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OPINION OF
THE SCIENTIFIC COMMITTEE ON ANIMAL NUTRITION
ON THE REVISION OF THE GUIDELINES
FOR THE ASSESSMENT OF ADDITIVES IN ANIMAL NUTRITION

(Adopted on 22 October 1999)

GUIDELINES FOR THE ASSESSMENT OF ADDITIVES IN FEEDINGSTUFFS

PART I: CHEMICALLY SPECIFIED ADDITIVES

GENERAL ASPECTS

This document is intended as a guideline for establishing dossiers on substances and preparations being submitted for authorisation as additives in feedingstuffs or a new usage of an authorised additive. The term "additive", as used in these guidelines, refers to the active chemically specified substances or the preparations containing active substances in the state in which they will be incorporated in premixtures and feedingstuffs. In part I of these guidelines the expression "Chemically specified substances" refers to those additives not being enzymes and micro-organisms.

The dossiers must enable an assessment to be made of the additives based on the present state of knowledge and make it possible to ensure their compliance with the fundamental principles laid down for their admission, which are the subject of the provisions of Article 3A of Council Directive 70/524/EEC. The dossiers should include detailed reports of the studies done, presented in the order and with the numbering proposed in these guidelines. They should include references and copies of all published scientific data relevant to the evaluation of the additive. An electronic version of the dossier should be made available for evaluation purposes.

The studies are intended to ensure the safety of use of the additive: a) to the target species at the proposed levels of incorporation in the feedingstuff; b) to those likely to be exposed to the additive by respiratory, other mucosal, eye or cutaneous contact while handling the additive as such or as incorporated into premixtures or feedingstuffs; c) to consumers who ingest food products from animals given the additive, which could contain residues of the additive, or its metabolites and for which the safety for the consumer is provided normally through the setting of Maximum Residue Limits (MRLs) and withdrawal periods; d) to the animals and the human-beings through the selection and spread of antimicrobial resistance genes; e) to the environment arising from the additive itself or by products derived from the additive and excreted by animals. As a general rule, studies to establish the identity, conditions of use, physico-chemical properties, methods of determination and efficacy of the additive, and also its metabolic fate and residues, physiological and toxicological effects on target species must be provided. When the additive is intended for a category of animals belonging to a defined species, efficacy and residue studies must be performed on this target category. The studies necessary for the evaluation of risks to human health or the environment will depend essentially on the nature of the additive and the circumstances of its use. In this respect, no strict rule is applicable. If necessary, additional information will be requested. Reasons must be given for the omission from the dossier of any data prescribed in these guidelines. Studies of mutagenicity, carcinogenicity and reproduction toxicity studies may be dispensed with if the chemical composition, practical experience, or other considerations can reasonably exclude these effects.

The studies have to be done and reported according to appropriate quality standards (e.g. Good Laboratory Practice (GLP) pursuant to Council Directive 87/18/EEC of 18 December 1986, on the harmonisation of laws, regulations and administrative provisions relating to the application of the principles of good laboratory practice and the verification of their applications for tests on chemical substances)¹.

The determination of physico-chemical, toxicological and eco-toxicological properties shall be performed in accordance with the methods established by Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances², as last amended by Commission Directive 93/105/EC of 25 of November 1993³, or with updated methods recognised by international scientific bodies. The use of other methods must be justified.

Each dossier shall contain an adequate summary. The dossiers relating to antibiotics, coccidiostats and other medicinal substances and to growth promoters, must be accompanied by a monograph, conforming to the model provided in Section V, enabling the additive concerned to be identified and characterised in accordance with Article 8 (1) of Directive 70/524/EEC.

For additives intended exclusively for pet food it may not always be necessary to subject additives to as exhaustive a program of chronic toxicity, mutagenicity, reproductive toxicity and carcinogenicity testing as that required for additives intended for feeding to livestock from which products for human consumption are derived. Residue studies in pet animals are not required.

Study of the metabolic fate of the additive, in food producing target animals and in laboratory species used for toxicity testing, is required in order to:

- a) ensure that there is adequate data on the toxicity of the parent additive and any metabolites produced in the target species to which the consumer might be exposed. To this end a comparison of the metabolic fates of the additive in the target and laboratory animal species used for the toxicity testing is important.
- b) identify and quantify the appropriate marker residue(s) to be used for setting the MRL for the marker residue and the withdrawal periods for the final product.

The Commission must be notified within a reasonable time by the Member State which has forwarded the dossier to it of any modification to the manufacturing process or the composition of an additive, its field of application or its conditions of use. This could necessitate the submission of documentation suitable for a new assessment. These requirements will be especially necessary for products derived from micro-organisms, the genetic characteristics of which have been modified or which arise as natural mutants. It is necessary for the applicant to continue to update the monograph once the authorisation is granted to take into account all new pertinent information to the additive.

¹ OJ N° L 15, 17.01.1987, p. 29

² OJ N° L 196, 16.08.1967, p. 1

³ OJ N° L 294, 30.11.1993, p. 21

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1. SECTION I - SUMMARY OF THE DATA IN THE DOSSIER

The summary must follow the order of the guidelines and address all the different parts with reference to the relevant pages of the dossier. It should contain a proposal covering all the conditions for the authorisation sought.

2. SECTION II - IDENTITY, CHARACTERISATION AND CONDITIONS OF USE OF THE ADDITIVE - METHODS OF CONTROL

2.1. Identity of the additive

2.1.1. Proposed proprietary name(s)

2.1.2. Type of additive according to its main function. When possible, evidence of mode(s) of action should be included. Any other uses of the active substance should be specified.

2.1.3. Qualitative and quantitative composition (active substance, other components, impurities, batch to batch variation). If the active substance is a mixture of active components, each of which is clearly definable, the main components must be described separately and the proportions in the mixture given.

2.1.4. Physical state, particle size distribution, particle shape, density, bulk density; for liquids: viscosity, surface tension

2.1.5. Manufacturing process including any specific processing procedures

2.2. Characterisation of the active substance(s):

2.2.1. Generic name, chemical name according to IUPAC nomenclature, other generic international names and abbreviations. Chemical Abstracts Service Number (CAS).

2.2.2. Structural formula, molecular formula and molecular weight.

For active substances being a fermentation product: microbial origin (name and place of culture collection recognised as an International Depository Authority, preferably in the European Union, where the strain is deposited and accession number and all relevant morphological, physiological, genetic and molecular characteristics for its identification). For genetically modified strains, information on the genetic modification must be provided.

2.2.3. Purity

Identification and quantification of occurring chemical and microbial impurities and toxic substances, confirmation of the absence of production organisms.

2.2.4. Relevant properties

Physical properties of the chemically specified substances: dissociation constant, pKa, electrostatic properties, melting point, boiling point, density, vapour pressure, solubility in water and organic solvents, Kow and Koc, mass and absorption spectra, NMR data, possible isomers and any other appropriate physical properties.

2.2.5. Manufacturing, purification processes, media used and, for fermentation products, batch to batch variation

2.3. Characterisation of the additive: Physico-chemical and technological properties

2.3.1. Stability of each formulation of the additive on exposure to environmental conditions such as light, temperature, pH, moisture and oxygen. Expected shelf life of the additive as marketed.

2.3.2. Stability of each formulation of the additive during the preparation and storage of premixtures and feedingstuffs, in particular stability to anticipated process/storage conditions (heat, moisture, pressure/shear and time). Possible degradation or decomposition products. Expected shelf life of the additive.

2.3.3. Other appropriate physico-chemical or technological properties in order to obtain and keep homogeneous mixtures in premixtures and feedingstuffs, antidusting and electrostatic properties, dispersability in liquids

2.3.4. Incompatibilities or interactions that could be expected with feedingstuffs, carriers, other approved additives or with medicinal products.

2.4. Conditions of use of the additive

2.4.1. Where an additive has significant technological as well as zootechnical effects it has to meet the requirements of both claims. The claims for each additive have to be identified and justified.

2.4.2. Proposed technological use in animal feedingstuff manufacture or if appropriate in raw materials.

2.4.3. Proposed mode of use in animal nutrition (e.g. animal species or categories and age group/production stage of animal, type of feedingstuff, and contra-indications).

2.4.4. Proposed method and level of inclusion in premixtures and feedingstuffs or raw materials if appropriate expressed as proportion of the additive and chemically specified substances by weight for premixtures, for feedingstuffs or raw materials if appropriate, with proposed duration of administration and withdrawal period.

- 2.4.5. Data from other known uses of the active substance (e.g. in foodstuffs, human or veterinary medicine, agriculture and industry) must be provided where this can aid safety assessment.
- 2.4.6. Proposed material safety data sheet as foreseen by Directive 91/155/EEC⁴ defining and laying down the detailed arrangements for the system of specific information relating to dangerous preparations in implementation of Article 10 of directive 88/379/EEC as amended by Directive 93/112/EEC⁵, and if necessary, proposed measures for the prevention of occupational risks and means of protection during manufacture, handling, use and disposal.

2.5. Control methods

- 2.5.1. Description of the methods used for the determination of the criteria listed under items 2.1.3., 2.1.4., 2.2.3., 2.2.4., 2.3.1., 2.3.2., 2.3.3. and 2.3.4.
- 2.5.2. Description of the qualitative and quantitative analytical methods for routine control of the active substance in premixtures and feedingstuffs.
- 2.5.3. Description of the qualitative and quantitative analytical methods for determining the marker residue(s)⁶ of the active substance in target tissues and animal produce.
- 2.5.4. The methods mentioned under 2.5.2 and 2.5.3 should be accompanied by information as to the sampling method used, percentage recovery, specificity, accuracy, precision, limits of detection, limits of quantification and validation procedure used. Reference standards of the active substance and/or of the marker residue(s) must be available. When devising methods, consideration must be given to the fact that their limits of quantification must be below the MRLs. Moreover, their suitability for routine analysis must be taken into account.

⁴ OJ N° L76, 22.03.1991, p. 35

⁵ OJ N° L314, 16.12.1993, p. 38

⁶ The marker residue is a residue whose concentration is in known relationship with the rate at which the concentration of the total residue in the target tissue depletes to the MRL

3. SECTION III - STUDIES CONCERNING THE EFFICACY OF THE ADDITIVE

3.1. Studies on the characteristics of feedingstuffs

These studies concern technological additives such as antioxidants, preservatives, binders, emulsifiers, stabilisers, gelling agents, pH modifiers etc., which are intended to improve or stabilise the characteristics of premixtures and feedingstuffs but have no direct biological effect on animal production.

Evidence of the efficacy of the additive must be provided, by means of appropriate criteria as reflected in recognised acceptable methods, under the intended conditions of use in comparison with appropriate control feedingstuffs. These investigations must be designed and performed so as to permit statistical evaluation.

Full information on the active substances, preparations, premixes and feedingstuffs examined, the reference number of the batches, the detailed treatment and testing conditions should be provided. Positive and negative effects, both technological and biological, should be described for each experiment.

3.2. Studies on the effects on animals

Studies on zootechnical additives must be performed in target species/animal categories for which the additive is intended in comparison with negative control groups and, possibly, with groups receiving feedingstuffs containing EU approved additives used at their recommended dosage (positive control).

Animals used should be healthy and preferably from a homogeneous group.

Studies must permit the evaluation of the efficacy of the additive according to farming practice in the European Union. Similar protocol designs should where possible be used for all trials so that data can eventually be tested for homogeneity and pooled (if tests so indicate) for statistical evaluation.

No single design is recommended, flexibility being provided to allow for scientific discretion in the design and conduct of the studies. The experimental design used must be justified according to the claim for the use of the additive and must include consideration of adequate statistical power.

3.2.1. For coccidiostats and other medicinal substances:

Importance should primarily be attached to evidence of the specific effects (e.g. species controlled, life-cycle stage(s) affected) and particularly to prophylactic properties (e.g. morbidity, mortality, oocyst count and lesion score).

Information on the effect on feed efficiency and liveweight gain should be provided.

The required efficacy data involve three stages of target animal experimentation:

- (a) controlled battery-cage experiments (single and mixed infections);
- (b) controlled floor pen studies (simulated use conditions);
- (c) controlled field trials (actual use conditions).

Simultaneously and when relevant, within trials on efficacy, additional data should be recorded to allow an evaluation of interference with growth and feed conversion (fattening birds, replacement layers and rabbits), effects on egg fertility and hatchability (breeding birds), and effects on egg production and egg quality (laying hens).

3.2.2. For other zootechnical additives:

Information should be provided on the effects on feed intake, liveweight gain, feed efficiency (preferably on a dry matter basis), product quality and yield and any other parameter of benefit to the animal, the environment, the producer or the consumer. The studies should include an indication of dose/response relationship where appropriate.

3.2.3. Experimental conditions

Trials should be reported individually, giving details of the controls and each experimental treatment. The trial protocol should be carefully drawn up with regard to general descriptive data as follows:

3.2.3.1. Herd: location and size; feeding and rearing conditions; for aquatic species, size and number of tanks or pens at the farm and water quality.

3.2.3.2. Animals: species (for aquatic species intended for human consumption identification shall be made by their colloquial name followed in parenthesis by the latin or Linnean description), breed, age, sex, identification procedure, physiological stage and general health.

3.2.3.3. Number of test and control groups, number of animals in each group. The number of animals involved in the trials must permit statistical analysis. The methods of statistical evaluation used should be stated. The report should include all animals or experimental units involved in the trials. Cases which cannot be assessed due to a lack or loss of data should be reported and their distribution within the groups of animals classified.

3.2.3.4. Diets: description of manufacture and quantitative composition of the diet(s) in terms of ingredients used, relevant nutrients (analysed values) and energy. Feed intake records.

- 3.2.3.5. Concentration of the active substance (and, where that is the case, substances used for comparative purposes) in the feedingstuffs should be established by a control analysis, using the appropriate recognised method. Reference number(s) of the batches.
- 3.2.3.6. Date and exact duration of testing. Date and nature of the examinations performed.
- 3.2.3.7. Dose determination studies: the purpose of these studies is to explain the rationale for the selection of a dose or dose range claimed to be optimally effective. Dose determination will be based on a control (untreated) and at least three non-zero levels in target animals.
- 3.2.3.8. The timing and prevalence of any undesirable consequences of treatment in individuals or groups must be reported (give details of the observation programme used in the study).
- 3.2.3.9. All additives studied under farm conditions must have good scientific evidence of safety for the user, consumer, animal and the environment. Where an additive does not meet the requirements for consumer safety any study undertaken should be designed to prevent animal products derived from the test animals from entering the human food chain.

3.3. Studies on the quality of animal produce

Animal products should be examined for organoleptic, nutritional, hygienic and technological qualities as appropriate.

3.4. Studies on the effects on the characteristics of animal wastes.

If the additive is intended to modify some characteristics of animal waste (e.g. nitrogen, phosphorus, odour, volume), then studies demonstrating these properties are required.

4. SECTION IV - STUDIES CONCERNING THE SAFETY OF USE OF THE ADDITIVE

The studies outlined in this section are intended to permit assessment of:

- the safety of use of the additive in the target species;
- any risk associated with the selection and/or transfer of resistance to antibiotics and increased persistence and shedding of enteropathogens;
- the risks to the consumer which could result from the consumption of food containing residues of the additive or its metabolites;
- the risks from respiratory, other mucosal, eye or cutaneous contact for persons likely to handle the additive as such or as incorporated into premixtures or feedingstuffs;
- the risks of adverse effects on the environment, from the additive itself or by products derived from the additive and excreted by animals;

Consideration should be given to known incompatibilities and/or interactions between the additive and veterinary medicines and/or components of the diet relevant to the species concerned.

These studies will normally be required in their entirety for each additive unless a specific exclusion or modification is specified in the directive.

A more limited submission will normally be accepted for a proposed extension of the authorised use to a minor species which is physiologically and metabolically proximate to one in which the use of the additive has already been approved. This reduced data set should demonstrate safety to the new species and lack of significant differences in metabolic fate and residues in edible tissues. The proposed MRL and withdrawal period for the minor species must be justified.

In order to assess the risks for the consumer and consequently the determination of the MRLs and the withdrawal period:

- the chemical structure of the active substance;
- the metabolism in the proposed target species;
- the nature of the residues in these target species;
- tissue depletion study of residues;
- data on the biological effects of the active substance together with its metabolites.

A knowledge of the bioavailability of the residues (both unbound and bound) may also be useful namely when many metabolites are produced and no marker residues is evidenced (see section 4.1.3.3.).

Furthermore, knowledge of the composition and of the physico-chemical and biological properties of the major excreted materials deriving from the additive are

required to define the extent of the studies necessary for assessment of the risk of adverse effects on the environment or persistence in the environment.(see paragraph 4.5.)

4.1. Studies on target species

4.1.1. Tolerance tests on target species/animal categories

The aim is to determine a safety margin (i.e. margin between the maximum proposed dose-level in feedingstuffs and the minimum level resulting in unfavourable effects). However a safety margin by a factor of at least ten is considered sufficient to require no further testing. Such a tolerance test must be conducted in the target species/animal categories preferably through the entire length of the production period although a test period of one month would normally be acceptable. This requires at least the assessment of clinical signs and other parameters to ascertain effects on target animal health. Depending on the toxicological profile additional parameters may also be required. Any adverse effects detected during efficacy trials should also be reported in this section.

Whenever the product is intended for use in animals which may be used for breeding, studies should be conducted to identify possible impairment of male or female general reproductive function or harmful effects on progeny resulting from the administration of the additive under investigation.

4.1.2. Microbiological safety of the additive.

4.1.2.1. All studies should be performed with the highest proposed dose level

4.1.2.2. If the active substance possesses antimicrobial activity at feed concentration level, the Minimum Inhibitory Concentration (MIC) should be determined in appropriate pathogenic and non-pathogenic, endogenous and exogenous bacteria, according to standardised procedures.

4.1.2.3. Tests to determine the ability of the additive to

- induce cross-resistance to relevant antibiotics
- select resistant bacterial strains under field conditions in the target species and, if so, investigations on the genetic mechanisms for transfer of the resistance genes.

4.1.2.4. Tests to determine the effect of the additive

- on a number of opportunistic pathogens present in the digestive tract (e.g. Enterobacteriaceae, Enterococci and Clostridia),
- on the shedding or excretion of relevant zoonotic micro-organisms, e.g. Salmonella spp, Campylobacter spp,

4.1.2.5. In cases where the active substance shows an antimicrobial action, field studies to monitor for bacterial resistance to the additive should be provided.

4.1.3. Metabolism and residue studies

4.1.3.1. The aim of the studies is:

- to establish the metabolic pathways of the active substance as a base for its toxicological evaluation;
- to identify residues and establish their kinetics in the tissues and products (milk, eggs);
- to identify the excreted substances as a pre-requisite for assessing their impact on the environment.

Occasionally, such as in the case of fermentation-derived additives, it could be necessary to extend these studies to other substances added to or derived during the fermentation process. Possession of a toxicity significant in relation to that of the active component(s) of the additive would exemplify this circumstance.

4.1.3.2. Metabolic fate

The planning and experimental design of the studies must take into account the anatomical, physiological (age, type, sex), zootechnical category and environmental peculiarities of the target population. When appropriate, the influence of the intestinal or ruminal microflora, enterohepatic circulation or caecotrophy must be considered. The dose regimen tested must be that intended for use, and possibly a multiple of that dose if justified. The active substance (including the labelled substance) must be incorporated into the feed unless there is justification for not doing so.

The studies required are the following:

- metabolic balance and kinetics in the plasma/blood following a single dose administration in order to assess the rate and extent of the absorption, distribution and excretion (urine, faeces, gills, bile, expired air, milk or eggs)
- identification of the major (>10%) metabolites in the excreta; except if a minor (<10%) metabolite would appear to be of toxicological concern,
- distribution of labelled material in tissues and products following a single dose administration to animals already in steady state equilibrium achieved with unlabelled additive.

The studies mentioned under 4.1.3.1. and 4.1.3.2. should include isotope tracer or alternative relevant methods.

4.1.3.3. Study of the residues

- identification of those residues (parent compound, metabolites, degradation products, bound residues⁷) which represent more than 10% of the total residue (except if a minor metabolite would appear to be of toxicological concern) in the tissues and products (milk, eggs) at metabolic equilibrium, i.e. following multi-dose administration of the labelled substance;
- kinetic study of the residues in the tissues (including milk and eggs when appropriate) during the depletion period following achievement of steady-state and using the highest level proposed metabolic profiling, identification of the target tissue⁸ and of the marker residue;
- depletion study of the marker residue from the target tissues (including milk and eggs when appropriate) after withdrawal of the additive following its repeated administration according to the proposed conditions of use and sufficient to have reached steady state, in order to set a withdrawal period on the basis of the fixed MRL.
- the withdrawal period for the additive must not be less than the time necessary for the concentration of the marker residue determined in the target tissue to fall below the MRL value (95% confidence limit). Spaced time points, suitably chosen by reference to the depletion phase of the active substance and its metabolites, and at least four animals per point depending on the species (size, genetic variability) should be considered as a minimum requirement⁹.

4.2. Studies on laboratory animals

These studies must be carried out with the active substance using internationally recognised standard test methods as described in Directive 67/548/EEC, and according to the principles of Good Laboratory Practice (GLP). Additional studies on particular metabolites produced by the target species may be necessary if these are not formed to a significant extent in the laboratory test species. Also where there is data in man this may need to be taken into consideration in deciding what additional studies to conduct.

⁷ Bound residues correspond to the tissue residual fraction that is not extractable using physico-chemical or biological means. They derive from the covalent binding of a metabolite of the compound with cellular macromolecules

⁸ The target tissue is the edible tissue selected to monitor for the total residue in the target animal. It is usually, but not necessarily, the last tissue in which residues deplete below the MRL

⁹ For the determination of a withdrawal period, the suggested minimum numbers of healthy animals sampled at each slaughter or time point are the following:

- Lactating cattle, 8, including animals at second or subsequent lactations (4 high yielding cattle at an early stage of lactation and 4 low yielding cattle at a late stage of lactation);
- Other large animals, 4 per sampling time;
- Poultry, 6 per sampling time;
- Laying birds, 10 eggs per time point;
- Fish, 10 per sampling time.

4.2.1. Acute toxicity

Acute oral toxicity studies should be carried in at least two mammalian species. One laboratory species may be replaced, if appropriate, by a target species. It will not be necessary to identify a precise LD50; an approximate determination of the minimum lethal dose is normally adequate. In order to reduce the number and suffering of the animals involved, the maximum dosage should not exceed 2,000 mg/kg b.w. and alternative methods (limit test, fixed dose method, acute toxicity class method) are recommended.

Studies on acute inhalation toxicity, skin and, where necessary, mucous membranes irritancy and also allergenic potential must be performed by appropriate tests for the assessment of possible workers' risks.

4.2.2. Genotoxicity studies including mutagenicity

In order to identify active substances and, if appropriate, their metabolites and degradation products with mutagenic and genotoxic properties, a selected combination of at least 3 different genotoxicity tests must be carried out. The test battery should normally include prokaryotic and eukaryotic systems tests including mammalian in vitro and in vivo tests systems. If appropriate the tests should be performed without and with mammalian metabolic activation.

Reasons for the choice of the tests with regard to their reliability to assess genotoxic effects on different genetic endpoints at the gene, chromosome and genome level should be given. Additional tests may be indicated depending on the outcome of the tests and taking into consideration the whole toxicity profile of the substance as well as the intended use. Tests must be carried out according to established and up to date validated procedures. When the test target is bone marrow, proof of exposure of the cells to the test substance is required in the case of a negative result.

4.2.3. Subchronic (90-day) oral toxicity studies

The duration of the tests must be at least 90 days. For additives intended for use in food producing animal species the studies should be carried out on two animal species, of which one should be a non-rodent species which may be the target species. For additives intended for use in animals not for human consumption the studies on the target species are sufficient: the active substance must be administered orally at least at three levels in addition to a control group to obtain a dose response.

The maximum dose should normally reveal evidence of harmful effects. The lowest dose level should not produce any evidence of toxicity.

4.2.4. Chronic oral toxicity studies (including carcinogenicity studies)

A chronic toxicity study, which may include examination of carcinogenicity must be carried out in at least one rodent species.

Carcinogenicity studies may not be necessary if the active substance and its metabolites:

- give consistently negative results in an appropriate range of genotoxicity tests,
- are not structurally related to known carcinogens, and
- give no effects indicative of potential (pre)neoplasia in (sub)chronic toxicity assays

4.2.5. Reproduction toxicity studies including teratogenicity

4.2.5.1. Two generation reproduction toxicity study

Studies of reproductive function must be carried out and extend over at least two filial generations (F1, F2) and may be combined with a teratogenicity study. The substance under investigation shall be administered to males and females at an appropriate time prior to mating. Administration should continue until the weaning of the F2 generation.

All relevant fertility, gestation, parturition, maternal behaviour, suckling, growth and development of the F1 offspring from fertilisation to maturity and the development of the F2 offspring to weaning must be carefully observed and reported.

4.2.5.2. Teratogenicity study

The teratogenicity study covers embryo and foetotoxicity. It must be carried out in at least two species.

4.2.6. Metabolism and disposition studies

Studies on absorption, distribution in the body fluids and tissues, excretion routes, must be performed. A metabolic study including the metabolic balance and identification of the main metabolites in the urine and faeces should be performed in animals of both sexes and the same strains as those used in the toxicological studies. A single dose of the labelled molecule (see 4.1.3) should be administered at steady state equilibrium reached using the unlabelled compound at a dose similar to the highest level proposed for use in the target animal.

4.2.7. Bioavailability of residues

The assessment of the risk to consumers related to certain residues contained in animal produce, namely bound residues, may take into account an additional safety factor based on the determination of their bioavailability using appropriate laboratory animals and recognised methods.

4.2.8. Other specific toxicological and pharmacological studies.

Further studies providing additional information useful for the assessment of the safety of the active substance and its residues should be conducted if there is any reason for concern.

4.2.9. Determination of a No-Observed- Effect Level (NOEL).

All the above findings together with all the relevant published data (including any suitable information on effects of the active substance in human) and information, where appropriate, on closely related chemical structures should be taken into consideration in identifying a NOEL expressed as mg/kg body weight per day.

However the NOEL to be used for the calculation of the ADI should be selected on the basis of toxicological or pharmacological effects as appropriate. For some additives, e.g. antibacterials, an ADI may be better established on the basis of effects on the human gut microflora. In the absence of internationally accepted and validated methods for describing gut flora, effects on selected and sensitive human gut bacterial strains may be more appropriate.

4.3. Safety evaluation for the human consumer

4.3.1. Proposal of the ADI for the additive

An ADI should where appropriate be proposed.

The ADI (expressed as mg of additive or additive related material per person per day) is derived by dividing the NOEL (No - Observed- Effect Level) by an appropriate safety factor and multiplying by a mean human body weight (b.w.) of 60 kg. This NOEL expressed as mg per kg b.w. per day may be selected using toxicological or pharmacological findings. In some cases an ADI based on the additives microbiological properties may be more relevant. The choice will depend on which property is most relevant in terms of health hazard to the consumer.

The safety factor used to determine the ADI for a particular additive should be selected, bearing in mind the following :

- the nature of the biological effect used to identify the NOEL
- the relevance of this effect to man and the reversibility of the effect
- the range and quality of the data used to identify the NOEL
- any knowledge of the effect(s) of the residue constituents.

It is customary to employ a safety factor of at least 100 in calculating the ADI (i.e. a factor of 10 to allow for potential interspecies variation and a further factor of 10 to allow for possible differences in response between individual humans). When data on the active substance are available for human-beings a lower safety factor may be acceptable.

4.3.2. Proposal of the MRL's of the additive

It is assumed in calculating the MRL that the intake of meat, milk and egg products is the sole source of potential human exposure. If this is not the case an allowance must be made for other sources.

A number of these substances have been used as feed additives and for other applications. In such cases the calculated MRL's would be expected to be the same. There may also be instances where on strict scientific considerations different MRL's are calculated for each use when the route, amount, dosage frequency and duration of dosing, are sufficiently different from those appropriate to use as feed additive, that there are evidences indicating that the kinetics and/or metabolism may result in different residue profiles. In such circumstances it is anticipated that the strictest MRL will be applied.

To establish an MRL the chemical nature of the drug-related material which is intended to be used to specify the tissue residue levels must be defined. This is termed the marker residue. This residue constituent must not necessarily be the toxicologically relevant residue but has to be chosen as a suitable indicator to represent the total significant residue. A suitable analytical method for this marker residue must also be available to ensure compliance with the MRL.

In establishing MRLs (expressed as µg/kg of marker residue per kg of edible wet tissue or product) on the basis of an ADI, the following daily human food consumption values should be applied :

	mammals	birds	fish
muscle	300 g	300 g	300 g *
liver	100 g	100 g	
kidney	50 g	10 g	
fat	50 g **	90 g***	
milk	1500 g		
egg	100 g		

* muscle and skin in natural proportions

** for pigs 50 g of fat and skin in natural proportions

*** fat and skin in natural proportions

The individual MRLs in different tissues should reflect the depletion kinetics of the residues within those tissues in the animal species intended for use. An analytical method with a limit of quantitative determination below the MRL is required (see section II point 2.5.3.).

If a substance could result in a residue in tissues and produce, the MRLs should be proposed in such a way that the total amount of toxicologically (or microbiologically) significant residue ingested ¹⁰ daily should be lower than the ADI (see table above).

The MRL should be set only after consideration and inclusion of any other potential sources of exposures of the consumer to components of the residues.

¹⁰ Proposed calculation: **[500 g meat** (consisting of 300 g muscle, 100 g liver, 50 g kidney, 50 g fat) **or 500 g poultry** (consisting of 300 g muscle, 100 g liver, 10 g kidney, 90 g fat) **or 300 g fish]** + **1500 g milk + 100 g egg.**

For certain additives residues could arise below the MRL values in milk, eggs or meat which could nonetheless interfere with food quality in particular food processing procedures, e.g.: use of milk in cheese making. For such additives, it may be appropriate to consider a "maximum (food product) processing compatible residue" in addition to establishing MRL values.

There are some circumstances where a MRL will not be required such as:

- no bioavailability of the residues and no harmful effect on the human gut including its microflora;
- complete degradation to nutrients or harmless substances in the target species;
- ADI "not specified" because of low toxicity in animal tests;
- where use is restricted entirely to feed for pet animals;
- where a substance is also used as a food additive¹¹, a MRL is normally not required if the marker residue is primarily the parent substance and it constitutes only an insignificant fraction of the ADI of the food additive.

4.3.3. Proposal of the withdrawal period for the additive

The withdrawal period will be set on the basis of the MRLs. The withdrawal time comprises the period after cessation of the administration of the proposed formulation of the additive which is necessary to enable the residue levels to fall below the MRLs (95% confidence limit).

To establish a withdrawal period a particular edible tissue may be identified as a surrogate for others, often termed the target tissue.

4.4. Worker safety assessment

The risk to workers from exposure to an additive requires assessment. The main routes of concern are inhalation and topical exposure. Farm workers are potentially exposed when handling or mixing the feed additives.

Experience in the manufacturing plant is often an important source of information in evaluating the risks to workers from exposure to the additive itself by both airborne and topical routes. Of particular concern are additives/additive-treated feed and/or animal excreta which are in, or may give rise to, a dry powdery form and feed additives which may have allergenic potential.

¹¹ According to Council Directive 89/107/EEC of 21 December 1988 on the approximation of the laws of the Member States concerning food additives for use as foodstuffs intended for human consumption (O.J. L 40 of 11.02.1989, p27)

4.4.1. Toxicological risk assessment for worker safety

4.4.1.1. Effects on the respiratory system

Evidence should be provided that airborne levels of dust will not constitute a hazard to the health of workers. This evidence should include where possible: inhalation tests in laboratory animals, published epidemiological data and /or the applicants own data on its workplant and/or irritancy and respiratory system sensitisation tests.

4.4.1.2. Effects on the eyes and skin

Where available, direct evidence of absence of irritancy and/or sensitisation should be provided from known human situations. This should be supplemented by findings from validated animal tests for skin and eye irritation, and for sensitisation potential using the appropriate additive.

4.4.1.3. Systemic toxicity

The toxicity data generated to meet safety requirements (including repeated dose toxicity, mutagenicity, carcinogenicity and reproductive testing) should be used to assess other aspects of worker safety. In doing so, it needs to be remembered that contamination of skin and/or inhalation of the additive are the most likely routes of exposure.

4.4.2. Control measures

If there is reasonable risk of inhalatory/systemic toxicity, irritancy or sensitisation, precautionary measures should be introduced. Product reformulation or modification of the procedures for production, use and/or disposal of the additive are preferred solutions. Use of personal protective devices alone should be regarded as a measure of last resort.

4.5. Environmental risk assessment

Consideration of the environmental impact of feed additives is important since the administration of feed additives is typically over a long period (even for lifetime), large groups of animals may be involved and many additives are poorly absorbed and therefore excreted intact to a considerable extent. Nonetheless, in a number of cases, the need for environmental assessment may be limited. It is inappropriate to set strict rules in this general guideline. To assist in determining the environmental impact of a feed additive, a stepwise approach should be followed, where in the first phase, additives which do not need further testing can be clearly identified. For other additives a second phase of studies (phase II A) are needed to provide additional information, based upon which further studies (Phase II B) may be considered necessary. Studies, when applicable, should be conducted according to Council Directive 67/548/EEC.

4.5.1. Phase I assessment

The purpose of Phase I assessment is to determine whether or not a significant environmental effect of an additive or its metabolites is likely, based largely on data already established for other purposes.

Exemption from Phase II assessment may be made on one of two criteria:

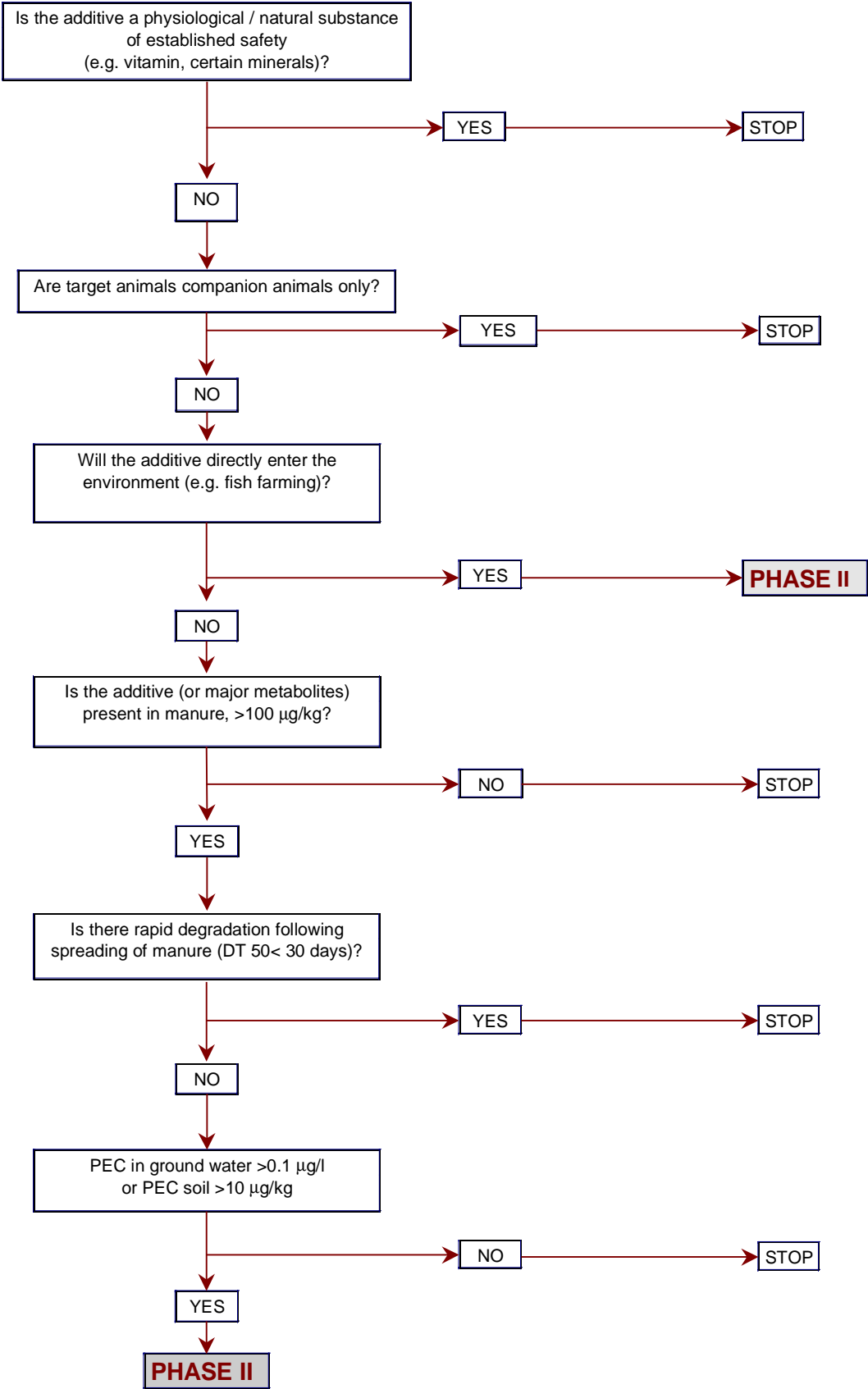
- a) The chemical nature and the biological effect of the additive and its use indicates that impact will be negligible: i.e. where the additive and/or its main (more than 20% of the total residues in the excreta) metabolite(s) are:
 - physiological substances (e.g.: a vitamin, or a mineral unless there is evident reason for concern (e.g. : copper),
 - additives intended for companion animals (excluding horses).
- b) The worst case Predicted Environmental Concentration (PEC) is too low to be of concern

The worst case PEC for soil is likely to arise as a result of manure generated during maximum excretion of the major residue constituents (the additive and/or its major metabolites), being spread on land. The PEC should be evaluated for each major residue constituent in manure and for each compartment of concern. For the terrestrial compartment, if the PEC does not exceed 100 µg/kg for the sum of the major residue constituents in manure, or if the major residue constituents in manure are readily degraded (Degradation Time DT 50 < 30 days) to natural constituents or to concentrations of less than 100 µg/kg, or if PEC in soil is less than 10µg/kg, then no further assessment is required.

The worst case PEC for water may arise either from direct transfer of spilled feed or excreta containing the additive and its metabolites into water bodies or from the leaching of material in excreta or soil into ground water. If the PEC for contamination of water bodies or ground water is reliably estimated to be less than 0.1 µg per litre no Phase II A assessment of the environmental impact of the additive on the aqueous compartment is likely to be necessary.

If the applicant cannot demonstrate that the proposed additive falls into any of these exemption categories or when the additive is directly released in the environment (e.g. aquaculture), Phase II assessment will normally be required.

ENVIRONMENTAL RISK FROM FEED ADDITIVES - DECISION TREE PHASE I



4.5.2. Phase II assessment

Phase II assessment is in two parts - Phase II A and Phase II B.

The bioaccumulation potential of the additive and/or its main metabolites and its influence on the predicted safety margin should be assessed. Bioaccumulation is not to be estimated to be potentially significant if e.g.: K_{ow} (partition coefficient) is <3 . Appropriate Phase IIB studies will generally be needed if such safety margins cannot be established.

4.5.2.1. Phase IIA

The purpose of Phase IIA assessment is to identify risk to the environment by :

- refining the calculation of the PEC(s);
- determining the relationship between exposure, the levels of additive and/or major metabolites and short term adverse effects in relevant surrogate animal and plant species for the environmental compartment(s) of concern;
- using these findings to determine the Predict a No Effect Concentration(s) (PNEC) value(s).

The following sequential procedure is recommended to determine the risk:

- (a) If not already completed in Phase I, a more refined PEC should be calculated for each environmental compartment of concern. In ascertaining the PEC account should be taken of:
 - the concentration of additive and/or its major metabolites in manure following administration of the additive to animals at the proposed dose level. This calculation should include consideration of excreta volumes and dosage rates;
 - the potential dilution of the excreted additive related material due to normal manure processing practice and storage prior to its application to land;
 - the adsorption/desorption of the additive and its metabolites onto soil, the persistence of residues in soil (DT_{50} and DT_{90});
 - other factors such as photolysis, hydrolysis, evaporation, degradation, dilution through ploughing etc.

The highest value for the PEC obtained by these calculations for each environmental compartment of concern should be adopted for level IIA risk assessment purposes.

If a high persistence in soil ($DT_{90} > 1$ year) at concentrations in excess of $10 \mu\text{g}/\text{kg}$ soil is anticipated at steady state, a level IIB assessment may be needed.

- (b) The levels producing serious short-term adverse effects for various trophic levels in the environmental compartments of concern (soil, water and air) must next be determined. These tests should follow OECD¹² or similar well-established guidelines. Suitable tests for the terrestrial environment include: toxicity to earthworms (50 % Lethal Concentration, LC₅₀ value), phytotoxicity (50 % Effective Concentration, EC₅₀ value) in terrestrial plants, effects on soil micro-organisms (e.g.: EC₅₀ for effects on methanogenesis and nitrogen fixation). For the aquatic environment: fish a 96 hour LC₅₀ study; *Daphnia magna* a 48 hour EC₅₀ study and algae an LC₅₀ study.
- (c) Calculation of the PNEC value for each compartment of concern should be carried out. This is derived normally by taking the lowest value observed (i.e.: the result in the most sensitive species) for an adverse effect in the above ecotoxicity tests and dividing by a safety factor of at least 100 depending on the indicator.
- (d) The calculated PEC and PNEC values should be compared. The acceptable ratio of the PEC to the PNEC value will depend on the nature of the test result used to determine the PNEC. Normally it will be between 1 and 0.1. If significantly lower ratios than these are identified, further ecotoxicological tests are unlikely to be necessary unless bioaccumulation is expected. Conversely, higher ratios will require some Phase IIB testing.

4.5.2.2. Phase IIB (more detailed toxicological studies)

For those additives where, following Phase II A assessment, doubt remains regarding their environmental impact, more detailed studies are required of the effects on biological species in the environmental compartment(s) in which phase II A studies indicate possible concern. In this situation, further tests are needed to determine the chronic and more specific effects on appropriate animal, plant and microbial species. It may be that in the Phase IIA assessment the PEC value has been over estimated. To demonstrate this it may be necessary to carry out measurements of the environmental concentrations and, of the persistence of the additive and/or its major metabolites in field use situations.

Suitable additional ecotoxicity tests are described in a number of publications, e.g.: in OECD guidelines. Three categories of environmental species may need to be considered, animals, plants and micro-organisms. Careful choice of such tests is necessary to ensure that they are appropriate to the situation in which the additive and/or its metabolites may be released and dispersed in the environment.

Where an adequate safety margin between the PEC and PNEC values cannot be established, identification of effective mitigating measures to limit the environmental impact must be provided.

The assessment of the impact on the terrestrial compartment may include:

¹² OECD Guidelines for Testing of Chemicals

A sublethal study of the effects on earthworms, further studies of the impact on soil microflora, phytotoxicity tests on a range of economically important plant species, studies on grassland invertebrates including insects and feral birds.

NB A separate evaluation of mammalian toxicity may not be necessary, as this aspect is likely to be addressed by mammalian toxicity testing conducted to determine the ADI.

The assessment of the impact on the aquatic compartment may include:

Chronic toxicity testing on the most sensitive aquatic organisms identified in the Phase IIA assessment, e.g.: the fish early life stage test, the Daphnia reproduction test and 72 hour algae tests.

5. SECTION V - FORM OF MONOGRAPH

The monograph is intended to provide a brief description of the product and the information necessary to establish its safety. Details of results and experimental methods described in the dossier are not required.

5.1. Identity of the additive

5.1.1. Proposed proprietary name(s)

5.1.2. Type of additive according to its main function. Any other uses of the active substance should be specified.

5.1.3. Qualitative and quantitative composition (active substance, other components, impurities, batch to batch variation). If the active substance is a mixture of active components, each of which is clearly definable, the main components must be described separately and the proportions in the mixture given.

5.1.4. Physical state, particle size distribution, particle shape, density, bulk density; for liquids : viscosity, surface tension

5.1.5. Manufacturing process including any specific processing procedures

5.2. Specifications concerning the active substance

5.2.1. Generic name, chemical name according to IUPAC nomenclature, other generic international names and abbreviations. Chemical Abstracts Service Number (CAS).

5.2.2. Structural formula, molecular formula and molecular weight. Qualitative and quantitative composition of the main components, if the active substance is a fermentation product.

5.2.3. Purity

Qualitative and quantitative composition of the active substances and occurring accompanying impurities, confirmation of the absence of living cells and toxic substances.

5.2.4. Relevant properties

Physical properties of the chemically specified substances: dissociation constant, pKa, electrostatic properties, melting point, boiling point, density, vapour pressure, solubility in water and organic solvents, K_{ow} and K_{oc} , mass and absorption spectra, NMR data, possible isomers and any other appropriate physical properties.

5.3. Physico-chemical, technological and biological properties of the additive.

5.3.1. Stability of the additive on exposure to environmental conditions such as light, temperature, pH, moisture and oxygen. Proposal of a shelf life.

- 5.3.2. Stability during the preparation of premixtures and feedingstuffs, in particular stability to anticipated process conditions (heat, moisture, pressure/shear and time). Possible degradation or decomposition products.
 - 5.3.3. Stability during the storage of premixtures and processed feedingstuffs under defined conditions. Proposal of a shelf life.
 - 5.3.4. Other appropriate physico-chemical, technological or biological properties such as dispersability under favourable conditions in order to obtain and keep homogeneous mixtures in premixtures and feedingstuffs, antidusting and antistatic properties, dispersability in liquids.
- 5.4. Control methods
- 5.4.1. Description of the methods used for the determination of the criteria listed under items 2.1.3., 2.1.4., 2.2.3., 2.2.4., 2.3.1., 2.3.2., 2.3.3., and 2.3.4.
 - 5.4.2. Description of the qualitative and quantitative analytical methods for determining the marker residue of the active substance in target tissues and animal produce.
 - 5.4.3. If the said methods have been published the literature references may suffice and the corresponding reprints should be given.
- 5.5. Biological properties of the additive
- 5.5.1. Particulars of the prophylactic effects for coccidiostats and other medicinal substances (e.g. morbidity, mortality, oocyst count and lesion score).
 - 5.5.2. For zootechnical additives other than those listed in 5.5.1. particulars of the effects on feed intake, liveweight gain, feed efficiency, animal growth, product composition and yield, body condition where appropriate, animal welfare and other parameters having a positive (or when appropriate negative) influence on animal production.
 - 5.5.3. For technological additives, relevant technological effects
 - 5.5.4. Any adverse effects, contra-indications or warnings, including biological interactions, with particulars of their justification. Any ADI or MRLs established for other uses of the active substance should be specified.
- 5.6. Details of the quantitative and qualitative residues in target tissues, if any, found in animal produce following envisaged use of the additive.
- 5.7. Other characteristics suitable for identification of the additive.

6. SECTION VI - FORM OF IDENTIFICATION NOTE

1. Identity of the additive
 - 1.1 Type of additive
 - 1.2 Physical state
 - 1.3 Qualitative and quantitative composition
 - 1.4 Community registration number (EC number)
 - 1.5 Packaging

2. Specifications concerning the active substance
 - 2.1 Generic name, chemical name, CAS Number
 - Generic name
 - Chemical name (IUPAC)
 - CAS Number
 - 2.2 Empirical formula

3. Physico-chemical, technological and biological properties of the additive
 - 3.1 Stability of additive
 - 3.2 Stability during the preparation of premixtures and feedingstuffs
 - 3.3 Stability during storage of premixtures and feedingstuffs
 - 3.4 Other properties

4. Conditions of use
 - 4.1 Species or category of animals, maximum age if specified
 - 4.2. Minimum and maximum content in feedingstuffs
 - 4.3. Contra-indications
 - 4.4. Warnings

5. Person responsible for putting into circulation

5.1 Name

5.2. Address

5.3. Registration number

6. Manufacturer

6.1. Name

6.2. Address

6.3. Approval number or registration number assigned to the establishment or the intermediary.

7. SECTION VII - RENEWAL OF AUTHORISATION OF ADDITIVES SUBMITTED TO A BRAND-SPECIFIC APPROVAL

1. General

An updated dossier and monograph should be prepared according to the most up to date guidelines and a list provided of all variations of any type since the authorisation for putting into circulation or the last renewal.

It has to be confirmed that the monograph and safety file has adapted to new information relevant to the additive and to changes in the guidelines.

Information should be provided on the authorisation status world-wide and sales volume (related to the number of animals fed the additive).

2. Identity of the active substance and of the additive

Evidence should be presented to show that the additive has not been changed or altered in composition, purity or activity in respect of the additive authorised, or where changes have been affected these should be justified. Any change of the manufacturing process should be reported.

3. Efficacy

Evidence should be presented to show that the additive retains the claimed efficacy under current conditions of animal production in the European Union. This may include general experience with the use of the additive and performance monitoring.

4. Microbiology

Special regard should be given to possible development of resistance to antimicrobials during the long-term use under practical conditions. The tests must thus be performed under field conditions in farms, where the additive has been routinely used for as long time as possible. A selection of common intestinal bacteria should be used as test organisms, and the selection should include relevant endogenous and exogenous Gram-positive as well as Gram-negative organisms.

If the tests show a change in the resistance pattern compared to the original figures, the resistant bacteria must be examined for cross-resistance to relevant antibiotics used for treatment of infectious diseases in man and animals. The most important are antibiotics belonging to the same group as the additive, but also other groups of antibiotics should be included in the trial.

Results of appropriate monitoring programmes should be reported.

5. Safety

Evidence should be presented that in the light of the current knowledge the additive remains safe under the approved conditions for target species, consumers, operators and the environment. A safety update for the period since the authorisation for putting into circulation or the last renewal with information on the following items should be presented:

- reports on adverse effects including accidents (previously unknown effects, severe effects of any type, increased incidence of known effects) for target animals, operators and the environment. The report on adverse effect should include the nature of the effect, number of affected individuals/organisms, outcome, conditions of use, causality assessment;
- reports on previously unknown interactions and cross-contaminations;
- data from residue monitoring where appropriate;
- any other information concerning the safety of the additive.

If no further information is provided on any of these factors, the reasons for this should be clearly identified.

8. SECTION VIII - NEW APPLICANT RELYING ON THE FIRST AUTHORISATION OF AN ADDITIVE ON EXPIRY OF THE 10-YEAR PERIOD OF DATA PROTECTION

An additive can be considered as identical for this purpose if the qualitative and quantitative composition and the purity of active and inactive components are essentially similar, the preparation is the same and the conditions of use are identical.

For such products it will normally not be necessary to repeat pharmacological, toxicological and efficacy studies and an abridged application can be submitted. This must include expert reports.

- Data have to be provided indicating that the specification range of the physical, chemical characteristics of the additive is essentially similar to that of the established product.
- It has to be confirmed that further scientific knowledge in the available literature on the additive has not changed the original assessment on efficacy and safety since the authorisation for putting into circulation of the original additive.
- In the case of additive with major similarities of composition, but which do not meet the strict requirements of essential similarity (e.g. different salts, free base or esters), bioequivalence (with respect to efficacy, safety and residue formation) with the established product has to be demonstrated by appropriate studies.
- The conformity of the withdrawal period with the MRL has to be established.