



European Centre for the Validation of Alternative Methods (ECVAM)
European Commission
Joint Research Centre
Institute for Health and Consumer Protection
21020 Ispra (Va) – Italy

Tel: +39 0332 789046
Fax: +39 0332 785336

Web site: <http://ecvam.jrc.it>

General Guidelines

for Submitting a Proposal to ECVAM

for the Evaluation of the Readiness of a Test Method to Enter
the ECVAM Prevalidation and/or Validation Process



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1. Introduction

1.1. Background to ECVAM

In 1986, the Council of Ministers adopted Directive 86/609/EEC (1) on the Approximation of Laws, Regulations and Administrative Provision of the Member States Regarding the Protection of Laboratory Animals for Experimental and other Scientific Purposes.

Article 7.2 of the Directive states that: “An experiment shall not be performed if another scientifically satisfactory method of obtaining the result sought, not entailing the use of an animal is reasonably and practicably available.”

Article 23 states that: “The Commission and Member States should encourage research into the development and validation of alternative techniques, which could provide the same level of information as that obtained in experiments using animals, but which involve fewer animals or which entail less painful procedures, and shall take such other steps as they consider appropriate to encourage research in this field.”

In October 1991, the European Commission responded to this article by means of a Communication to the Council and the Parliament (2), which established the European Centre for the Validation of Alternative Methods (ECVAM).

ECVAM main duties are:

- To coordinate the validation of alternative test methods at the European Union level.
- To act as a focal point for the exchange of information on the development of alternative test methods.
- To set up, maintain and manage a database on alternative procedures.
- To promote dialogue between legislators, industries, biomedical scientists, consumer organisations and animal welfare groups, with a view to the



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development, validation and international recognition of alternative test methods.

ECVAM focuses its main activities on the validation of alternative test methods that can replace any existing or anticipated regulatory procedure involving animal use or can reduce the number of animals required in such procedures.

For further information on the activities of ECVAM consult the ECVAM web site at the internet address <http://ecvam.jrc.it>.

1.2. Background to the document

This document summarises the information that the proposer of a test method should send to ECVAM in order for a preliminary assessment to be made of the readiness of the test method to enter the ECVAM prevalidation and/or validation process.

Five main stages in the evolution of a new test, from development to regulatory acceptance, have been identified, named: test development, prevalidation, validation, independent assessment and progression toward regulatory acceptance. The formal validation process is an essential prerequisite an alternative test method should undergo before being evaluated for incorporation into regulation.

ECVAM encourages the submission of alternative tests methods for evaluation of their compliance with general requirements for validation and their readiness to enter a formal prevalidation and/or validation process.

ECVAM is willing to support and advice test method developers during development and/or refinement phases as well as proposers during submission preparation for evaluation. Priority will be given to evaluate and support the validation process of test methods, which can replace or reduce the number of animals required in *in vivo* regulatory toxicological procedures. This document has been therefore produced with the aim to provide test method developers and proposers with basic general guidelines on what type of information and documentation should be submitted to ECVAM for a preliminary assessment of the validation state of the test method.



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2. General principles

2.1. *Alternative toxicological test methods*

An alternative toxicological test method is any method that can be used to *replace*, or *reduce* the use of animals for experimental purposes or *refine* procedures to make such experiments less painful or stressful (the “Three Rs” principle).

2.2. *Principles of validation and types of validation study*

Validation is the process by which the relevance and reliability of a test method are established for a particular purpose (3-4).

Relevance refers to the scientific value of the tests methods, whereas *reliability* refers to the reproducibility of the results within and between laboratories and over time.

The *purpose* of the method is the intended application of the procedure.

Three types of validation study have been defined:

a *Prevalidation study*: is a small-scale inter-laboratory study, carried out to ensure that the protocol of a test method is sufficiently optimised and standardised for inclusion in a formal validation study and to obtain a preliminary assessment of its relevance and reliability (5). The prevalidation process is divided into three consecutive phases namely, *protocol refinement* (Phase I), *protocol transfer* (Phase II), and *protocol performance* (Phase III). It involves the testing of a limited number of coded substances in at least three laboratories.

a *Validation study* is a large-scale inter-laboratory study, designed to assess the relevance and reliability of an optimised method for a particular purpose.

a *Catch-up validation study* is a prevalidation study in which the structural and performance criteria of a method are compared with those of a similar method, which has already undergone formal validation and has been accepted as scientifically valid.



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2.3. *Prediction models*

An alternative test method for the replacement of an animal test is a combination of a test system and a prediction model (PM).

The PM is an unambiguous algorithm for converting *in vitro* data into predictions of pharmacotoxicological endpoints in animals or humans (6-7).



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3. Submission guidelines

3.1. Introduction

The proposer of a test method should adhere to the following guidelines in submitting the application for the preliminary evaluation of the test method.

The information provided in the Proposal Evaluation Form should allow for an early comprehensive evaluation of the existing information and data. In the case the preliminary assessment of the test reliability and relevance is positive, the proposer of a test method will be asked to share more detailed information with ECVAM to perform a deeper assessment. The amount and type of information required for the assessment of the relevance and state-of-the-art of a test method may vary depending on the method and its proposed use.

3.2. Mechanistic basis of the test method and endpoint(s) evaluated

In this section the proposer should provide a short description of the mechanistic basis of the test method and an explanation of the endpoint(s) assessed. The closeness and differences of the *in vitro* endpoint(s) evaluated compared to the *in vivo* pharmacotoxicological endpoint(s) in the species of interest (animal or human) should be illustrated, if such information is available.

3.3. Purpose of the test method and proposed practical application

This section should comprise an explanation of the area for which the test method has been developed or refined and its intended application.

Three main types of purposes have been defined:

Non-regulatory alternative procedures: test methods, which might be used for providing mechanistic information or for screening and prioritisation purposes.

Alternative test methods for inclusion as part of hierarchical approach in regulatory guidelines: such alternative procedures do not allow a replacement of existing regulatory procedure but could provide information useful to reduce the number of



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animals required in regulatory testing or refine the procedure applied to reduce pain and distress.

Test methods for the replacement of existing regulatory guidelines: in such case a test method is able to provide the same level of information as that obtained in an *in vivo* regulatory toxicological test.

3.4. Relevance of the test method

In the case of a test method for the prediction of an *in vivo* pharmacotoxicological endpoint, any preliminary evidence supporting the predictive ability of the test method should be described in this section. This refers to any early assessment of the relationship, whether mechanistic or correlative, existing between the data generated by the test method and the effect(s) measured in the species of interest.

3.5. Need of the test method in relation to existing in vivo or other in vitro methods

This section should include an explanation of the need of the test method in relation to existing regulatory testing guidelines or other available *in vitro* methods.

3.6. Definition of the proposed test method

In this section the proposer should define his test method according to the following definitions:

- a *refinement test* is a test method which alleviates or minimises potential pain, suffering and distress to animals
- a *reduction test* is a test method which obtains the same level of information from the use of fewer animals
- a *replacement test* is any test method which permits a given purpose to be achieved without using living vertebrate animals
- a *complement test* is a test that provides information that adds to or helps interpret the results of other tests.



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3.7. Preliminary self-assessment of the protocol optimisation level

In this section the proposer should provide a list of information to allow a preliminary evaluation of the optimisation level of the protocol associated to the test method.

3.8. Information on the prediction model applied

If a prediction model has been developed, in this section the proposer should give preliminary information on the type of prediction model applied and on its predictive ability.

4. Additional information

4.1. Laboratories sufficiently experienced in the proposed test method

In this section the proposer should provide the name of other laboratories or partners that are already sufficiently experienced in performing the test method.

4.2. References to patents and relevant publications

In this section the proposer should quote the most relevant publications referring to the proposed test method. Any confidential information or existing patents should be listed in the table.

5. Information on proposers

This section should include the data of the person in charge of the proposal submission. This will be the person ECVAM will respond to or contact for further information or for convocation to define further actions.



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The articles quoted may be downloaded from the ECVAM web site at the internet address <http://ecvam.jrc.it>.