



VALIDATION AND BARRIERS TO VALIDATION

EPAA Discussion Paper

Final version 24 October 2007

1. Introduction

Political expectations in the area of development, validation and acceptance of alternative methods to animal testing are high. Several political initiatives have been launched for the protection of animals used in science and the promotion of alternative non-animal approaches to scientific testing, such as the Directive 86/609/EEC on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes, the Directive 76/768/EEC on the approximation of the laws of the Member States relating to cosmetics products, the Community Action Plan on the Protection and Welfare of Animals 2006-2010 [SEC(2006)65] or voluntary frameworks as, e.g., the European Partnership for Alternative Approaches to Animal Testing.

Alternatives are not only beneficial for animal welfare but the availability of such methods also encourages the development of new markets for 3R approaches. Alternative methods have the potential to increase the credibility and accuracy of test results as well as the predictive capacity for the safety of human beings and the environment. In fact, in certain cases human cell models are better in predicting human health effects than the animal model. Furthermore, alternative methods usually have higher throughput and lesser costs than animal studies. Lastly, they represent a business opportunity for a number of companies and contract research laboratories. The development and validation of new methods and strategies could therefore also contribute to increasing competitiveness of the European industry.

It is important in this context to underline the three types of alternative methods. The current replacement alternatives cover typically only one specific end-point whereas some animal methods, which remain necessary for the time being, examine integrated effects at species level. An increasing number of alternative methods are being developed, however, mostly to be used in the context of integrated or tiered assessment strategies highlighting the importance of Reduction and Refinement methods.

The 3Rs:

1. **Reduction alternative:** any means of lowering the number of animals used to obtain information of a given amount and precision.
2. **Refinement alternative:** any development that refines procedures to lessen or eliminate pain or distress to animals, or enhances animal well-being.
3. **Replacement alternative:** any scientific method employing non-sentient material, which may replace methods using sentient, living animals.

2. Validation Process in ECVAM

The European Centre for the Validation of Alternative Methods (ECVAM) was created by a Communication from the Commission to the Council and the Parliament on 29 October 1991 [SEC(91)1794], Communication of the European Commission to Council and the European Parliament), pointing to a requirement in Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes, which requires that the Commission and the Member States should actively support the development, validation and acceptance of methods which could reduce, refine or replace the use of animals in experiments (3Rs).

An **alternative test method** is the combination of a **test system** and a **prediction model**. The test system comprises a process or procedure including a specific protocol used to obtain information on a substance. The prediction model is a formula used to convert the results generated by the test system into a prediction of the toxic effects. It is developed on the basis of robust reference data or standards.

Definitions of validation terms (see Bouvier et al. 2007):

Validation is the process by which the reliability and relevance of a particular approach, method, process or assessment is established for a defined purpose.

A **validated test method** is a test method for which the reliability and relevance for a specific purpose have been established in one or more validation studies

Reliability measures of the extent to which a test method can be performed reproducibly within and between laboratories and over time, when performed by using the same protocol. It is assessed by calculating intra-laboratory and inter-laboratory reproducibility and intra-laboratory repeatability.

The **relevance** of a test method describes whether it is meaningful and useful for a particular purpose. It is the extent to which the measurement result and uncertainty can accurately be interpreted as reflecting or predicting the biological effect of interest.

The **predictive capacity** is the extent to which the measurement result and uncertainty can accurately be interpreted as reflecting or predicting the biological effect of interest.

The **negative predictive value** is the proportion of correct negative responses among substances indicated as negative by a test method. Negative predictive value is a function of the sensitivity and specificity of the test method and of the prevalence of negatives among the substances tested.

The **positive predictive value** is the proportion of correct positive responses among materials indicated as positive by a test method. It is one indicator of test method accuracy. Positive predictive value is a function of the sensitivity and specificity of the test method and of the prevalence of positives among the substances tested.

In 1995, based upon experience from several large-scale validation studies, and in consultation with various international experts, ECVAM published recommendations concerning the practical and logistical aspects of validating alternative test methods in prospective studies (ECVAM workshop report 5).

Based on several large-scale validation studies, five main stages were identified: test development; prevalidation; validation involving a formal between-laboratory study with the testing of coded chemicals; independent assessment; and progression toward regulatory acceptance.

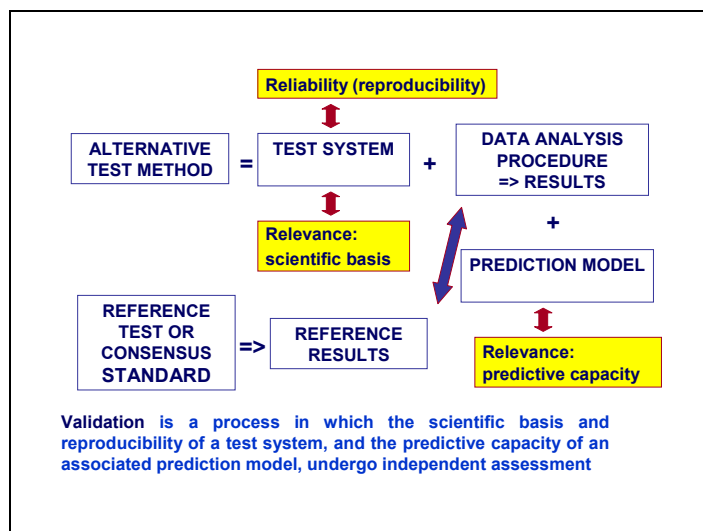


Figure 1 Definition of Validation (Hartung 2007)

The objective of the prevalidation scheme implemented by ECVAM is to ensure that a candidate method fulfils all criteria to be included in a formal validation study. This approach contributes to improving the use of human and financial resources and increases the likelihood to meet expectations of the scientific, regulatory and animal welfare community.

In the context of this paper, an alternative method is scientifically validated in the

EU if a statement of ECVAM Scientific Advisory Committee (ESAC) on the validity of the method has been issued. It is important to note, however, validation by ECVAM is only one of the routes for alternative methods to be accepted by authorities.

More recently, ECVAM has developed a modular approach for the validation of alternative methods (Fig. 2). Its objective is to make the process more flexible while maintaining high standards. The various aspects of validation are broken down into independent modules, and the information necessary to complete each module is defined.

Noteworthy, these data can be obtained in a prospective study or in a retrospective (weight of evidence) evaluation or a combination of both. A prospective study represents the preferred and default process if no sufficient information on the test and its applicability are available.

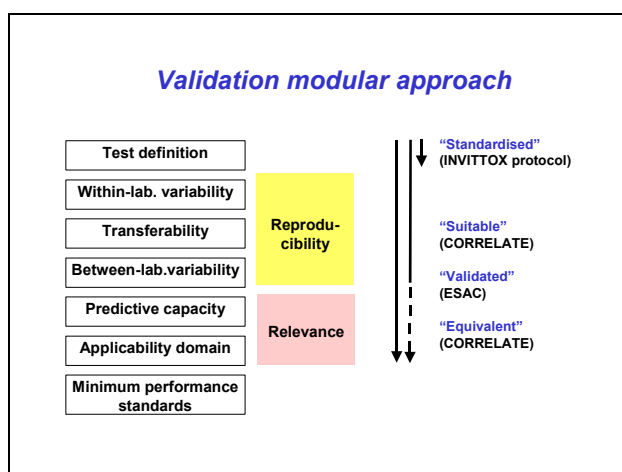


Figure 2 The modular approach used at ECVAM

The focus of the independent peer review is therefore on the data required to assess test validity, and not on the sequence of the process. The peer review starts once the information to satisfy all the modules is complete. In this way, the between-laboratory variability and predictive capacity of a test can be assessed independently.

Thinking in terms of validation modules, will broaden the applicability of the validation process to a variety of tests and procedures, including test based on new technologies (e.g.

genomics or proteomics), computer-based models (for example, (quantitative) structure–activity relationship models), and expert systems.

ECVAM intends that the modular approach can be applied to retrospective and

prospective validation studies. This permits the assessment of test validity by completing the missing information via the relevant validation procedure: prospective validation, retrospective validation, catch-up validation, or a combination of these procedures.

In this context, it is important to note that the requirements for formal validation may vary depending on the type of application and use.

3. Barriers to Validation

There are a number of barriers hampering a quick and successful validation in ECVAM. In different EPAA discussions, within the working group on validation and acceptance and during an interdisciplinary EPAA workshop on barriers to validation in September 2006, a number of problem areas have been identified.

- 3.1. **Lack of clarity as to the purposes and uses** of the method, resulting sometimes in ineffective untailed validation process
- 3.2. **Lack of appropriate information** - Examination of relevance and reliability of alternative methods requires complete and unbiased high-quality animal or human reference data. This includes information on reference test methods, positive and negative substances (reference standards) and about performance characteristics of animal test(s), if a reference method is available or defined
- 3.3. **Lack of mechanistic understanding of the health or environmental effects.**
- 3.4. **Lack of test materials** - Unavailability of substances used to generate reference data is also a barrier; substances may not be available or not available in sufficient quantities (e.g. human tissues or cells, difficulty to use human embryonic stem cells).

The following issues can be addressed by the EPAA:

- *In vivo* and *in vitro* data needed for validation are not always publicly available: information could be provided when possible
 - No regulatory reference test is available: There are toxicological endpoints for which no regulatory reference test exists, e.g. respiratory irritation, respiratory sensitization, and immunotoxicity.
 - Several regulatory tests exist: If several regulatory animal tests exist, which one should be chosen as reference test?
 - Publication bias (higher number of positives): Since scientific articles often deal with the positive (toxic) substance, there is bias to positive substances.
 - Notification bias (higher number of negatives): Many negative substances have been notified. Thus, there is a bias to negative substances in these databases.
 - Use of *in vivo* data as the only reference standard when human data are not available
- 3.5. **Duration of validation procedure** - Validation in ECVAM can take between 2 and 5 years, depending mainly on the maturity of the method, i.e. how much standardization and refinement is still required. This is due to the duration of public procurement procedures for involvement of external laboratories (9-12 months) on the one hand, and on the length of peer review

processes and time needed to complete data sets and substances.

The duration of the peer review process could be addressed by the EPAA:

- Availability of experts
- Availability of all documents

3.6. **New methodologies** – Processes for validation of new or complex methodologies and approaches (testing strategies, *in silico*, transgenic models, and omics) do not exist yet. They need to be developed and assessed, where formal validation is necessary.

3.7. **Budget constraints** – Lack of financial resources can slow down the validation process if operational and staff support is insufficient to deal with alternative methods submitted for validation. Sufficient funding for running costs and validation studies needs to be provided.

The European Partnership can address this Issue.

4. How to overcome barriers to validation? Solutions under consideration

For the most obvious shortcomings, pilot solutions are being implemented.

4.1. Availability of data and substances

The European Partnership started to develop solutions to address the predominant problem faced by ECVAM, which is lack of *in vitro* and *in vivo* data to assess the reliability and relevance of alternative assays and strategies. Several steps within a **framework for collaboration** were identified and are tested through a series of pilot assays:

- Definition of needs in terms of substances and data required by the management team of the validation study
- Establishment of a simple and standardised information request containing minimum background necessary for quick decision making and smooth processing within company
- Appointment of contact person within each company, able to technically assess the request and equipped with “standard guidance” on processing requests aimed at supporting validation of 3Rs.

Indeed, for the time being, ECVAM information requests do not reach the relevant company person. A designation of “3Rs validation” contact points in the associations and companies could improve the current situation.

The efficiency of this pilot system will be assessed, adjusted where appropriate and generalised to all information requests for validation purposes.

Invitation by the Management team to have preliminary discussions with EPAA could lead interested companies to share data relevant for the study.

4.2. Business confidentiality aspects

ECVAM has developed an IPR survey form for new submissions of alternative methods in order to identify possible problems of confidentiality of data in the early beginning of validation. If there is an important IPR problem, the issue needs to be addressed appropriately taking into account concerns of industry on the one hand

and the need for rapid validation of alternative test methods on the other hand.

Confidentiality agreements provide a possible solution to IPR-related barriers. This instrument should be envisaged for all commercially sensitive data that are subject to peer review during the validation process. Such an agreement could be restricted to the most sensitive data.

To avoid business confidentiality issue, using **well-known reference compounds** would in vast majority of cases provide a suitable solution. In this case, the establishment of **mechanisms as described in section 4.1 and contact points** might suffice to ensure industry feedback to ECVAM information requests.

4.3. Shortening administrative delays

ECVAM has launched a number of activities to speed up validation and to make the validation process more efficient. The establishment of a Reference Laboratory for Alternative Tests – CORRELATE – will serve to accelerate the process of method evaluation and the planned early involvement of regulators via the currently established ERAP (European Regulatory Advisory Panel) will help to determine regulatory use and will simplify validation and subsequent legal acceptance.

5. Conclusions

Alternatives are not only beneficial for animal welfare and quality assurance of test methods but the availability of these methods also encourages the development of new markets for such methods. Alternative methods can contribute to increasing the credibility and accuracy of tests as well as the safety of human beings.

EPAA has a great potential to make the process of validation more efficient by enhancing the collaboration between ECVAM and industry in order to make available testing data and substances.

The inventory of barriers can be summarised as follows:

	Within the scope of EPAA	Beyond the scope of EPAA
Scientific	Definition of the purpose of use of the method Lack of or information about reference method Unavailability of data and substances Lack of established validation process for new generation/technology approaches Lack of regulatory reference test Publication and notification bias (high number of positives/negatives)	Unknown performance characteristics of animal tests Unavailability of substances (out of stock) Unavailability of human tissues or cells Impurities of substances Lack of quality assurance Unstable test material
Non-scientific	Time to complete data set IP or business confidentiality aspects Duration of peer review process (timing and logistic aspects) Budget constraints	Duration of public procurement process Proprietary aspects related to the test

A number of activities either led by ECVAM or by EPAA, is going to alleviate some of

the most obvious shortcomings:

- Framework for collaboration to enable support to future validation projects by providing clear guidance, establishing networks of contacts within all industry sectors and mechanisms for efficient support in the validation process.
- Confidentiality agreements and other mechanisms to address intellectual property and business confidentiality issues
- The establishment of a Reference Laboratory for Alternative Tests, CORRELATE to accelerate the evaluation process. CORRELATE addresses the assessment of “me-too” developments and method suitability.
- Early involvement of regulators via the European Regulatory Advisory Panel (ERAP) should help to determine regulatory use and to simplify validation and subsequent legal acceptance. The ERAP will be a platform for consultation and advise involving various regulatory agencies at EU and member state level.

This inventory will be further debated within EPAA as from second half of 2007 in order to design an action plan involving all necessary stakeholders and to put in place clear implementation within the remaining 3-year life span of the Partnership.

References

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3. OECD TGD N°34 : Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment (2005)
4. Conclusions from EPAA workshop on barriers to validation 25-26 September 2006: www.epaa.eu.com
5. T. Hartung (2007) Food for thought on validation ALTEX 24, 67-73