaches to achieve replacement, reduction and refinement of animal use and, if possible, at the same time increase predictive power and robustness of regulatory testing is expected to occur at different levels. Examples of 3Rs testing approaches are included in the veterinary and human reflection papers [1, 2].

5.3. Criteria for regulatory acceptance of 3Rs testing approaches

Following criteria should be followed before consideration of a 3Rs testing approach for regulatory acceptance:

- Availability of defined test methodology including standard protocols with clear defined/scientifically sound endpoints.

- Relevance, where relevance describes the relationship of the test method to the effect of interest and whether it is meaningful and useful for a particular purpose (context of use). It is the extent to which the test correctly measures or predicts the biological effect of interest. Relevance incorporates consideration of the accuracy (e.g. concordance with comparable validated test method with established performance standards) of a test method [10].
- Context of use includes a description of the circumstances under which the 3Rs testing approach is applicable in the assessment of human or veterinary medicinal products and the limitations within which the available data adequately support use of the 3Rs testing approach. It should for instance be demonstrated that the new or substitute testing method or testing strategy provides either new data that fill a recognised gap or data that are at least as useful as, and preferably better than those obtained using existing methods.
- Reliability/robustness; a measure of the extent that a test method can be performed reproducibly over time when using the same protocol.

5.4. Demonstration of scientific validity of modified and new approaches

The amount of information needed and the criteria applied to a new method will depend on a number of factors, including:

- the regulatory and scientific rationale for the use of the method;
- the type of method being evaluated (e.g. existing test, new method);
- the proposed uses of the method (e.g. mechanistic, total or partial replacement, as part of a testing strategy);
- the mechanistic basis for the test and its relationship to the effect(s) of concern (e.g. whether it is a mechanistic/functional and/or an empirical relationship);
- the history of use of the test method, if any, within the scientific and regulatory communities.

Flexible approaches to demonstrate a method's scientific validity for regulatory use are acceptable including formal validation by recognised centres for validation for alternative methods European Union Reference Laboratory for alternatives to animal testing (e.g. EURL ECVAM) and EDQM (see section 5.4.1) or approaches as described in section 5.4.2 and 5.4.3.

5.4.1. Formal validation

Examples of formal validation processes for 3Rs test methods are described by the EURL ECVAM and by the EDQM. Formal validation generally directly implies the intention to seek regulatory acceptance.

EURL ECVAM's validation criteria are comparable to the criteria subsequently defined by the (US) Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and the OECD [10–13]. The evolution of a regulatory test is subdivided in five stages that reflect the sequence of steps to be performed for a prospective validation exercise: evaluation of candidate method (to see if suitable/ready for validation), pre-validation (protocol refinement, transfer and performance), validation, independent peer review and recommendation for consideration in a regulatory context (e.g. development of new or updated OECD test guidelines).

In a prospective validation study, an inter-laboratory blind trial (involving at least three laboratories) is conducted to assess whether tests can be shown to be relevant and reliable for one or more specific

purposes. This inter-laboratory trial is followed by data analyses and an evaluation of the outcome of the study in comparison with predefined performance criteria [6, 13–16].

The modular approach to the EURL ECVAM principles on test validity allows for flexibility by breaking down the various stages in validation into independent modules and defining for each module the information needed for assessing test validity. This allows for retrospective validation studies to be conducted or for a combination of retrospective and prospective studies [16].

At the level of the EDQM, the Biological Standardisation programme (BSP) aims at validating new methods for the quality control of biological medicinal products (including immunological veterinary medicinal products) with the goal of including them in European Pharmacopoeia monographs (for details see footnote[e]). It is overseen by a steering committee consisting of the chairs of the relevant European Pharmacopoeia groups of experts, representatives from the relevant European Medicines Agency (EMA) working parties, co-opted scientific experts and an observer from the World Health Organization (WHO). The programme takes methods of interest which have been validated on a local scale (single laboratory/limited products) and proceeds with a wider generic validation to demonstrate the potential applicability in other laboratories and with other similar products on the market. Similar to the EURL ECVAM procedure the process involves multiple phases including preparatory method refinement, small scale transfer studies and finally large scale international collaborative studies with manufacturers and national control laboratories. The study reports are presented to the relevant European Pharmacopoeia expert group for consideration for inclusion of the method in the European Pharmacopoeia and are made publicly available.

5.4.2. Alternative routes of demonstration of scientific validity

3Rs testing approaches that have sufficient demonstration of scientific validity according to the criteria described (see section 5.3) but have not been assessed in a formal validation process can however also be accepted in a regulatory submission and/or included in regulatory guidelines/documents wherever possible. In this case, the data are evaluated on a case–by-case basis by the competent authorities (e.g. EMA and National Competent Authorities [NCAs]).

5.4.3. Submission of data collected in new 3Rs approaches

Voluntary submission of data obtained by using a new 3Rs testing approach can be made in parallel with data generated using existing methods. Data generated with the new 3Rs testing approaches will not be used as part of the regulatory decision making process and should be evaluated independently and solely for the purpose of evaluation of the novel 3Rs testing approaches for possible future regulatory acceptance. This will allow the generation of data to support validation and/or context of use before consideration of 3Rs testing approaches for regulatory acceptance.

The application of this process should be considered on a case-by-case basis, e.g.:

- Where necessary, as an alternative route for demonstration of scientific validity of a 3Rs testing approach where the validation status is considered insufficient for regulatory purposes.
- Context of use evaluation might be needed for 3Rs testing approaches that have been formally validated for a different context of use.

[e] http://www.edqm.eu/en/Biological-Standardisation-Programme-mission-60.html
http://www.edqm.eu/en/BSP-Work-Programme-609.html
https://www.edqm.eu/en/BSP-programme-for-3Rs-1534.html

The evaluation of the data generated in this context could be part for example of a (V)ICH guideline process such as the guideline ICH S1 Regulatory Notice Document on changes to core guideline on rodent carcinogenicity testing of pharmaceuticals [10] or through the EMA Scientific Advice as described below (section 5.5.).

5.5. EMA procedures for seeking advice on proposals for regulatory acceptance of 3Rs testing approaches

Sponsors can request scientific advice from the EMA at any stage of development of a human or veterinary medicinal product, whether the medicine is eligible for the centralised authorisation procedure or not in accordance with established procedures [8].

In addition to the above scientific advice procedure, the EMA can provide specific scientific advice to support qualification of innovative drug development methods (section 5.5.1.). This qualification process, applicable only to human medicines development, is the most appropriate route to seek regulatory acceptance of a novel 3Rs methodology.

For veterinary medicinal products, similar innovative approaches may be submitted in accordance with existing scientific CVMP guidance for companies requesting scientific advice (EMA/CVMP/172329/2004-Rev.3) for an assessment on a case-by-case basis.

5.5.1. Scientific Advice Working Party (SAWP) qualification of novel methodologies for human medicinal products

A novel 3Rs testing approach may be submitted to the EMA in accordance with the procedure described in the guideline on Qualification of Novel Methodologies for Drug Development [9] and is assessed by the SAWP.

The documentation to support the novel methodology is submitted by the applicant to the EMA general qualification inbox qualification@ema.europa.eu. The submitted data will be assessed by a "qualification team" (led by a representative from the CHMP and/or the SAWP). The applicant has the opportunity to involve also other agencies such as FDA (United States' Food and Drug Administration) and/or PMDA (Japan's Pharmaceutical and medical device agency).

This qualification process leads either to a CHMP qualification opinion or to a CHMP qualification advice on the acceptability of the approach for a specific use. In agreement with the applicant, a public consultation will be pursued prior to a final qualification opinion in order to take the views of the scientific community into consideration. Following the publication of the final qualification opinion, the method becomes available for use in the scientific community.

5.6. Assessment of submitted proposal

Assessment of the new 3Rs testing approaches will be performed according to the criteria as defined in 5.3 in collaboration with the relevant 3Rs experts from CHMP and/or CVMP working parties.

The outcome of the assessment can entail following recommendations:

1. New 3Rs testing approaches are based on sufficient data and can be recommended for regulatory acceptance, e.g. inclusion in a scientific guideline or qualification opinion (see section 5.5.1).
2. New 3Rs testing approaches need further real-life data collection period (see section 5.4.3).

3. New 3Rs testing approaches are based on data sufficient to demonstrate suitability for a particular case and can be used in the context of approval of an individual dossier but additional data collection is required to demonstrate suitability for inclusion as general guidance for all relevant products.

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