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

PART 3
TOXICOLOGICAL AND PHARMACOLOGICAL TESTS
I. Introduction

1. The particulars and documents accompanying the application for marketing authorization pursuant to Articles 8(3)(i) and 10(1) shall be given in accordance with the requirements below.
Member States shall ensure that the safety tests are carried out in conformity with the provisions relating to good laboratory practice laid down by Council Directives 87/18/EEC(4) and 88/320/EEC(5).

The toxicological and pharmacological tests must show:
(a) the potential toxicity of the product and any dangerous or undesirable toxic effects that may occur under the proposed conditions of use in human beings; these should be evaluated in relation to the pathological condition concerned;

(b) the pharmacological properties of the product, in both qualitative and quantitative relationship to the proposed use in human beings. All results must be reliable and of general applicability. Whenever appropriate, mathematical and statistical procedures shall be used in designing the experimental methods and in evaluating the results.
Additionally, it is necessary for clinicians to be given information about the therapeutic potential of the product.

2. Where a medicinal product is intended for topical use, systemic absorption must be investigated, due account also being taken of the possible use of the product on broken skin and absorption through other relevant surfaces. Only if it is proved that systemic absorption under these conditions is negligible may repeated dose systemic toxicity tests, foetal toxicity tests and studies of reproductive function be omitted.

If, however, systemic absorption is demonstrated during therapeutic experimentation, toxicity tests shall be carried out on animals, including where necessary, foetal toxicity tests.
In all cases, tests of local tolerance after repeated application shall be carried out with particular care and include histological examinations; the possibility of sensitization shall be investigated and any carcinogenic potential investigated in the cases referred to in Section II E of this Part.

3. For biological medicinal products such as immunological medicinal products and medicinal products derived from human blood or plasma, the requirements of this Part may have to be adapted for individual products; therefore the testing programme carried out shall be justified by the applicant.

In establishing the testing programme, the following shall be taken into consideration:
- all tests requiring repeated administration of the product shall be designed to take account of the possible induction of, and interference by, antibodies;
- examination of reproductive function, of embryo/foetal and perinatal toxicity, of mutagenic potential and of carcinogenic potential shall be considered. Where components other than the active substance(s) are incriminated, validation of their removal may replace the study.

4. For radiopharmaceuticals, it is appreciated that toxicity may be associated with a radiation dose. In diagnosis, this is a consequence of the use of radiopharmaceuticals; in therapy, it is the wanted property. The evaluation of safety and efficacy of radiopharmaceuticals shall, therefore, address requirements for medicinal products and radiation dosimetry aspects. Organ/tissue exposure to radiation shall be documented. Absorbed radiation dose estimates

shall be calculated according to a specified, internationally recognized system by a particular route of administration.

5. The toxicology and pharmacokinetics of an excipient used for the first time in the pharmaceutical field shall be investigated.

6. Where there is a possibility of significant degradation during storage of the medicinal product, the toxicology of degradation products must be considered.

II. PERFORMANCE OF TESTS

A. Toxicity

1. Single dose toxicity

An acute test is a qualitative and quantitative study of the toxic reactions which may result from a single administration of the active substance or substances contained in the medicinal product, in the proportions and physico-chemical state in which they are present in the actual product.

The acute toxicity test must be carried out in two or more mammalian species of known strain unless a single species can be justified. At least two different routes of administration shall normally be used, one being identical with or similar to that proposed for use in human beings and the other ensuring systemic exposure to the substance.

This study will cover the signs observed, including local reactions. The period during which the test animals are observed shall be fixed by the investigator as being adequate to reveal tissue or organ damage or recovery, usually for a period of 14 days but not less than 7 days, but without exposing the animals to prolonged suffering. Animals dying during the observation period should be subject to autopsy as also should all animals surviving to the end of the observation period. Histopathological examinations should be considered on any organ showing macroscopic changes at autopsy. The maximum amount of information should be obtained from the animals used in the study.

The single dose toxicity tests should be conducted in such a way that signs of acute toxicity are revealed and the mode of death assessed as far as reasonably possible. In suitable species, a quantitative evaluation of the approximate lethal dose and information on the dose effect relationship should be obtained, but a high level of precision is not required.

These studies may give some indication of the likely effects of acute overdosage in man and may be useful for the design of toxicity studies requiring repeated dosing on the suitable animal species.

In the case of active substances in combination, the study must be carried out in such a way as to check whether or not there is enhancement of toxicity or if novel toxic effects occur.

2. Repeated dose toxicity (sub-acute or chronic toxicity)

Repeated dose toxicity tests are intended to reveal any physiological and/or anatomo-pathological changes induced by repeated administration of the active substance or combination of active substances under examination, and to determine how these changes are related to dosage.

Generally, it is desirable that two tests be performed: one short-term, lasting two to four weeks, the other long-term. The duration of the latter shall depend on the conditions of clinical use. Its purpose shall be to determine by experiment the non-toxic dose range of the product and normally it shall last three to six months.

In respect of medicinal products to be administered once only to humans, a single test lasting two to four weeks shall be performed.

If however, having regard to the proposed duration of use in human beings, the investigator sees fit to carry out experiments of greater or lesser duration than indicated above, he must give adequate reasons for doing so. Reasons should also be given for the dosages chosen.

Repeated dose toxicity tests shall be carried out on two species of mammals one of which must be a non-rodent. The choice of route(s) of administration employed shall depend on the intended therapeutic use and the possibilities of systemic absorption. The method and frequency of dosage shall be clearly stated.

The maximum dose should be chosen so as to bring harmful effects to light. The lower doses will then enable the animal's tolerance of the product to be determined. Wherever possible, and always in experiments on small rodents, the design of the experiment and the control procedures must be suited to the scale of the problem being tackled and enable fiducial limits to be determined.

The evaluation of the toxic effects shall be based on observation of behaviour, growth, haematological and biochemical tests, especially those relating to the excretory mechanism, and also on autopsy reports and accompanying histological data. The choice and range of each group of tests will depend on the species of animal used and the state of scientific knowledge at the time.

In the case of new combinations of known substances that have been investigated in accordance with the provisions of this Directive, the chronic long-term tests may, except where acute and sub-acute toxicity tests have demonstrated potentiation or novel toxic effects, be suitably modified by the investigator who shall submit his reasons for such modification.

B. Examination of reproductive function

If the results of other tests reveal anything suggesting harmful effects on progeny or impairment of male or female reproductive function, this shall be investigated by appropriate tests.

C. Embryo/foetal and perinatal toxicity

This investigation comprises a demonstration of the toxic and especially the teratogenic effects observed in the issue of conception when the medicinal product under investigation has been administered to the female during pregnancy.

Although up to the present these tests have had only a limited predictive value in regard to the application of the results to human beings, they are thought to provide important information where the results show effects such as resorptions and other anomalies.

Omission of these tests, either because the medicinal product will not normally be used by women capable of child-bearing or for other reasons, must be adequately justified.

Embryo/foetal toxicity studies shall normally be conducted on two mammalian species, one of which should be other than a rodent. Peri- and postnatal studies shall be conducted in at least one species. Where metabolism of a medicinal product in a particular species is known to be similar to that in man, it is desirable to include this species. Also, it is desirable that one of the species is the same as in the repeated dose toxicity studies.

The details of the test (number of animals, amounts administered, timing of administration and criteria for evaluation of results) shall depend on the state of scientific knowledge at the time

when the application is lodged, and the level of statistical significance that the results must attain.

D. Mutagenic potential

The purpose of the study of mutagenic potential is to reveal the changes which a substance may cause in the genetic material of individuals or cells and which have the effect of making successors permanently and hereditarily different from their predecessors. This study is obligatory for any new substance.

The number and types of results and the criteria for their evaluation shall depend on the state of scientific knowledge at the time when the application is lodged.

E. Carcinogenic potential

Tests to reveal carcinogenic effects shall normally be required:
(a) in respect of substances having a close chemical analogy with known carcinogenic or cocarcinogenic compounds;

(b) in respect of substances which have given rise to suspicious changes during the long-term toxicological tests;

(c) in respect of substances which have given rise to suspicious results in the mutagenic-potential tests or in other short-term carcinogenicity tests.
Such tests may also be required in respect of substances to be included in medicinal products likely to be administered regularly over a prolonged period of a patient's life.
The state of scientific knowledge at the time when the application is lodged shall be taken into account when determining the details of the tests.

F. Pharmacodynamics

This heading covers the variations caused by the medicinal product in the functions of the physiological systems, whether these functions are normal or experimentally modified.
This study shall follow two distinct lines of approach.

Firstly, the actions on which the recommended application in therapeutic practice is based shall be adequately described. The results shall be expressed in quantitative terms using, (e.g. dose-effect curves, time-effect curves etc.), and wherever possible, compared with data relating to a substance whose activity is known. Where a higher therapeutic potency is being claimed for a substance, the difference shall be demonstrated and shown to be statistically significant.

Secondly, the investigator shall provide a general pharmacological characterization of the substance, with special reference to adverse reactions. In general, the main functions of the physiological systems should be investigated. The depth of this investigation must be increased as the doses liable to produce adverse reactions approach those producing the main effect for which the substance is being proposed.

The experimental techniques, unless they are standard procedures, must be described in such detail as to allow them to be reproduced, and the investigator must establish their validity. The experimental results shall be set out clearly and, when relevant to the test, their statistical significance quoted.

Unless good reasons are given to the contrary, any quantitative modification of responses resulting from repeated administration of the substance shall be investigated.

Tests on combinations of active substances may be prompted either by pharmacological premisses or by indications of therapeutic effect.

In the first case, the pharmacodynamic study shall demonstrate those interactions which might make the combination of value in therapeutic use.

In the second case, where scientific justification for the combination is sought through therapeutic experimentation, the investigation shall determine whether the effects expected from the combination can be demonstrated in animals, and the importance of any collateral effects shall at least be investigated.

If a combination includes a novel active substance, the latter must previously have been studied in depth.

G. Pharmacokinetics

Pharmacokinetics means the study of the fate of the active substance within the organism, and covers the study of the absorption, distribution, biotransformation and excretion of the substance.

The study of these different phases may be carried out both by means of physical, chemical or biological methods, and by observation of the actual pharmacodynamic activity of the substance itself.

Information on distribution and elimination (i.e. biotransformation and excretion) shall be necessary in all cases where such data are indispensable to determine the dosage for humans, and in respect of chemotherapeutic substances (antibiotics, etc.) and substances whose use depends on their non-pharmacodynamic effects (e.g. numerous diagnostic agents, etc.).

Pharmacokinetic investigation of pharmacologically active substances is necessary.

In the case of new combinations of known substances which have been investigated in accordance with the provisions of this Directive, pharmacokinetic studies may not be required, if the toxicity tests and therapeutic experimentation justify their omission.

H. Local tolerance

The purpose of local tolerance studies is to ascertain whether medicinal products (both active substances and excipients) are tolerated at sites in the body which may come into contact with the medicinal product as a result of its administration in clinical use. The testing strategy shall be such that any mechanical effects of administration or purely physico-chemical actions of the product can be distinguished from toxicological or pharmacodynamic ones.

I. Well-established medicinal use

For the purpose of demonstrating, pursuant to Article 10(1)(a)(ii), that the component(s) of a medicinal product have a well established use, with an acceptable level of safety, the following specific rules shall apply:

(a) Factors which have to be taken into account in order to establish a "well established medicinal use" of components of medicinal products are the time over which a substance has been used, quantitative aspects of the use of the substance, the degree of scientific interest in the use of the substance (reflected in the published scientific literature) and the coherence of scientific assessments. Therefore different periods of time may be necessary for establishing "well established use" of different substances. In any case, however, the period of time required for establishing a "well established medicinal use" of a component of a medicinal

product must not be less than one decade from the first systematic and documented use of that substance as a medicinal product in the Community.

(b) The documentation submitted by the applicant should cover all aspects of the safety assessment and must include or refer to a review of the relevant literature, taking into account pre- and postmarketing studies and published scientific literature concerning experience in the form of epidemiological studies and in particular of comparative epidemiological studies. All documentation, both favourable and unfavourable, should be communicated.

(c) Particular attention must be paid to any missing information and justification must be given why demonstration of an acceptable level of safety can be supported although some studies are lacking.

(d) The Expert report must explain the relevance of any data submitted which concern a product different from the product intended for marketing. A judgment must be made whether the product studied can be considered as similar to the product which will be granted a marketing authorisation in spite of the existing differences.

(e) Post-marketing experience with other products containing the same components is of particular importance and applicants should put a special emphasis on this issue.