

[^{F1}ANNEX I

CHEMICAL, PHARMACEUTICAL AND ANALYTICAL STANDARDS, SAFETY AND RESIDUE TESTS, PRE-CLINICAL AND CLINICAL TRIALS IN RESPECT OF TESTING OF VETERINARY MEDICINAL PRODUCTS

Textual Amendments

- F1** Substituted by [Commission Directive 2009/9/EC of 10 February 2009 amending Directive 2001/82/EC of the European Parliament and of the Council on the Community code relating to medicinal products for veterinary use \(Text with EEA relevance\)](#).

TITLE I

REQUIREMENTS FOR VETERINARY MEDICINAL PRODUCTS OTHER THAN IMMUNOLOGICAL VETERINARY MEDICINAL PRODUCTS

PART 3:

SAFETY AND RESIDUES TESTS

The particulars and documents which shall accompany the application for marketing authorisation pursuant to the second and fourth indents of Article 12(3)(j) shall be submitted in accordance with the requirements below.

- A. Safety tests

CHAPTER I:

PERFORMANCE OF TESTS

The safety documentation shall show:

- (a) the potential toxicity of the veterinary medicinal product and any dangerous or undesirable effects which may occur under the proposed conditions of use in animals; these should be evaluated in relation to the severity of the pathological condition concerned;
- (b) the potential harmful effects to man of residues of the veterinary medicinal product or substance in foodstuffs obtained from treated animals and what difficulties these residues may create in the industrial processing of foodstuffs;
- (c) the potential risks which may result from the exposure of human beings to the veterinary medicinal product, for example during its administration to the animal;
- (d) the potential risks for the environment resulting from the use of the veterinary medicinal product.

All results shall be reliable and valid generally. Whenever appropriate, mathematical and statistical procedures shall be used in designing the experimental methods and in evaluating the

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results. Additionally, information shall be provided regarding the therapeutic potential of the product and about the hazards connected with its use.

In some cases it may be necessary to test the metabolites of the parent compound where these represent the residues of concern.

An excipient used in the pharmaceutical field for the first time shall be treated like an active substance.

1. **Precise identification of the product and of its active substance(s)**

- international non-proprietary name (INN),
- International Union of Pure and Applied Chemistry Name (IUPAC),
- Chemical Abstract Service (CAS) number,
- therapeutic, pharmacological and chemical classification,
- synonyms and abbreviations,
- structural formula,
- molecular formula,
- molecular weight,
- degree of impurity,
- qualitative and quantitative composition of impurities,
- description of physical properties,
- melting point,
- boiling point,
- vapour pressure,
- solubility in water and organic solvents expressed in g/l, with indication of temperature,
- density,
- spectra of refraction, rotation, etc,
- formulation of the product.

2. **Pharmacology**

Pharmacological studies are of fundamental importance in clarifying the mechanisms by which the veterinary medicinal product produces its therapeutic effects and therefore pharmacological studies conducted in experimental and target species of animal shall be included in Part 4.

However, pharmacological studies may also assist in the understanding of toxicological phenomena. Moreover, where a veterinary medicinal product produces pharmacological effects in the absence of a toxic response, or at doses lower than those required to elicit toxicity, these pharmacological effects shall be taken into account during the evaluation of the safety of the veterinary medicinal product.

Therefore the safety documentation shall always be preceded by details of pharmacological investigations undertaken in laboratory animals and all relevant information observed during clinical studies in the target animal.

2.1. **Pharmacodynamics**

Information on the mechanism of action of the active substance(s) shall be provided, together with information on primary and secondary pharmacodynamic effects in order to assist in the understanding of any adverse effects in the animal studies.

2.2. **Pharmacokinetics**

Data on the fate of the active substance and its metabolites in the species used in the toxicological studies shall be provided, covering absorption, distribution, metabolism and excretion (ADME). The data shall be related to the dose/effect findings in the pharmacological and toxicological studies, to determine adequate exposure. Comparison with the pharmacokinetic data obtained in the studies on the target species, Part 4, Chapter I, Section A.2, shall be included in Part 4 in order to determine the relevance of the results obtained in the toxicology studies for the toxicity to the target species.

3. Toxicology

The documentation on toxicology shall follow the guidance published by the Agency on the general approach to testing and guidance on particular studies. This guidance includes:

1. basic tests required for all new veterinary medicinal products for use in food-producing animals in order to assess the safety of any residues present in food for human consumption;
2. additional tests that may be required depending on specific toxicological concerns such as those associated with the structure, class, and mode of action of the active substance(s);
3. special tests which might assist in the interpretation of data obtained in the basic or additional tests.

The studies shall be conducted with the active substance(s), not with the formulated product. Where studies of the formulated product are required, this is specified in the text below.

3.1. Single-dose toxicity

Single-dose toxicity studies may be used to predict:

- the possible effects of acute overdosage in the target species,
- the possible effects of accidental administration to humans,
- the doses which may usefully be employed in the repeat dose studies.

Single-dose toxicity studies should reveal the acute toxic effects of the substance and the time course for their onset and remission.

The studies to be carried out shall be selected with a view to providing information on user safety, e.g. if substantial exposure by inhalation or dermal contact of the user of the veterinary medicinal product is anticipated, those routes of exposure shall be studied.

3.2. Repeat-dose toxicity

Repeat-dose toxicity tests are intended to reveal any physiological and/or 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.

In the case of pharmacologically active substances or veterinary medicinal products intended solely for use in non-food-producing animals, a repeat-dose toxicity study in one species of experimental animal shall normally be sufficient. This study may be replaced by a study conducted in the target animal. The frequency and route of administration, and the duration of the study shall be chosen having regard to the proposed conditions of clinical use. The investigator shall give his reasons for the extent and duration of the trials and the dosages chosen.

In the case of substances or veterinary medicinal products intended for use in food-producing animals, repeat-dose (90 day) toxicity testing shall be performed in a rodent and a non-rodent

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species in order to identify target organs and toxicological endpoints and identify the appropriate species and the dose levels to be used in chronic toxicity testing, if appropriate.

The investigator shall give his reasons for the choice of species, having regard to the available knowledge of the metabolism of the product in animals and man. The test substance shall be administered orally. The investigator shall clearly state and give his reasons for the method and frequency of administration and the length of the trials.

The maximum dose should normally be selected so as to bring harmful effects to light. The lowest dose level should not produce any evidence of toxicity.

Evaluation of the toxic effects shall be based on observation of behaviour, growth, haematology and physiological tests, especially those relating to the excretory organs, and also on autopsy reports and accompanying histological data. The choice and range of each group of tests depends on the species of animal used and the state of scientific knowledge at the time.

In the case of new combinations of known substances which have been investigated in accordance with the provisions of this Directive, the repeat-dose tests may, except where toxicity tests have demonstrated potentiation or novel toxic effects, be suitably modified by the investigator, who shall submit his reasons for such modifications.

3.3. Tolerance in the target species

A summary shall be provided of any signs of intolerance which have been observed during studies conducted, usually with the final formulation, in the target species in accordance with the requirements of Part 4, Chapter I, Section B. The studies concerned, the dosages at which the intolerance occurred and the species and breeds concerned shall be identified. Details of any unexpected physiological changes shall also be provided. The full reports of these studies shall be included in Part 4.

3.4. Reproductive toxicity including developmental toxicity

3.4.1. Study of the effects on reproduction

The purpose of this study is to identify possible impairment of male or female reproductive function or harmful effects on progeny resulting from the administration of the veterinary medicinal products or substance under investigation.

In the case of pharmacologically active substances or veterinary medicinal products intended for use in food-producing animals, the study of the effects on reproduction shall be performed in the form of a multi-generation reproduction study, designed to detect any effect on mammalian reproduction. These include effects on male and female fertility, mating, conception, implantation, ability to maintain pregnancy to term, parturition, lactation, survival, growth and development of the offspring from birth through to weaning, sexual maturity and the subsequent reproductive function of the offspring as adults. At least three dose levels shall be used. The maximum dose should be selected so as to bring harmful effects to light. The lowest dose level should not produce any evidence of toxicity.

3.4.2. Study of developmental toxicity

In the case of pharmacologically active substances or veterinary medicinal products intended for use in food-producing animals, tests on developmental toxicity shall be performed. These tests shall be designed to detect any adverse effects on the pregnant female and development of the embryo and foetus consequent to exposure of the female from implantation through gestation to the day before predicted birth. Such adverse effects include enhanced toxicity relative to that observed in non-pregnant females, embryo-foetal death, altered foetal growth, and structural changes to the foetus. A developmental toxicity test in the rat is required. Depending on the

results, a study in a second species may have to be performed, in accordance with established guidance.

In the case of pharmacologically active substances or veterinary medicinal products not intended for use in food producing animals, a study of developmental toxicity shall be performed in at least one species, which may be the target species, if the product is intended for use in female animals which may be used for breeding. However, where the use of the veterinary medicinal product would result in significant exposure to users, standard developmental toxicity studies shall be performed.

3.5. Genotoxicity

Tests for genotoxic potential shall be performed to reveal changes which a substance may cause in the genetic material of cells. Any substance intended to be included in a veterinary medicinal product for the first time must be assessed for genotoxic properties.

A standard battery of *in vitro* and *in vivo* genotoxicity tests in accordance with established guidance shall usually be carried out on the active substance(s). In some cases, it may also be necessary to test one or more metabolites that occur as residues in foodstuffs.

3.6. Carcinogenicity

The decision on whether carcinogenicity testing is required shall take into account the results of genotoxicity tests, structure-activity relationships and the findings in systemic toxicity tests that may be relevant to neoplastic lesions in longer term studies.

Any known species specificity of the mechanism of toxicity shall be considered, as well as any differences in metabolism between the test species, target animal species, and human beings.

Where carcinogenicity testing is necessary, generally a two-year rat study and an 18-month mouse study are required. With appropriate scientific justification, carcinogenicity studies may be carried out in one rodent species, preferably the rat.

3.7. Exceptions

Where a veterinary medicinal product is intended for topical use, systemic absorption shall be investigated in the target animal species. If it is proved that systemic absorption is negligible, the repeated dose toxicity tests, the tests for reproductive toxicity and the carcinogenicity tests may be omitted, unless:

- under the intended conditions of use laid down, oral ingestion of the veterinary medicinal product by the animal is to be expected, or
- under the intended conditions of use laid down, exposure of the user of the veterinary medicinal product by other routes than the dermal route is to be expected, or
- the active substance or metabolites may enter foodstuffs obtained from the treated animal.

4. Other requirements

4.1. Special studies

For particular groups of substances or if the effects observed during repeated dose studies in animals include changes indicative of e.g. immunotoxicity, neurotoxicity- or, endocrine dysfunction, further testing shall be required, e.g. sensitisation studies or delayed neurotoxicity tests. Depending on the nature of the product, it may be necessary to conduct additional studies to assess the underlying mechanism of the toxic effect or the irritation potential. Such studies shall usually be conducted with the final formulation.

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The state of scientific knowledge and established guidance shall be taken into account when designing such studies and evaluating their results.

4.2. Microbiological properties of residues

4.2.1. Potential effects on the human gut flora

The potential microbiological risk presented by residues of antimicrobial compounds for the human intestinal flora shall be investigated in accordance with established guidance.

4.2.2. Potential effects on the microorganisms used for industrial food processing

In certain cases, it may be necessary to carry out tests to determine whether microbiologically active residues may interfere in technological processes in the industrial processing of foodstuff.

4.3. Observations in humans

Information shall be provided showing whether the pharmacologically active substances of the veterinary medicinal product are used as medicinal products in human therapy; if this is so, a compilation shall be made of all the effects observed (including adverse reactions) in humans and of their cause, to the extent that they may be important for the assessment of the safety of the veterinary medicinal product, where appropriate including results from published studies; where constituents of the veterinary medicinal products are themselves not used or are no longer used as medicinal products in human therapy, the reasons shall be stated.

4.4. Development of resistance

Data on the potential emergence of resistant bacteria of relevance for human health are necessary in the case of veterinary medicinal products. The mechanism of the development of such resistance is particularly important in this regard. Where necessary, measures to limit resistance development from the intended use of the veterinary medicinal product shall be proposed.

Resistance relevant for clinical use of the product shall be addressed in accordance with Part 4. Where relevant, cross reference shall be made to the data set out in Part 4.

5. **User safety**

This section shall include a discussion of the effects found in the preceding sections and relate this to the type and extent of human exposure to the product with a view to formulating appropriate user warnings and other risk management measures.

6. **Environmental risk assessment**

6.1. Environmental risk assessment of veterinary medicinal products not containing or consisting of genetically modified organisms

An environmental risk assessment shall be performed to assess the potential harmful effects, which the use of the veterinary medicinal product may cause to the environment and to identify the risk of such effects. The assessment shall also identify any precautionary measures which may be necessary to reduce such risk.

This assessment shall normally be conducted in two phases. The first phase of the assessment shall always be performed. The details of the assessment shall be provided in accordance with accepted guidance. It shall indicate the potential exposure of the environment to the product and the level of risk associated with any such exposure taking into account in particular the following items:

- the target animal species, and the proposed pattern of use,

- the method of administration, in particular the likely extent to which the product will enter directly into environmental systems,
- the possible excretion of the product, its active substances or relevant metabolites into the environment by treated animals; persistence in such excreta,
- the disposal of unused veterinary medicinal product or other waste product.

In the second phase, further specific investigation of the fate and effects of the product on particular ecosystems shall be conducted, in accordance with established guidance. The extent of exposure of the product to the environment, and the available information about the physical/chemical, pharmacological and/or toxicological properties of the substance(s) concerned, including metabolites in case of an identified risk, which has been obtained during the conduct of the other tests and trials required by this Directive, shall be taken into consideration.

6.2. Environmental risk assessment for veterinary medicinal products containing or consisting of genetically modified organisms

In the case of a veterinary medicinal product containing or consisting of genetically modified organisms the application shall also be accompanied by the documents required under Article 2 and Part C of Directive 2001/18/EC.

CHAPTER II:

PRESENTATION OF PARTICULARS AND DOCUMENTS

The dossier of safety tests shall include the following:

- an index of all studies included in the dossier,
- a statement confirming that all data known by the applicant at the time of submission, whether favourable or unfavourable, are included,
- a justification for the omission of any type of study,
- an explanation of the inclusion of an alternative type of study,
- a discussion of the contribution that any study that pre-dates studies performed in line with good laboratory practice (GLP) according to Directive 2004/10/EC can make to the overall risk assessment.

Each study report shall include:

- a copy of the study plan (protocol),
- a statement of compliance with good laboratory practice, where applicable,
- a description of the methods, apparatus and materials used,
- a description and justification of the test system,
- a description of the results obtained, in sufficient detail to allow the results to be critically evaluated independently of their interpretation by the author,
- a statistical analysis of the results where appropriate,
- a discussion of the results, with comment on observed and no-observed-effect levels, and on any unusual findings,
- a detailed description and a thorough discussion of the results of the study of the safety profile of the active substance, and its relevance for the evaluation of potential risks presented by residues to humans.

B. Residue tests

CHAPTER I:

PERFORMANCE OF TESTS

1. Introduction

For the purposes of this Annex, the definitions of Council Regulation (EEC) No 2377/90⁽¹⁾ shall apply.

The purpose of studying the depletion of residues from the edible tissues or of eggs, milk and honey derived from treated animals is to determine under what conditions and to what extent residues may persist in foodstuffs produced from these animals. In addition, the studies shall enable the determination of a withdrawal period.

In the case of veterinary medicinal products intended for use in food-producing animals, the residue documentation shall show:

1. to what extent, and how long, do residues of the veterinary medicinal product or its metabolites persist in the edible tissues of the treated animal or in milk, eggs and/or honey obtained therefrom;
2. that in order to prevent any risk to the health of the consumer of foodstuffs from treated animals, or difficulties in the industrial processing of foodstuffs, it is possible to establish realistic withdrawal periods which can be observed under practical farming conditions;
3. that the analytical method(s) used in the residues depletion study are sufficiently validated to provide the necessary reassurance that the residues data submitted are suitable as the basis for a withdrawal period.

2. Metabolism and residue kinetics

2.1. Pharmacokinetics (absorption, distribution, metabolism, excretion)

A summary of the pharmacokinetic data shall be submitted with cross reference to the pharmacokinetic studies in target species submitted in Part 4. The full study report does not need to be submitted.

The purpose of pharmacokinetic studies with respect to residues of veterinary medicinal products is to evaluate the absorption, distribution, metabolism and excretion of the product in the target species.

The final product, or a formulation, which has comparable characteristics in terms of bioavailability as the final product, shall be administered to the target animal species at the maximum recommended dose.

Having regard to the method of administration, the extent of absorption of the veterinary medicinal product shall be fully described. If it is demonstrated that systemic absorption of products for topical application is negligible, further residue studies will not be required.

The distribution of the veterinary medicinal product in the target animal shall be described; the possibility of plasma protein binding or passage into milk or eggs and of the accumulation of lipophilic compounds shall be considered.

The pathways for the excretion of the product from the target animal shall be described. The major metabolites shall be identified and characterised.

2.2. Depletion of residues

The purpose of these studies, which measure the rate at which residues deplete in the target animal after the last administration of the medicinal product, is to permit the determination of withdrawal periods.

At a sufficient number of times after the test animal has received the final dose of the veterinary medicinal product, the quantities of residues present shall be determined by validated analytical methods; the technical procedures and the reliability and sensitivity of the methods employed shall be specified.

3. **Residue analytical method**

The analytical method(s) used in the residues depletion study (studies) and its (their) validation shall be described in detail.

The following characteristics shall be described:

- specificity,
- accuracy,
- precision,
- limit of detection,
- limit of quantification,
- practicability and applicability under normal laboratory conditions,
- susceptibility to interference,
- stability of incurred residues.

The suitability of the analytical method proposed shall be evaluated in the light of the state of scientific and technical knowledge at the time the application is submitted.

The analytical method shall be presented in an internationally agreed format.

CHAPTER II:

PRESENTATION OF PARTICULARS AND DOCUMENTS

1. **Identification of the product**

An identification of the veterinary medicinal product(s) used in the testing shall be provided, including:

- composition,
- the physical and chemical (potency and purity) test results for the relevant batch(es),
- batch identification,
- relationship to the final product,
- specific activity and radio-purity of labelled substances,
- position of labelled atoms in the molecule.

The dossier of residue tests shall include:

- an index of all studies included in the dossier,
- a statement confirming that all data known by the applicant at the time of submission, whether favourable or unfavourable, are included,
- a justification for the omission of any type of study,
- an explanation of the inclusion of an alternative type of study,
- a discussion of the contribution that any study that pre-dates GLP can make to the overall risk assessment,

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- a withdrawal period proposal.

Each study report shall include:

- a copy of the study plan (protocol),
- a statement of compliance with good laboratory practice, where applicable,
- a description of the methods, apparatus and materials used,
- a description of the results obtained, in sufficient detail to allow the results to be critically evaluated independently of their interpretation by the author,
- a statistical analysis of the results where appropriate,
- a discussion of the results,
- an objective discussion of the results obtained, and proposals concerning the withdrawal periods necessary to ensure that no residues which might constitute a hazard for consumers are present in foodstuffs obtained from treated animals.]

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(1) [^{F1}OJ L 224, 18.8.1990, p. 1.]

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