COUNCIL DIRECTIVE

of 28 September 1981

on the approximation of the laws of the Member States relating to analytical, pharmaco-toxicological and clinical standards and protocols in respect of the testing of veterinary medicinal products

(81/852/EEC)

THE COUNCIL OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community, and in particular Article 100 thereof,

Having regard to the proposal from the Commission (1),

Whereas the approximation begun by Council Directive 81/851/EEC of 28 September 1981 on the approximation of the laws of the Member States relating to veterinary medicinal products (2) should continue and the principles laid down in that Directive should be implemented;

Whereas, among existing disparities, those relating to the control of veterinary medicinal products are of fundamental importance; whereas point 10 of the second paragraph of Article 5 of Directive 81/851/EEC requires that applications for authorization to place a veterinary medicinal product on the market should be accompanied by particulars and documents relating to the results of tests and trials carried out on the product concerned;

Whereas standards and protocols for the performance of tests and trials on veterinary medicinal products are an effective means of control of these products and, hence, of protecting public health and can facilitate the movement of these products by laying down uniform rules applicable to tests and trials and the compilation of dossiers;

Whereas the adoption of the same standards and protocols by all the Member States will enable the competent authorities to arrive at their decisions on the basis of uniform tests and by reference to uniform criteria and will, therefore, help to obviate differences in evaluation;

Whereas the physico-chemical, biological or microbiological tests provided for in point 10 of the second paragraph of Article 5 of Directive 81/851/EEC are closely related to points 3, 4, 6 and 9 of the said paragraph; whereas it is necessary, therefore, to specify the data to be provided under those points;

Whereas the waiting period referred to in point 8 of the second paragraph of the said Article 5 of Directive 81/851/EEC must be determined in accordance with the results of the tests and trials provided for in point 10 thereof;

Whereas the concepts of harmfulness and therapeutic efficacy referred to in Article 11 of Directive 81/851/EEC can be examined only in relation to one another and have only a relative significance, depending on the progress of scientific knowledge and the use for which the medicinal product is intended; whereas the particulars and documents which must accompany an application for authorization to place a veterinary medicinal product on the market must demonstrate that potential hazards are outweighed by the therapeutic efficacy of the product; whereas, failing such demonstration, the application must be rejected;

Whereas it is the quality of the tests and trials which is pre-eminent; whereas the tests and trials carried out pursuant to these provisions must, therefore, be taken into consideration, irrespective of the nationality of the experts who perform them and of the country in which they are carried out,

HAS ADOPTED THIS DIRECTIVE:

Article 1

Member States shall take all appropriate measures to ensure that the particulars and documents which shall accompany applications for authorization to place a veterinary medicinal product on the market, pursuant to points 3, 4, 6, 8, 9 and 10 of the second paragraph of Article 5 of Directive 81/851/EEC, are submitted by the persons concerned in accordance with the Annex to this Directive.

Where, pursuant to point 10 (a) or (b) of the second paragraph of Article 5 of the abovementioned Direc-

(1) OJ No C 152, 5. 7. 1976, p. 11.
(2) See page 1 of this Official Journal.

tive, references to published data are submitted, the provisions of this Directive shall apply in like manner.

Article 2

The Committee for Veterinary Medicinal Products referred to in Article 16 of Directive 81/851/EEC may examine any question relating to the application of this Directive.

Article 3

Member States shall bring into force the provisions necessary to comply with this Directive within 24 months following its notification and shall forthwith inform the Commission thereof.

Member States shall ensure that the texts of the main provisions of national law which they adopt in the field covered by this Directive are communicated to the Commission.

Article 4

This Directive is addressed to the Member States.

Done at Brussels, 28 September 1981.

For the Council
The President
P. WALKER

ANNEX

PART 1

ANALYTICAL (PHYSICO-CHEMICAL, BIOLOGICAL OR MICROBIOLOGICAL) TESTS OF VETERINARY MEDICINAL PRODUCTS

A. QUALITATIVE AND QUANTITATIVE PARTICULARS OF THE CONSTITUENTS

The particulars and documents which shall accompany applications for marketing authorization pursuant to point 3 of the second paragraph of Article 5 of Directive 81/851/EEC shall be submitted in accordance with the following requirements; if any amendments are made to these requirements for reasons of scientific progress, all grounds shall be stated.

1. Qualitative particulars of all the constituents of the medicinal product means the designation or description of:

   — the active ingredient(s),

   — the constituent(s) of the excipients, whatever their nature or the quantity used, including colouring agents, preservatives, stabilizers, thickeners, emulsifiers, anti-agglutinating agents, flavouring and aromatic substances, propellents, etc.,

   — the constituents of the pharmaceutical form intended to be ingested or otherwise administered to animals.

   These particulars shall be supplemented by any relevant data concerning the container and, where appropriate, its manner of closure.

2. The usual terminology to be used in describing the constituents of proprietary medicinal products means, notwithstanding the application of the other provisions of point 3 of the second paragraph of Article 5 of Directive 81/851/EEC:

   — in respect of substances which appear in the European Pharmacopoeia or, failing this, in the national pharmacopoeia of one of the Member States, the main title at the head of the monograph in question, which will be obligatory for all such substances, with a reference to the pharmacopoeia concerned,

   — in respect of other substances, the international non-proprietary name recommended by the WHO, which may be accompanied by another non-proprietary name, or, failing this, the exact scientific designation; substances not having an international non-proprietary name or an exact scientific designation shall be described by a statement indicating how and from what they were prepared, together with any other necessary relevant details,

   — in respect of colouring matters, designation by the 'E' code assigned to them in Council Directive 78/25/EEC of 12 December 1977 on the approximation of the laws of the Member States relating to the colouring matters which may be added to medicinal products [1], as last amended by Directive 81/464/EEC [2].

3. In order to give 'quantitative particulars' of the active constituents of the medicinal product, it is necessary, depending on the pharmaceutical form concerned, to specify the weight or the number of international units, either per dosage-unit or per unit of weight or volume of each active ingredient and with regard to the constituents of the excipient, the weight or the volume of each of them, with due allowance for the details provided in section B below.

   This information shall be supplemented:

   — in respect of proprietary medicinal products to be administered in drops, by the weight of each active ingredient contained in the number of drops corresponding to the recommended dose,

   — in respect of syrups, emulsions, granule or preparations and other pharmaceutical forms to be administered in measured quantities, by the weight of each active ingredient per measured quantity.

   Active ingredients present in the form of compounds or derivatives shall be described quantitatively by their total weight and, if necessary or relevant, by the weight of the active moiety or moieties of the molecule (in the case of chloramphenicol palmitate, for example, the weight of the ester and that of the corresponding chloramphenicol shall be given).

   The biological units of activity of substances which have not been defined chemically, and on which there is insufficient bibliographical information, shall be expressed in such a way as to provide unambiguous information on the activity of the substances, e.g. by stating the physiological effect on which the method of determining the dose is based.

(1) OJ No L 11, 14. 1. 1978, p. 18.
(2) OJ No L 183, 4. 7. 1981, p. 33.

B. DESCRIPTION OF METHOD OF PREPARATION

The 'brief description of the method of preparation' accompanying the application for marketing authorization pursuant to point 4 of the second paragraph of Article 5 of Directive 81/851/EEC shall be so drafted as to provide an adequate synopsis of the nature of the operations employed.

For this purpose, it shall include at least:

— mention of the various stages of manufacture, so that an assessment can be made of whether the processes employed in producing the pharmaceutical form might have produced an adverse change in the constituents,

— in the case of continuous manufacture, full details concerning precautions taken to ensure the homogeneity of the final product,

— the actual manufacturing formula, with the quantitative particulars of all the substances used, the quantities of excipients, however, being given in approximate terms in so far as the pharmaceutical form makes this necessary; mention shall be made of any substances which may disappear in the course of manufacture,

— a statement of the stages of manufacture at which sampling is carried out for in-process control tests, where other data in the documents supporting the application show such tests to be necessary for quality control of the proprietary medicinal product.

C. CONTROL OF STARTING MATERIALS

For the purposes of this paragraph, 'starting materials' means all the constituents of the proprietary medicinal product and, if necessary, of its container, as referred to in point A, paragraph 1.

The particulars and documents accompanying the application for marketing authorization pursuant to points 9 and 10 of the second paragraph of Article 5 of Directive 81/851/EEC must include the results of the tests relating to quality control of all the constituents used. These shall be submitted in accordance with the following provisions.

1. **Starting materials listed in pharmacopoeias**

The monographs of the European Pharmacopoeia shall be applicable to all substances appearing in it.

In respect of other substances, each Member State may require observance of its own national pharmacopoeia with regard to products manufactured in its territory.

Constituents fulfilling the requirements of the European Pharmacopoeia or the pharmacopoeia of one of the Member States shall be deemed to comply sufficiently with point 9 of the second paragraph of Article 5 of Directive 81/851/EEC. In this case, the description of the analytical methods may be replaced by a detailed reference to the pharmacopoeia in question.

However, where a starting material in the European Pharmacopoeia or in the pharmacopoeia of a Member State has been prepared by a method liable to leave impurities not mentioned in the pharmacopoeia monograph these impurities and their maximum tolerance levels must be declared and a suitable test method advanced.

Reference to pharmacopoeias of third countries may be permitted in cases where the substance is described neither in the European Pharmacopoeia nor in the national pharmacopoeia concerned; in that case the monograph shall be submitted, accompanied where necessary by a translation for which the applicant shall be responsible.

Colouring matters shall, in all cases, satisfy the requirements of Directive 78/25/EEC.

The routine tests to be carried out on each batch of starting materials shall be declared in an application for authorization to market. These tests must make it possible to provide evidence that each batch of starting material satisfies the quality requirements of the relevant pharmacopoeial monograph.

Should a specification contained in a monograph of the European Pharmacopoeia or in the national pharmacopoeia of a Member State be insufficient to ensure the quality of the substance, the competent authorities may request more appropriate specifications from the person responsible for placing the product on the market.

2. **Starting materials not in a pharmacopoeia**

Constituents which are not given in any pharmacopoeia shall be described in the form of a monograph under the following headings:

(a) 'The name of the substance', meeting the requirements of point 2 of paragraph A, shall be supplemented by any trade or scientific synonyms;

(b) 'The description of the substance', set down in a form similar to that used in a descriptive item in the European Pharmacopoeia, shall be accompanied by any necessary explanatory evidence, especially concerning the molecular structure where appropriate; it shall, in such cases be accompanied by a brief indication of the method of synthetic preparation. Where substances can be described only by their method of preparation, the description shall be sufficiently detailed to characterize a substance which is constant in both its composition and its effects;

(c) 'Methods of identification' may be divided into complete techniques as used for the development of the medicinal product and tests which ought to be carried out as a routine matter.

(d) 'Purity tests' shall be described in relation to the sum total of predictable impurities, especially those which may have a harmful effect, and, if necessary, those which, having regard to the combination of substances to which the application refers, may adversely effect the stability of the medicinal product or distort analytical results.

(e) 'The assay technique(s)' shall be described in sufficiently precise detail to be reproducible in control tests carried out at the request of the competent authority; any special apparatus and equipment which may be used shall be described in adequate detail, possibly accompanied by a diagram. The formulae of the laboratory reagents shall, if necessary, be supplemented by a description of the method of preparation.

The standard error of the method, its reliability and the acceptability limits of the results shall be specified and, if necessary, justified in the lights of feasibility and the progress of scientific knowledge.

With regard to complex substances of plant or animal origin, a distinction shall be made between the case where multiple pharmacological effects render a chemical, physical or biological control of the principal constituents necessary, and the case of substances containing one or more groups of principals having similar activity, in respect of which an overall method of assay may be accepted.

(f) 'Any special precautions which may be necessary during storage' of the starting material and, if necessary, its storage life shall be given.

D. CONTROL TESTS DURING MANUFACTURE

The particulars and documents which shall accompany an application for marketing authorization, pursuant to points 9 and 10 of the second paragraph of Article 5 of Directive 81/851/EEC, shall include particulars relating to the product control tests which may be carried out at an intermediate stage of the manufacturing process, with a view to ensuring the consistency of the technical characteristics and the production process.

These tests are essential to verify the conformity of the medicinal product with the formula when, exceptionally, an applicant proposes an analytical technique for testing the finished product which does not include the assay of all the active ingredients (or of all the excipient constituents which are subject to the same requirements as the active ingredients).

The same applies where quality control of the finished product depends on in-process control tests, particularly if the medicinal product is essentially defined by its method of preparation.

E. CONTROL TESTS ON THE FINISHED PRODUCT

The particulars and documents which shall accompany the application for marketing authorization pursuant to points 9 and 10 of the second paragraph of Article 5 of Directive 81/851/EEC, shall include particulars relating to control tests on the finished product. They shall be submitted in accordance with the following requirements.

1. General characteristics of the various pharmaceutical forms

Certain tests of the general characteristics of a product which can be carried out in the course of the manufacturing process shall be included among the tests on the finished product.

As a guideline, and subject to the possible requirements of the European Pharmacopoeia or the national pharmacopoeias of Member States, the general characteristics which are to be verified for various pharmaceutical forms are given in point 5.

These tests shall relate, wherever necessary, to the control of average weights and maximum deviations, to mechanical, physical or microbiological tests, organoleptic characteristics such as clarity, colour, taste, physical characteristics such as density, pH, refractive index, etc. For each of these characteristics, standards and tolerances shall be specified by the applicant in each particular case.

2. Identification and assay of active ingredient(s)

The description of the techniques for analyzing the finished product shall set out, in sufficiently precise detail, to enable them to be reproduced readily, the methods used for identification and assay of the active ingredient(s) in either a representative average sample from the production batch or a number of dosage-units considered individually.

In every case, the methods shall correspond to the state of scientific progress at the time and give details and explanations of the standard errors, the reliability of the analytical method and the maximum acceptable deviations.

In certain exceptional cases of particularly complex mixtures, where assay of active ingredients which are very numerous or present in very low amounts would necessitate an intricate investigation difficult to carry out in respect of each production batch, the assay of one or more active ingredients in the finished product may be omitted, on the express condition that such assays are made at intermediate stages in the production process. This relaxation may not be extended to the characterization of the substances concerned. This simplified technique shall then be supplemented, if possible, by a method of quantitative evalution, enabling the competent authorities to have the conformity of the medicinal product with its formula verified after it has been placed on the market.

An assay of biological activity is obligatory when physicochemical methods cannot provide adequate information on the quality of the product.

Where the particulars given in paragraph B show that a significant overage of an active ingredient was employed in the manufacture of the medicinal product, the descrip-

tion of the control tests on the finished product shall include, where appropriate, the chemical and even the toxico-pharmacological investigation of the changes that this substance has undergone, and possibly the characterization or assay of the degradation products.

3. Identification and assay of excipient constituents

An upper-limit test is obligatory in respect of excipient constituents which are subject to rules relating to toxic substances or which are used as preservatives; furthermore, constituents liable to affect physiological functions shall be assayed.

The method proposed for identifying colouring agents shall make it possible to check whether such agents are permitted under Directive 78/25/EEC.

In so far as is necessary, characterization tests shall be carried out on the other constituents of the excipient.

4. Safety tests

Apart from the toxico-pharmacological tests submitted with the application for marketing authorization, particulars of safety tests (abnormal toxicity) or local tolerance in animals shall be included in the analytical particulars wherever such tests shall be undertaken as a matter of routine in order to verify the quality of the medicinal product.

5. General characteristics of medicinal products to be verified systematically, depending on the pharmaceutical form of each product

The following requirements are given as an indication and without prejudice to any requirements of the European Pharmacopoeia or national pharmacopoeias of Member States.

— *Tablets and pills:* colour, weight and acceptable variations in unit weight; if necessary, disintegration time with the method used to determine this.

— *Coated tablets:* colour, disintegration time with the method used to determine this; weight of finished tablet; weight of core and acceptable variations in unit weight.

— *Capsules and gelatine capsules:* colour, disintegration time with the method used to determine this; appearance and weight of content, with accecptable variations in unit weight.

— *Enteric-coated preparations (tablets, capsules, gelatine capsules, granular preparations):* in addition to the requirements of the particular pharmaceutical form, details of the resistance time and the disintegration time in variable conditions of acidity (at different pH levels), with the method used to determine them.

— *Preparations with special protective coating (tablets, capsules, gelatine capsules, granular preparations):* in addition to the requirements of the particular pharmaceutical form, verification of the effectiveness of the coating for the desired purpose.

— *Preparations with gradual release of the active principle:* in addition to the requirements of the particular pharmaceutical form, requirements relating to gradual release, with the method used to determine this.

— *Cachets, packets and sachets:* nature and weight of contents and acceptable variations in unit weight.

— *Injectable preparations:* colour, volume of contents and acceptable variations of this volume; pH, clarity of solution, size limit of particulate matter in the case of suspensions; sterility tests, with description of test methods and, if necessary, a pyrogen test with description of method.

— *Ampoules with solid content:* quantity of medicinal product per ampoule and permitted variations in weight; sterility requirements and tests.

— *Ampoules to be taken orally:* colour, appearance, volume of content and acceptable variations.

— *Ointments, creams, etc.:* colour and consistency; weight and acceptable margin of variation; nature of container; in certain cases, microbiological control tests.

— *Suspensions:* colour; where settlement occurs, the ease of re-suspendability.

— *Emulsions:* colour; type; stability.

— *Suppositories, bougies and preparations for intra-uterine administration:* colour, weight and acceptable variations in unit weight; melting temperature or disintegration time and description of the method.

— *Aerosols:* description of container and valve with details of output; particle size-limit, where the product is intended to be inhaled.

— *Collyria, eye ointments, eye lotions:* colour; appearance; sterility controls, with description of the method used; where appropriate, clarity and size-limit of particulate matter in the case of suspension, pH determination.

— *Syrups, solutions, etc.:* colour, appearance.

— *Pre-mixes for medicated feedingstuffs:* in addition to the requirements specific to each pharmaceutical form, all relevant information on the characteristics of the pre-mix enabling a sufficiently homogeneous and stable medicated feedingstuff to be prepared.

— *Preparations for administration within the udder via the teat canal:* colour, consistency; weight of content and,

in the case of products presented in single injectable dose formulations, usable weight with acceptable deviation; sterility test; pH determination.

F. STABILITY TESTS

The particulars and documents which shall accompany the application for marketing authorization pursuant to points 6 and 9 of the second paragraph of Article 5 of Directive 81/851/EEC shall be submitted in accordance with the following requirements.

A description shall be given of the investigations by which the shelf life proposed by the applicant has been determined; in the case of pre-mixes for medicated feedingstuffs, information should also be given as necessary on the shelf life of the medicated feedingstuffs manufactured from these pre-mixes, in accordance with the recommended instructions for use.

Where a finished product is liable to give rise to toxic degradation products, the applicant shall declare these and indicate characterization or assay methods.

The conclusions shall contain the results of analyses, justifying the proposed shelf life under normal, or, where appropriate, under special storage conditions.

A study of the interaction between medicinal product and container shall be submitted wherever the risk of such interaction is regarded as possible, especially where injectable preparations or aerosols for internal use are concerned.

PART 2

TOXICOLOGICAL AND PHARMACOLOGICAL TESTS

The protection of the animal as a living creature shall be taken into account. However, it is recognized that for veterinary medicinal products a degree of toxicity and hazard for the animal are acceptable, provided that such toxicity has no consequences for man and that the treatment of the animal is justified on therapeutic and/or economic grounds.

The particulars and documents which shall accompany the application for marketing authorization pursuant to point 10 of the second paragraph of Article 5 of Directive 81/851/EEC shall be submitted in accordance with the requirements of Chapters I and II.

CHAPTER I

PERFORMANCE OF TESTS

A. INTRODUCTION

The toxicological and pharmacological tests shall show:

1. The potential toxicity of the 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 gravity of the pathological condition concerned.

2. The pharmacological properties of the medicinal product, in both qualitative and quantitative relationship to the proposed use in animals.

3. To what extent and for how long after use of the medicinal product in animals there are residues in food products obtained from the animals, what their possible harmful effects are on man and what difficulties they create in the industrial processing of food.

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

B. TOXICITY STUDY

1. **Single-dose toxicity**

Single-dose toxicity testing means 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 in which they are present in the actual product.

Wherever it is considered necessary, the product in its actual pharmaceutical form shall be subjected to an acute toxicity test.

The single-dose toxicity test shall be carried out in at least two mammalian species of known strain, and at least two different routes of administration shall normally be used. The study with two mammalian species may be replaced by a study with one mammalian species and an animal species of another class for which the medicinal product is

intended. One of the routes of administration shall be identical with, or similar to, that proposed for use in the animal for which the medicinal product is intended and the other shall ensure systemic absorption of the substance. The study shall be carried out on equal numbers of male and female animals.

This study shall describe the symptoms observed, including local reactions. The LD_{50} value with its confidence limits (95 %) shall, where possible, be noted. The period during which the test animals are observed shall be fixed by the investigator and shall not be less than one week.

In the case of active substances in combination, the study shall be carried out in such a way as to check whether or not potentiation or novel toxic effects occur.

2. **Repeated-dose toxicity**

Repeated-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.

Generally, it is desirable that at least one test be performed, the duration of which depends on the conditions of clinical use; its purpose is to determine by experiment the non-toxic dose range of the product examined during the trial. The investigator shall give reasons for the extent and duration of the trials and the dosages chosen.

If, however, having regard to, in particular, the directions for use of the medicinal product, the investigator responsible sees fit not to carry out this examination, he shall give adequate reasons for his decision.

Repeated-dose toxicity tests shall be carried out on two species of mammals, one of which shall be a non-rodent. The study with two mammalian species may be replaced by a study with one mammalian species and another animal species for which the medicinal product is intended. The choice of the route(s) of administration shall take account of the routes for therapeutic use and the likelihood of systemic absorption. The method and frequency of administration and the length of the trials shall be clearly stated.

The maximum dose should be selected so as to bring harmful effects to light. The lower doses will then enable the animal's tolerance of the new product to be determined.

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 possibly 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 repeated-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. Substances which have been shown to be safe by wide usage over at least three years in clinical treatment of human beings or animals, and by the result of controlled trials shall be regarded as known substances which have already been investigated in accordance with these standards and protocols.

3. **Tolerance in the target species of animal**

The purpose of this study, which shall be carried out with all animal species for which the medicinal product is intended, is to carry out in all such animal species local and general tolerance trials designed to establish a tolerated dosage wide enough to allow an adequate safety margin and the clinical symptoms of intolerance using the recommended route or routes, in so far as this may be achieved by increasing the therapeutic dose. The report on the trials shall contain as many details as possible of the expected pharmacological effects and the adverse side-effects; the latter shall be assessed with due regard to the fact that the experimental animals may be of very high value.

The medicinal product shall be administered via the routes most likely to produce the appearance of the pharmacological effects sought.

Where the trials are carried out with animals of high unit price, the sequential method described in the Appendix hereto may be used.

4. **Foetal toxicity**

This investigation comprises an examination of the toxic and abortifacient effects observed in the conceived issue when the medicinal product under investigation is also intended to be administered to the female during pregnancy. If, during studies on the effects of residues, signs of foetal toxicity are observed, or if other observations made independently of such studies give rise to doubt in this respect, trials with the intended species of animal may be required. These may be carried out as part of the clinical trials.

5. **Examination of reproductive function**

If the results of the other tests reveal anything suggesting impairment of male or female reproductive function or

harmful effects on progeny, the reproductive function shall be investigated by appropriate tests.

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

C. STUDY OF PHARMACOLOGICAL PROPERTIES

1. **Pharmacodynamics**

Pharmacodynamics means the study of the variations caused by the medicinal product in the functions of the organs, whether these functions are normal or experimentally modified.

This study shall follow two distinct lines of approach.

First, the actions on which the recommended application in practice is based shall be adequately described. The results shall be expressed in quantitative terms (using, for example, dose-effect curves, time-effect curves, etc.) and, wherever possible, in comparison with a substance the activity of which is well 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 give a general pharmacological assessment of the substance, with special reference to the possibility of side-effects. In general, the main functions should be investigated. This investigation shall be intensified as the doses liable to produce side-effects approach those producing the therapeutic effects for which the substance is being proposed.

The experimental techniques, unless they are standard procedures, shall be described in such detail as to allow them to be reproduced, and the investigator shall establish their validity. The experimental results shall be set out clearly and, for certain types of tests, 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 also be investigated.

Medicinal combinations may be prompted either on pharmacological grounds or by clinical indications. In the first case, the pharmacodynamic study shall demonstrate those interactions which might make the combination itself of value in clinical use. In the second case, where scientific justification for the medicinal combination is sought through clinical experimentation, the investigation shall determine whether the effects expected from the combination can be demonstrated in animals and, at least, the importance of any side-effects shall be checked. If a combination includes a novel active substance, the latter shall have been previously studied in depth.

2. **Pharmacokinetics**

Pharmacokinetics means the study of the life of the substances within the body, and covers the study of the absorption, distribution, biotransformation (or metabolism) and elimination of the products.

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 medicinal product.

Information on distribution and elimination is required in respect of chemotherapeutic substances (antibiotics, etc.) and substances the use of which depends on their non-pharmacodynamic effects and in all cases where it is essential to determine the dosage for animals or to determine residues in foodstuffs.

In the case of new combinations of known substances which have been investigated in accordance with the provisions of this Directive, pharmacokinetic studies are not required if the toxicity tests and clinical trials justify their omission. The same applies to substances which have been shown by controlled trials to be efficacious and safe by wide usage over a period of at least three years in the clinical treatment of human beings or animals.

D. STUDY OF RESIDUES

For the purposes of this Directive, 'residues' means all active ingredients or metabolites thereof which remain in meat or other foodstuffs produced from the animal to which the medicinal product in question has been administered.

The purpose of studying residues is to determine whether and, if so, under what conditions and to what extent residues persist in foodstuffs produced from treated animals and to ascertain the withdrawal periods to be adhered to in order to obviate any hazard to human health and/or difficulties in the industrial processing of foodstuffs.

Assessment of the hazard due to residues entails establishing whether residues are present in the animals treated under recommended conditions of use and investigating the effects of those residues.

1. **Determination of residues**

The determination of residues shall be carried out with regard to, in particular, the results of the pharmacokinetic tests. At varying times after the test animal has received the final dose of the medicinal product, the quantities of residues present shall be determined by appropriate physical, chemical or biological methods; the technical procedures

and the reliability and sensitivity of the methods employed shall be specified. If of practical value, the results shall be checked as far as possible, at least by examining the sick animals for which the medicinal product is recommended.

Checking procedures shall be proposed which can be carried out in the course of a routine examination and which have a level of sensitivity such as to enable residue concentrations likely to affect health in animal-based foodstuffs to be determined with certainty.

2. **Investigation of the effects of residues**

(a) *Toxicity of orally administered residues*

Toxicity studies to determine the safety of orally administered residues shall be performed differently according to whether it is a medicinal product which is eliminated without transformation or one which is metabolized. In the first case, the investigator may work directly with the medicinal product. In the second case, he shall work on the principal metabolites chiefly found in foodstuffs. If the metabolites cannot be isolated or synthesized, the toxicity study shall be carried out by a different method; in such cases recourse may be had to a study of 'relay' toxicity.

The trials shall be carried out, using the oral route, on two mammalian species, one of which shall be a non-rodent and shall normally last from three to six months. If the medicinal product or metabolite is worked on directly, the doses shall be fixed with due regard to the residues actually present and shall be so selected that the highest dose causes harmful effects to appear as far as possible, while the lower doses then enable the limit of tolerance in animals to be found. If the study of 'relay toxicity' is adopted, the upward gradation of the doses will be limited by the quantity of residues actually present.

Evaluation of the toxic effects shall be based on observation of behaviour, growth, haematology and physiological tests, especially those relating to the excretory organs, as well as 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.

(b) *Other effects of orally administered residues*

Effects of residues on the reproductive functions shall be tested in rodents of both sexes.

Tests to reveal carcinogenic effects are indispensable:

1. in respect of substances having a close chemical analogy with known carcinogenic or co-carcinogenic compounds;

2. in respect of substances which have given rise to suspect signs and symptoms during the repeated-dose toxicity study;

3. when the test for mutagenic effects produces results indicating a possibility of carcinogenic effects.

Tests to reveal teratogenic effects are indispensable:

1. in respect of substances having a close chemical analogy with known teratogenic products;

2. in respect of substances which have given rise to abnormal signs and symptoms during the study of effects on the reproductive functions;

3. in respect of new molecules with a chemical structure not analogous to known products.

The study of teratogenic effects shall be carried out with at least two animal species: rabbits, using a breed sensitive to substances known to possess foetal toxicity, and rats or mice (specifying the strain). The details of the test (number of animals, doses, time at which administered and criteria for evaluation of results) depend on the state of scientific knowledge at the time when the application is lodged and the level of statistical significance which the results shall attain.

Furthermore, a study of mutagenic effects carried out by means of a test (such as the Ames test) suitable for the assessment of hazards is necessary.

A study of allergic phenomena is desirable.

(c) *Difficulties affecting the industrial processing of foodstuffs*

In certain cases, it may be necessary to carry out tests to determine whether residues cause difficulties affecting technological processes in industrial foodstuff processing.

3. **Exceptions**

Toxicity studies (a) to (c) are not required if it has been established that the medicinal product is rapidly and completely eliminated or if it is used only occasionally. In such cases, the withdrawal period shall be fixed according to the data available, so that there is no risk of danger to consumers of foodstuffs.

E. MEDICINAL PRODUCTS FOR TOPICAL USE

Where a medicinal product is intended for topical use, systemic absorption shall be investigated in the target species of animal. If it is proved that systemic absorption is negligible, the repeated-dose toxicity tests, the foetal toxicity tests and the studies of reproductive function referred to at points B.2, B.4 and B.5 may be omitted.

If the medicinal product is absorbed systemically in a significant quantity from the point of view of residues or from that of pharmacodynamics (concentration) or if, under the conditions of use laid down, oral ingestion of the medicinal product by the animal is to be expected, the medicinal product shall be investigated in accordance with the requirements of points B to D.

In all cases, tests of local tolerance after repeated administration shall be carried out and shall include histological examinations. Where a medicinal product which is not systemically absorbed may enter a food product obtained from the treated animal (intra-mammary preparations, etc.), the assay of residues in accordance with point D shall be carried out each time.

F. RESISTANCE

Data on the emergence of resistant organisms are necessary in the case of medicinal products (notably antimicrobials) used for the prevention or treatment of infectious disease in animals.

CHAPTER II

PRESENTATION OF PARTICULARS AND DOCUMENTS

As in any scientific work, the dossier of toxicological and pharmacological tests shall include the following:

(a) an introduction defining the subject, accompanied by any useful bibliographical references;

(b) a detailed experimental protocol giving the reasons for any omission of certain tests listed above, a description of the methods, apparatus and materials used, details of the species, breed or strain of animals, where they were obtained, their number and the conditions under which they were housed and fed, stating *inter alia* whether they were free from specific pathogens (SPF) or traditional pathogens;

(c) all the results obtained, whether favourable or unfavourable. The original data should be described in sufficient detail to allow the results to be critically evaluated independently of their interpretation by the author. By way of explanation and illustration, the results may be accompanied by reproductions of kymograms, photomicrographs, etc.;

(d) a statistical analysis of the results, where such is called for by the test programme, and variance within the data;

(e) an objective discussion of the results obtained, leading to conclusions on the toxicological and pharmacological properties of the substance, on its safety margin in the test animal and the target animal and its possible side-effects, on its fields of application, on its active dose levels and any possible incompatibilities;

(f) information showing whether the constituents of the medicinal product are used as medicinal products in human therapy; if this is so, a report should be made on all the effects observed (including side-effects) in man and on their cause, to the extent that they may be important for the assessment of the veterinary medicinal product, where appropriate in the light of trial results or bibliographical documents; where constituents of the medicinal products are themselves not used or are no longer used as medicinal products in human therapy, the reasons should be stated;

(g) a detailed description and a thorough discussion of the results of the study on the presence of residues in food and an assessment of the hazards which they constitute for man. Account should be taken of all the factors which may be of importance, particularly with regard to customary diet and levels of contamination by foreign matter present in the environment. In the case of each recommended use, this description shall be followed by proposals concerning the withdrawal periods which, allowing for an adequate safety margin, shall be so established as to ensure that no further residue remains in food or, if this is impossible, to ensure that any danger to man is eliminated by applying internationally recognized assessment criteria: dose devoid of effect in animals, acceptable daily dose (ADD), safety margin of 1:100 or </> 1:100 according to available information, etc.;

(h) all information necessary to acquaint the clinician as fully as possible with the utility of the proposed product. The discussion will be supplemented by suggestions as to side-effects and possible treatment for acute toxic reactions in animals to which the product is to be administered;

(i) a summary together with precise bibliographical references.

PART 3

CLINICAL TRIALS

The particulars and documents which shall accompany applications for marketing authorizations pursuant to point 10 of the second paragraph of Article 5 of Directive 81/851/EEC shall be submitted in accordance with the provisions of Chapters I and II below.

CHAPTER I

CONDUCT OF TRIALS

The purpose of clinical trials is to demonstrate or to ascertain the therapeutic effect of the medicinal product, to specify its indications and contra-indications according to species, age, its directions for use, any side-effects which it may have and its safety under normal condition of use.

Clinical trials shall be preceded by adequate pharmacological and toxicity tests carried out in accordance with the provisions of this Directive and, where they are practicable, by tests carried out preferably on the one or more animal species for which the medicinal product is intended. The investigator shall acquaint himself with the conclusions of these preliminary trials.

As far as possible, clinical trials shall be carried out with control animals (controlled clinical trials); if it is economically justifiable, the therapeutic effect obtained should be compared both with a placebo and with absence of treatment and/or with the effect of a medicinal product of known therapeutic value which has already been used. All the results obtained, whether positive or negative, shall be reported.

The methods used to make the diagnosis shall be specified. The results shall be set out by making use of quantitative or conventional criteria (system of crosses, etc.).

CHAPTER II

PARTICULARS AND DOCUMENTS

Particulars concerning clinical trials shall be sufficiently detailed to enable an objective judgement to be made.

1. **Records of clinical observations**

All the particulars shall be supplied by each of the investigators on individual record-sheets in the case of individual treatment and collective record-sheets in the case of collective treatment.

The particulars supplied shall take the following form:

(a) name; address, function and university qualifications of investigator;

(b) place and date of treatment; name and address of owner of the animals;

(c) in the case of individual treatment and collective treatment, if the latter has been given, full identification of the trial animals, names or registered numbers, species, breeds or strains, age, weight, sex (in the case of females, specify whether pregnant or in milk and, in the case of birds, in lay, etc.);

(d) method of rearing and feeding, stating the nature and quantity of any additives contained in the feed;

(e) case history (as full as possible), occurrence and course of any inter-current diseases;

(f) diagnosis and means used to make it;

(g) symptoms and severity of the disease, if possible according to conventional criteria (system of crosses, etc.);

(h) dosage of the medicinal product, method, route and frequency of administration and precautions, if any, taken during administration (duration of injection, etc.);

(i) duration of treatment and period of subsequent observation;

(j) all details concerning medicinal products (other than that being assayed) which have been administered during the period of examination, either prior to or concurrently with the test product and, in the latter case, details of the interactions observed;

(k) all results of the clinical trials (including unfavourable or negative results) with a full statement of the clinical observations and the results of the objective tests of activity (laboratory analyses, physiological tests), required to evaluate the application; the techniques used must be specified, and the significance of any variations in the results explained (for example: variance in method, variance between individuals or the effects of the medication); demonstration of the pharmacodynamic effect in animals shall not in itself suffice to justify conclusions concerning any therapeutic effect;

(l) all particulars of the observed side-effects, whether harmful or not, and of any measures taken in consequence; the cause-and-effect relationship shall be investigated if possible;

(m) effect on animals' performances (for example: egg-laying, milk production and reproductive function);

(n) a conclusion on each individual case or, where collective treatment is concerned, on each collective case.

Omission of one or more of items (a) to (n) shall be explained.

Where, in respect of particular therapeutic indications, the applicant can show that he is unable to provide comprehensive data on therapeutic effect because:

(a) the indications for which the medicinal product in question is intended are encountered so rarely that the applicant cannot reasonably be expected to provide comprehensive evidence;

(b) in the present state of scientific knowledge, comprehensive information cannot be provided,

the marketing authorization may be granted subject to the following conditions:

(a) the medicinal product in question is to be supplied on veterinary prescription only and may, in certain cases, be administered only under strict veterinary supervision;

(b) the package insert and any other information must draw the attention of the veterinary practitioner to the fact that, in certain specified respects, the particulars available concerning the medicinal product in question are as yet inadequate.

The person responsible for placing the veterinary medicinal product on the market shall make all necessary arrangements to ensure that the original documents, which formed the basis of the data supplied, are kept for at least five years as from the date of transmission of the dossier to the competent authority.

2. **Summary and conclusions**

The clinical observations referred to in paragraph 1 shall be summarized in a synopsis of the trials and the results thereof, indicating in particular:

(a) the number of animals treated either individually or collectively, with a breakdown according to species, breed or strain, age and sex;

(b) the number of animals withdrawn prematurely from the trials and the reasons for such withdrawal;

(c) in the case of control animals, whether they have:

— received no treatment;

— received a placebo;

— received another medicinal product of known effect;

(d) the frequency of observed side-effects;

(e) observations as to the effect on performance (for example: egg-laying, milk production and reproductive function), where the medicinal product is intended for animals in which yield is an important factor;

(f) details concerning test animals which may be at increased risk owing to their age, their mode of rearing or feeding, or the purpose for which they are intended, or animals the physiological or pathological condition of which requires special consideration;

(g) a statistical evaluation of the results, when this is called for by the test programme.

Finally, the investigator shall draw general conclusions from the experimental evidence, expressing his opinion on the harmlessness of the medicinal product under normal conditions of use, its therapeutic effect and any useful information relating to indications and contra-indications, dosage and average duration of treatment and, where appropriate, any interactions observed with other medicinal products or feed additives as well as any special precautions to be taken during treatment and the clinical symptoms of overdosage.

Appendix

SEQUENTIAL METHOD

This method consists of calculating a non-lethal theoretical dose for the animal concerned on the basis of the pharmacologically effective doses, determined during the experimental trials with the medicinal product, bearing in mind the maximum tolerated doses observed during the single-dose toxicity study, in accordance with point B.1. That dose is then administered to an animal which shall be watched very carefully in order that as much information as possible may be obtained regarding the effects of the medicinal product. If the animal displays no symptoms of non-tolerance, the test shall recommence with another animal, using a higher dose the strength of which is left to the investigator's discretion. If the animal easily tolerates this new dose, the test shall be continued with a further higher dose. The dose which shall not be exceeded shall be that obtaining at the point where symptoms of toxicity appear. If the animal dies, the test shall recommence with a lower dose, and so on. In every case, the aim is to determine a single dosage which enables a favourable pharmacological effect to be obtained without harming the animal.