The European Agency for the Evaluation of Medicinal Products
Human Medicines Evaluation Unit

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COMMITTEE FOR PROPRIETARY MEDICINAL PRODUCTS (CPMP)

REPLACEMENT OF ANIMAL STUDIES BY IN VITRO MODELS

(Position adopted by the CPMP on 19 February 1997)

Background information

The CPMP at its meeting on 18-19 February 1997 adopted a document entitled "Replacement of animal studies by in vitro models".

The elaboration of this paper started some years ago. After several discussions at the level of the Safety Working Party, the paper was brought to a provisional conclusion during the Safety Working Party meeting held in June 1996. The document was afterwards subject to a reconsultation with the European Centre for the Validation of Alternative Methods (ECVAM), which is part of the Environment Institute of the EC Joint Research Centre of Ispra. Finally, it was presented for adoption at the February meeting of the CPMP.

The CPMP's considerations on this issue are now put at the disposal of interested parties. Any comments that you may have should be sent to Dr. Ulla Liminga at the EMEA Secretariat.

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REPLACEMENT OF ANIMAL STUDIES BY IN VITRO MODELS

INTRODUCTION

Studies aimed at assessing the safety of a potential new medicinal product before its first administration to man, before carrying out therapeutic trials in wider population and finally, before placing the product on the market, are a scientific and ethical necessity. These studies are carried out in laboratory animals (except for certain genotoxicity tests) in compliance with Directives 65/65/EEC as amended, 75/318/EEC as amended, 75/319/EEC as amended, and their associated Notes for Guidance, with recommendations 83/571/EEC and 87/176/EEC.

Ethical considerations of animal protection and welfare, as well as practical considerations require that the use of laboratory animals is limited as much as possible. In Directive 86/609/EEC, which is fully applicable to the safety assessment of medicinal products, the harmonisation of legislative, regulatory and administrative provisions in the European Union relating to the protection of animals used for experimental and other scientific purposes is addressed. It has permitted the clarification of a variety of points regarding the procurement of animals, their housing, their sacrifice etc. Moreover, Article 23 of the Directive encourages research for techniques likely to provide the same level of information as that obtained by studies carried out in animals. For this purpose, the European Centre for the Validation of Alternative Methods (ECVAM) was set up as a result of a communication from the Commission to the Council and the European Parliament in October 1991 (see annex 1).

Examples of requirements which already have improved the well-being of animals in various aspects, are the obligation of compliance with GLP for all safety studies and the Recommendation 87/176/EEC which has limited the number of animals required for single-dose toxicity studies as a high degree of precision in the assessment of the lethal dose is no longer necessary. In addition, the ICH process where already existing guidelines are harmonised and new guidance is created will have consequences leading to the reduction of the use of animals

SCOPE

This paper addresses the feasibility of replacing in vivo animal studies by in vitro investigations in the preclinical development of medicinal products. Furthermore, considerations regarding validation procedures for in vitro methods and their incorporation into the CPMP Notes for Guidance are presented.

FEASIBILITY OF REPLACING IN VIVO ANIMAL STUDIES

The use of laboratory animals should be limited without jeopardising human safety. Based on todays knowledge, this can be acheived by Reduction, Refinement or Replacement of animal studies, known as the '3Rs rule'.

Reduction:

- by avoiding study replication.
- by avoiding studies in animal models which have been shown to be irrelevant for human extrapolation
- by developing complementary *ex vivo* and *in vitro* investigation methods
- by increasing the biotechnical quality of tests
- by obtaining more relevant information in fewer animals

Refinement:

- by using new methodologies derived from scientific and technical progress and more human end-points. Furthermore, qualitative elements should be taken into account, linked

to the animals' quality of life during the experiments and the necessity to reduce the risk and degree of suffering.

Replacement:

- by developing alternative methods to animal experimentation such as in vivo and in vitro techniques, using where possible human cells.

To progress in the spirit of Directive 86/609/EEC, it is necessary to take two essential elements into account which should not be contradictory:

- The scientific development of in vitro models, which has been pursued for several years. In vitro models may be one of the most efficient means for reducing or replacing the use of laboratory animals.
- The necessity to obtain from the in vitro methods, a level of information on safety which is at least equal to that obtained from standard in vivo studies.

By first taking these two elements into account, a strategy of validating in vitro methods can be defined as a process by which the reliability and the relevance of an in vitro method are established for a particular purpose. The purpose of this process is to provide independent conformation that an in vitro method provides relevant information.

I. PROCEDURE FOR VALIDATING IN VITRO TESTS

Any in vitro test or series of tests must undergo a procedure aiming at establishing relevance and reliability, before they can be considered as valid. This procedure must be set up according to the following steps:

Phase 1: Test development and definition

Any test will have to follow the defined objective (for example screening test or test for detecting a defined toxic effect) and the sponsoring laboratory will have to describe the operating procedures thoroughly, in order to enable other laboratories to reproduce the test. All data relating to the specificity, sensitivity and reproducibility of the test must be supplied. The consistency of the test must be demonstrated on a limited but conclusive number of reference substances, including negative and positive comparators. Publishing the proposed test in an international peer reviewed scientific journal may be considered as a positive factor for evaluating its potential utility.

Phase 2: Test optimisation

Any in vitro test will have to be assessed by a multi-center study, involving laboratories from different countries. The utility, reliability, robustness and practicability of the test must be described in a common assessment report, stressing in particular the proposed technical improvements compared with the original method. The laboratories taking part in this study will have to define and evaluate a limited and conclusive number of reference substances, including negative and positive comparators. It is recommended to assess the reference substances by using a blinded design. Publishing this multi-center test in an international peer reviewed scientific journal is essential for evaluating the test.

Phase 3: Validation

Once a proposed test has been described and assessed in its final configuration via phases 1 and 2, it will have to be submitted to a multi-center study, including a larger number of laboratories from different countries, aiming at comparing the relevance of the proposed test to the accepted standard in vivo methods. This phase will have to include an increased number of appropriately chosen relevant products. This performance comparison can to a limited extent be carried out in phases 1 and 2, bearing in mind that a premature comparison, especially before the exact testing conditions are defined, is likely to cause unnecessary sacrifice to laboratory animals. The publication of the results of the multi-center study in an international peer reviewed scientific journal is essential for the assessment of the test and for its use at the international level.

Phase 4: Setting-up or taking part in an international data bank

To improve knowledge of the performance of the proposed test, the creation of an international data bank is indispensable, in particular if the test should be performed on a routine basis. The results obtained in the validation study should be incorporated if relevant.

In general, it is recommended that collaboration between laboratories should start at a very early stage, in order to avoid any unnecessary delays and waste of time.

With regard to the validation of already known tests or series of tests, an abridged multi-center procedure can be considered on a case by case basis, taking into account any existing published data. It is also recommended to consider the results obtained in other areas (chemicals, pesticides), where a number of in vitro tests have been developed.

II. PROCEDURE FOR INCORPORATING IN VITRO TESTS INTO THE REGULATORY REQUIREMENTS

When an in vitro method has successfully reached the end of the validation procedure, the appropriate data should be submitted to an expert group appointed by the CPMP for an independent reassessment. These experts should not have been involved in the development of the test.

The expert group should especially consider the following:

- the availability of a rationale, as well as a clear statement of scientific need
- a determination of the relationship of the endpoint(s) of the test method to the *in vivo* biological effect and to the toxicity of interest
- the limitations of the method
- the availability of a formal protocol (preferentially in the public domain), that is sufficiently detailed and includes data analyses and decision criteria
- a demonstration of intra-test variability, repeatability and reproducibility of the test method within and amongst laboratories, including level of variability and how this varies with time.

The expert group might formulate the following recommendations:

1. The proposed test can be considered as valid. This provided that the test adequately predicts the endpoint of interest or that it provides data for toxicity assessment that are at least as useful as, and perhaps better than those obtained by using existing methods.
2. The proposed test is not acceptably validated and can not be recommended for routine use.

Within an international harmonisation context, it is suitable that the dossier regarding the assessment of a test or series of in vitro tests should be submitted to all regulatory authorities. The assessment by independent, high-level scientific bodies is also recommended.

III. AREAS FOR WHICH THE ACCEPTANCE OF IN VITRO TESTS CAN BE CONSIDERED

Assessment of toxicity by single dose administration: Series of cell cultures of various tissues could be used for an initial ranging of toxicity levels. Nevertheless, the use of in vitro tests alone is not considered to provide sufficient information.

Assessment of toxicity by repeated dose administration: When studies in laboratory animals have shown one or several target organs, it is recommended to use in vitro methods (cell cultures for example) rather than animal studies for further evaluation. However, the replacement of in vivo studies by in vitro models is not foreseeable.

Evaluation of toxicity on reproductive functions: since the assessment of adverse effects on human reproductive function is most often based on animal data alone, the introduction of in vitro methods requires special consideration. Alternative methods for the assessment of

embryo/fetotoxicity have been described. Possibly, such tests could, after refinement, be used for screening purposes and in mechanistic studies. For the male reproductive system, in vitro cell cultures based on different cell populations can readily be used for comparative and mechanisitc studies.

Evaluation of genotoxicity: in vitro tests are widely used.

Evaluation of carcinogenic potential: Research for alternative in vitro and in vivo tests of shorter duration which can be used for the evaluation of carcinogenic potential is encouraged. The present discussion at the international level on the abandonment of one species for carcinogenicity testing should be noted.

Evaluation of local tolerance: the standard methods used in the laboratory animal have been highly criticised, especially with regard to the assessment of ocular tolerance. Many methods, some of which are already being used for screening purposes are available.

Pharmacokinetics, toxicokinetics and metabolism: many ex vivo and in vitro models are currently used for metabolic studies (perfused organs, cultured cells, microsomes etc.). These are useful when choosing the animal species to be used in in vivo studies. They could also replace animal experiments in studies on mechanisms of action. The use of live animals is unavoidable for studies of tissue distribution and for determining systemic exposure. However, such studies should include only a limited number of animals and should not aim at an unnecessary accuracy of the results.

Safety pharmacology: many ex vivo and in vitro models are currently used and should be systematically preferred whenever possible.

Biological standardisation: in vitro and physio-chemical methods are accepted.

GENERAL GUIDANCE FOR THE PRECLINICAL DEVELOPMENT OF MEDICINAL PRODUCTS

Those involved in the development of medicinal products should bear in mind that preclinical safety studies carried out to support the use of a new medicinal product should correspond to a real necessity and not be done according to a check-list. In order to facilitate such an approach, the following question-based guidance could be applied:

- What information is the animal study expected to provide?
- Is this information essential in the reassurance on safety for this particular medicinal product?
- If this information is essential, could it be obtained by experiments not involving intact animals?
- If information is essential and can only be obtained from live animals, is it possible to decrease numbers of animals used without compromising the usefulness of the data?
- Is there overall justification for the final protocol?

ANNEX 1

The European Centre for the Validation of Alternative Methods (ECVAM) is a part of the Environment Institute of the EC Joint Research Centre of Ispra (Italy) and is founded:

- To coordinate the validation of alternative test methods at community level.
- To act as a focal point for the exchange of information on the development of alternative test methods
- To establish, maintain and manage a database on alternative procedures, with associated user services.
- To promote dialogue between legislators, industrial companies, biomedical scientists, consumer organisations and animal welfare groups with a view to the development, validation and international recognition of alternative test methods.