GUIDELINES FOR THE ASSESSMENT OF ADDITIVES IN FEEDINGSTUFFS

Amendments proposed to modify the
Guidelines for the Assessment of Additives in Animal Nutrition
[Council Directive 87/153/EEC of 7 March 1987[1]].
to make this document also applicable for
microorganisms and/or enzyme preparations
intended for nutritional purposes

Provisional Opinion Expressed July 10 1992.

GENERAL ASPECTS

These guidelines are intended as a guide for establishing dossiers on substances and preparations being submitted for authorization as additives in feedingstuffs. These 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 7 (2) of Council Directive 70/524/EEC of 23 November 1970 concerning additives in feedingstuffs[2].

All the studies outlined in these guidelines may be required and, if necessary, additional information will be requested. 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 metabolism, biological and toxicological effects on target species must be provided. 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.

It may not always be necessary to subject additives intended exclusively for pet food to be as exhaustive a programme of chronic toxicity, mutagenicity and carcinogenicity testing as that required for additives intended for feeding to livestock from which product for human consumption is derived. To determine chronic toxicity, studies on two target species or on one target species and rats for a period of one year will generally suffice. Mutagenesis and carcinogenesis studies can generally be dispensed with if the chemical composition, practical experience, or other considerations do not indicate the likelihood of changes. It is possible to dispense with the analysis of residues in pet animals.

Knowledge of the metabolism of the additive in food producing stock, of the residues and their bioavailability is essential. In particular it must enable the extent of the toxicological studies to be performed on laboratory animals in order to assess the risks, if any, to the consumer to be determined. This evaluation cannot be based solely on data confined to determining the direct effects of the additives on laboratory animals. The latter do not provide specific information on the actual effects of residues resulting from the metabolism in the species for which the additive is intended.

Any application for authorization of an additive or a new usage for an additive shall be supported by a dossier which should include detailed reports presented in the order and with the numbering proposed in these guidelines. Reasons must be given for the omission from the

[1] O.J. No. L64 (07.03.87) p.19.

[2] O.J. No. L270 (14.12.70) p.1, and O.J. No. L319 (08.12.84) p.13

dossier of any data prescribed in these guidelines. Publications to which reference is made must be attached to it. The reports of experiments must include the plan and reference number of the experiment, detailed description of the tests, results and their analysis, and also the name, address and signature of the person responsible for the study. A statement from the person responsible for Good Laboratory Practice regarding observance of such practice is to be attached to the reports.

The determination of physico-chemical, toxicological and ecotoxicological properties shall be performed in accordance with the methods established by Commission Directive 84/449/EEC of 25 April 1984 adapting to technical progress for the sixth time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances[3], or with methods internationally recognized by scientific bodies. The use of other methods should be justified.

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

The term 'additive', as used in these guidelines, refers to the active substances or the preparations containing active substances in the state in which they will be incorporated in premixtures and feedingstuffs.

The Commission must be notified within a reasonable time by the Member State which 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.

3 O.J. No. L251 (10.09.84) p.1.

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SECTION I

SUMMARY OF THE DATA IN THE DOSSIER

SECTION II

1. Identity of the additive

1.1 Proposed proprietary name(s)
1.2 Type of additive according to its main function
1.3 Qualitative and quantitative composition (active substance, other components, impurities)
1.4 Physical state, particle size
1.5 Manufacturing process including any specific processing procedures

N.B. If the active substance is a mixture of active components, each clearly definable, main components must be described separately and the proportions of the mixture given.

2. Specifications concerning the active substance

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

If the active substance is a micro-organism: name and taxonomic description according to the international Codes of Nomenclature. Other internationally recognized Manuals of Systematics can also be used[4].

For enzyme preparations : name according to main enzymic activities as described by IUB/IUPAC, EINECS and CAS Number.

As a general principle micro-organisms and/or enzyme preparations should be derived from non-pathogenic and non-toxicogenic microbial sources.

2.2 Formula, empirical and structural, and molecular weight. Qualitative and quantitative composition of the main components, if the active substance is a fermentation product.

For micro-organisms : name and place of culture collection, if possible one in an EEC collection, where the strain is deposited and depositing number, genetic modification and all relevant properties for its identification. In addition, origin, appropriate morphological and physiological characteristics, developmental stages, relevant factors that may be involved in its biological activity (as an additive), resistance pattern, DNA-DNA homology data and plasmid profile. Number of colony forming units (CFU) for each species .

For enzyme preparations : the biological origin, the activities towards relevant chemically pure model substrates and other physico-chemical characteristics.

2.3. Degree of purity

For micro-organisms : genetic stability and purity of strains cultivated.

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[4] Such as Bergey's Manual of Determinative Bacteriology. "The Yeasts, a taxonomic study" by Lodder, "A Dictionary of Fungi" by Ainsworth and Bisley or "The Genus Aspergillus" by Raper and Fennel.

For enzyme preparations : purity (checking the level of contaminating micro-organisms, heavy metals, absence of mycotoxins and antibacterial activity),and composition of the non-enzymic component derived from the source material.

Qualitative and quantitative composition of the impurities.

2.4. Relevant properties

For chemicals: Electrostatic properties, melting point, boiling point, decomposition temperature, density, vapour pressure, solubility in water and organic solvents, mass and absorption spectra and any other appropriate physical properties.

For micro-organisms : relevant biological properties.
For enzyme preparations: optimal pH, temperature and other appropriate properties.

2.5. Manufacturing, purification processes and media used. Variation in the composition of the batches in the course of production.

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

3.1. Stability (for micro-organisms loss of biological activity e.g. viability) on exposure to environmental conditions such as light, temperature, pH, moisture and oxygen. Expiry date.

3.2. Stability (for micro-organisms loss of biological activity, e.g. viability) during the preparation of premixtures and feedingstuffs, in particular stability to heat, pressure and moisture. Possible decomposition products.

3.3. Stability (for micro-organisms loss of biological activity, e.g. viability) during the storage of premixtures and feedingstuffs (storage time under defined conditions). Expiry date.

3.4. Other appropriate physico-chemical, technological or biological properties such as ability to obtain homogeneous mixtures in premixtures and feedingstuffs, dust-forming properties, and for micro-organisms and/or enzyme preparations, assessment of resistance to degradation or loss of biological activity in the digestive tract or by systems of simulation *in vitro*.

3.5. Physico-chemical or biological incompatibilities or interactions (e.g. with feedingstuffs, other additives or with medicinal products).

4. Conditions of use of the additive.

4.1. Proposed use in animal nutrition (e.g. species and category of animal, type of feedingstuff, period of administration and withdrawal period).

4.2. Contra-indications.

4.3. Proposed dosing in premixture and feedingstuffs (expressed as a percentage of the active substance by weight or appropriate units of biological activity such as CFU/per gram of product for micro-organisms or relevant activity units for enzyme preparations, for premixtures; and in mg/kg for feedingstuffs).

4.4. Other known uses of the active substance or the preparation (e.g. in foodstuffs, human or veterinary medicine, agriculture and industry). For each use give the proprietary names, indications and contra-indications.

4.5. If necessary, measures for the prevention of risks and means of protection during manufacture and handling.

5. Control methods.

5.1. Description of the methods used for the determination of the criteria listed under items 1.3., 2.3., 2.4., 2.5., 3.1., 3.2., 3.3., 3.4. and 4.3.

5.2. Description of the qualitative and quantitative analytical methods for routine control of the additive in premixtures and feedingstuffs.

5.3. Description of the qualitative and quantitative analytical methods for determining residues of additives in animal produce.

N.B. The methods specified and the results should be accompanied by information as to percentage recovery, specificity, limits of detection, possible interferences, reproducibility and to the sampling method used. Reference standards of the preparation and of the active substance must be available.

In the case of micro-organisms state methods of detection, enumeration, identification and relevant markers.

SECTION III

STUDIES CONCERNING THE EFFICACY OF THE ADDITIVE

1. Studies concerning improvements in the characteristics of feedingstuffs.

   These studies concern technological additives such as antioxidants, preservatives, binders, emulsifiers, stabilizers and gelling agents, which are intended to improve or stabilise the characteristics of premixtures and feedingstuffs. Some micro-organisms and/or enzyme preparations could also be considered as technological additives, if they improve relevant feed characteristics.

   Evidence of the efficacy of the additive should be provided by means of appropriate criteria under the intended conditions of use in comparison with negative control feedingstuffs and, possibly, feedingstuffs containing technological additives of known effectiveness.

   The precise nature of the active substances, preparations, premises and feedingstuffs examined, the reference number of the batches, the concentration of the active substances in premixtures and feedingstuffs, the testing conditions (e.g. temperature and humidity) and also the dates and duration of testing, the adverse and negative effects which occurred during testing shall be specified for each experiment.

2. Studies concerning the effects of additives on animal production.

   These studies concern zootechnical additives which have effects on animal production. The following studies including dose-response relationship, should be performed on each target species in comparison with negative control groups and, possibly, groups receiving feedingstuffs containing additives of known effectiveness. If the active substance is a mixture of effective components, the presence of each component must be justified.

2.1. For coccidiostats and other medicinal substances, importance should primarily be attached to evidence of the specific effects and particularly prophylactic properties (e.g. morbidity, oocyst count and lesion score). Information on the effect on feed efficiency, animal growth and marketable quantity and quality of the animal produce may be added.

2.2. For other zootechnical additives (including micro-organisms and/or enzyme preparations) information should be provided on the effects on: nutritional efficiency, animal growth, animal products characteristics and yield, animal welfare and other parameters having a positive influence on animal production.

2.3. Experimental conditions :

   The test performed must be described and the results presented individually in detail. The statistical evaluation and the methods employed should be reported. The following data must be provided :

2.3.1. Species, breed, age and sex of the animals, identification procedure.

2.3.2. Number of test and control groups, number of animals in each group. The number of animals of both sexes must be sufficient for statistical purposes.

2.3.3. Concentration of the active substance using the appropriate recognized measure in the feedingstuffs established by a control analysis. Reference number of the batches. Nutritional composition of the diet in terms of quality and quantity.

2.3.4. Location of each experiment. Animal health, physiological, feeding and rearing conditions as usually practised in the Community. Feed control and measures taken to avoid contamination of control groups during the experiment (particularly for micro-organisms through self contamination by the micro-organism).

2.3.5. Date and exact duration of testing.Date and nature of the examinations performed

2.3.6. Unfavourable effects and other incidents which occurred during the experiment and time of their appearance.

3. Studies concerning the quality of animal produce.

Studies on the organoleptic, nutritional, microbial, hygienic and technological qualities of edible produce from animals fed with feedingstuffs containing the additive. Studies of characteristics of the animal produce and effects on the final composition should be provided.

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,
- the risks from inhalation or from cutaneous, mucosal or eye contact for persons likely to handle the additive as such or as incorporated into premixtures of feedingstuffs,
- the risks to the consumer which could result from the consumption of food containing residues of the additive, or its metabolites,
- the risks of pollution or survival of the environment from the additive itself or by products derived from the additive and excreted by animals,
- the risks to non-target species.

These studies will be required in their entirety or in part depending on the nature of the additive and the conditions proposed for its use; in the case of micro-organisms and/or enzyme preparations appropriate safety tests must be performed (including tolerance tests and a translocation test). If the active substance is chemically specified the knowledge of their metabolism in the various target species and also of the composition and the bioavailability of the tissue residues will be essential for determining the extent of studies on laboratory animals to assess the risks for the consumer. Furthermore, knowledge of the composition and of the physico-chemical and biological properties of the excreted residues deriving from the additive will be indispensable to define the extent of the studies necessary for assessment of the risk of pollution or survival of the environment.

1. Studies on target species

1.1. Toxicological studies of the additive.
Tolerance tests.
Study of the biological, toxicological, macroscopic and histological effects. Determination of the safety margin between the maximum proposed dose-level and the level resulting in unfavourable effects. It may be sufficient to indicate a minimum or approximate value for this margin if it can be shown that the level resulting in unfavourable effects greatly exceeds the maximum proposed dose-level.

1.2. Microbiological studies of the additive.

1.2.1. If the active substance is chemically specified studies of the antimicrobial spectrum of action of the additive by determination of the Minimum Inhibitory Concentration (MIC) in various pathogenic and non-pathogenic Gram-negative and Gram-positive species of bacteria should be provided.

1.2.2. Studies on the cross-resistance to therapeutic antibiotics by determination of the MIC in mutants produced, which exhibit chromosomal resistance to the additive. In the case of the use of micro-organisms which are resistant to therapeutic antibiotics a plasmid profile should be provided.

1.2.3. Tests to find out whether the additive is capable of selecting resistance factors. These tests are to be performed under field conditions in the animal species for which the additive is primarily intended. Subsequently, it should be determined whether R factors which may have been found carry multiple resistance and are transmissible.

1.2.4. Tests to determine the effect of the additive

- on the microflora of the digestive tract
- on the colonisation of the digestive tract
- on the shedding or excretion of pathogenic micro-organisms.

1.2.5. If the active substance shows an antimicrobial action field studies to monitor the percentage of bacteria resistant to the additive should be provided. These are to be carried out at major intervals before, during and after the use of the additive.

1.2.6. If the additive is a micro-organism, it should be determined if it is resistant to antibiotics.

1.2.7. If the additive is a genetically modified micro-organism, the specifically adopted guidelines should be followed .

1.2.8. If the additive (e.g. enzyme preparations) is produced by a micro-organism the level of contamination by the viable producer organism (if any) should be determined.

1.3 Studies of the metabolism and residues [5][6]
(when the active substance is chemically specified)

1.3.1. Study of metabolism

- metabolic balance: rate and extent of absorption and elimination of the active substance,
- identification of the metabolic pathways and main metabolites,
- distribution and excretion (biliary, urinary, faecal) of the metabolites,
- if appropriate influence of the intestinal or ruminal microflora, of enterohepatic cycle, of caecotrophy, on the metabolism.

1.3.2. Analytical studies of the residues : qualitative and quantitative composition of the residues (active substance, metabolites) in the various animal food products at metabolic equilibrium and under practical conditions of use of the additive.

1.3.3. Kinetic study of the residues (following repeated administration of the additive according to the proposed use) : persistence of the active substance and the main metabolites in the various organs and tissues after withdrawal of the supplemented feedingstuff.

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[5] The studies mentioned under 1.3.1., 1.3.3., and 1.3.4. should be carried out with labelled molecules or other appropriate methods, in each case the choice of the method utilised should be justified. The labelling should be suitable for the purpose intended.

[6] If the active substance is produced by fermentation, these studies should be extended to related substances derived from the production.

1.3.4. Study of the bioavailability of the residues in animal food products before and after storage and cooking (see 3.7.).

1.3.5. Methods of monitoring : qualitative and quantitative methods of determination used in the studies mentioned under items 1.3.1. to 1.3.4. with information as to percentage recovery, specificity and limits of detection. The methods of determination of the residues must be sufficiently sensitive to permit detection of residues at levels which are toxicologically negligible.

2. Study on excreted residues
(when the active substance is chemically specified)

2.1. Nature and concentration of the residues derived from the additive (active substance, metabolites) in the excreta.

2.2. Persistence (half-life value) and kinetics of elimination of these residues in slurries, farm yard manure and litter.

2.3. Effects on methanogenesis.

2.4. Degradation, persistence (half-life value) and kinetics of elimination in soils (contrasting soil types).

2.5. Effects on soil fauna and microbial processes of transformation (e.g. decomposition of plant and animal residues).

2.6. Effects on terrestrial plants (e.g. seed germination, plant growth and plant up-take). These studies should be carried out under controlled conditions and field conditions, using different plant species.

2.7. Solubility and stability in water of the products derived from the additive (active substance, metabolites).

2.8. Effects on aquatic life.

2.8.1. Effects on flora (e.g. *Chlorella)*.

2.8.2. Toxicity in non-vertebrates (*e.g. Daphnia magna).*

2.8.3. Toxicity in fish (at least two wild species found in the Community territory).

3. Studies on laboratory animals
(when the active substance is a non pathogenic micro-organism found naturally these type of studies may not be necessary).

These studies must be carried out with the active substance and its major metabolites or products, if the latter are also present in edible animal produce and are bioavailable. As far as possible attempts should be made to select laboratory animals which may be expected to digest and metabolize the additive in a similar way to man or the target species.

Full detailed descriptions must be provided of the tests performed. These should cover the animal species and strains employed, the size and number of test and control groups, the dose levels administered, the composition of the diet and the results of feed

analyses, the rearing conditions, the exact duration of the tests, the dates of the various examinations performed and mortality. Full details must be given of the macroscopic pathological and histopathological findings in all animals tested
with an indication of the time of appearance of all pathological lesions. All results, including statistical assessment, must be presented in detail.

3.1. Acute toxicity

3.1.1. Acute oral toxicity studies must be carried out on two animal species (preferably the rat should be one). The maximum dosage should not be higher than 2,000 mg/kg body weight. Detailed observations should be reported of the biological effects observed during a period of at least two weeks after ingestion.

3.1.2. Studies on acute inhalational toxicity, skin and, where necessary, mucous membranes irritancy and also allergenic potential must be performed by appropriate tests for the assessment of possible risks associated with the handling of the additive.

3.2. Mutagenicity

In order to identify active substances or their metabolites or products that posses mutagenic properties a selected combination of mutagenicity tests, based on different genetic endpoints, must be carried out, except if the active substance is a micro-organism. Tests must be performed, in the presence and absence of a microsomal mammalian preparation for a metabolic activation.

The following package of tests is recommended :
(a) a test for gene mutations in a prokaryotic system,
(b) a test for gene mutations in an *in vitro* eukaryotic system or a sex-linked recessive lethal test in *Drosophila melanogaster*,
(c) a test for chromosomal damage *in vitro* and *in vivo.*

The battery of tests suggested above does not imply, however, that other tests are inappropriate or that other tests, in particular *in vivo* tests, would not be acceptable as alternatives.

In all cases reasons for the choice of tests should be given. Tests must be carried out according to established validated procedures. Depending on the outcome of the tests and taking into consideration the whole toxicity profile of the substance as well as the intended use, additional investigations may be indicated.

3.3. Pharmacokinetic aspects

If the active substance is chemically specified balance studies and identification of metabolites must be performed using suitable labelled molecules or other appropriate techniques and should cover both single and multiple dose administration of the active substance over appropriate periods. Metabolism studies must also include investigation of the pharmacokinetics of the active substance and of the major metabolites. Consideration must be given to the differences in the way that various species metabolize the active substance when selecting the most relevant species for subsequent toxicological investigations.

3.4. Subchronic toxicity

These studies must be carried out in general on two animal species (preferably the rat should be one). The second species may in some instances be a target species. The test substance may be administered orally and a dose-response relationship must be established. The duration in rodents must be at least 90 days.

In certain cases investigations extending over six months to two years in non-rodents (the commonly used non-rodent is the dog, preferably of a defined breed) may be desirable to establish the variation in sensitivity of different animal species to the test substance.

These studies are not relevant for micro-organisms, in the case of enzymic preparations appropriate tests should be provided.

3.5. Chronic toxicity/carcinogenicity

Chronic toxicity studies must be carried out on one species (preferably the rat), carcinogenicity studies preferably on two species of rodent. The substance must be administered orally at several dose levels. A combined chronic toxicity/carcinogenicity study with in-utero exposure is also acceptable. Experiments must extend for a minimum of 24 months in rats and 18 months in mice. If continued beyond the minimum period, the test must be terminated when survival in any but the highest dose level groups has fallen to 20%.

Full clinical chemistry, haematological and urine examinations must be carried out at appropriate intervals throughout the experiment. Full macroscopic and histological examinations must be carried out on all animals dying during the test and on all survivors at the termination of the study.

These studies are not relevant for micro-organisms, in the case of enzymic preparations appropriate tests should be provided.

3.6. Reproductive toxicity

Studies on reproduction must be carried out preferably on the rat. They must extend over at least two filial generations and may be combined with embryotoxicity including teratogenic studies. All relevant fertility, gestation, parturition, peri- and postnatal parameters must be carefully observed and reported. Specific teratogenic studies must be carried out in at least two suitable species.

3.7. Toxicology of metabolites

Information for the calculation of residue concentration is required as a basis for assessing the risk for man.
The basis for calculation of the proposed withdrawal period must be made available.
The studies mentioned in 1.3.4. must be carried out in laboratory animals.

3.8. Other relevant studies.

Any further special study providing additional information useful for the assessment of test substance may be made available (e.g. bioavailability, neurotoxicity or immunotoxicity).

SECTION V

FORM OF MONOGRAPH

1. Identity of the additive

1.1. Type of additive according to its main function
1.2. Qualitative and quantitative composition (active substance, other components, impurities)
1.3. Physical state, particle size
1.4. Possible specific processing.

N.B. If the active substance is a mixture of active components, each clearly definable, main components must be described separately and the proportions of the mixture given.

2. Specifications concerning the active substance

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

If the active substance is a micro-organism: name and taxonomic description according to the international Codes of Nomenclature. Other internationally recognized Manuals of Systematics can also be used[7].

For enzyme preparations : name according to main enzymic activities as described by IUB/IUPAC, EINECS and CAS Number.

2.2. Formula, empirical and structural, and molecular weight. Qualitative and quantitative composition of the main components, if the active substance is a fermentation product.

For micro-organisms : name and place of culture collection, if possible one in an EEC collection, where the strain is deposited and depositing number, genetic modification and all relevant properties for its identification.

For enzyme preparations : the biological origin, the activities towards relevant chemically pure model sustrates and other physico-chemical characteristics.

2.3. Degree of purity

For micro-organisms : genetic stability and purity of strains cultivated.
For enzyme preparations : purity (checking the level of contaminating micro-organisms, heavy metals, absence of mycotoxins and antibacterial activity),and composition of the non-enzymic component derived from the source material.

Qualitative and quantitative composition of the impurities

2.4. Relevant properties

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[7] Such as Bergey's Manual of Determinative Bacteriology. "The Yeasts, a taxonomic study" by Lodder, "A Dictionary of Fungi" by Ainsworth and Bisley or "The Genus Aspergillus" by Raper and Fennel.

For chemicals: Electrostatic properties, melting point, boiling point, decomposition temperature, density, vapour pressure, solubility in water and organic solvents, mass and absorption spectra and any other appropriate physical properties.

For micro-organisms : relevant biological properties.
For enzyme preparations: optimal pH, temperature and other appropriate properties.

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

3.1. Stability (for micro-organisms loss of biological activity, e.g. viability) on exposure to environmental conditions such as light, temperature, pH, moisture and oxygen. Expiry date.

3.2. Stability (for micro-organisms loss of biological activity, e.g. viability) during the preparation of premixtures and feedingstuffs, in particular stability to heat, pressure and moisture. Possible decomposition products.

3.3. Stability (for micro-organisms loss of biological activity, e.g. viability) during the storage of premixtures and feedingstuffs (storage time under defined conditions). Expiry date.

3.4. Other appropriate physico-chemical, technological or biological properties such as ability to obtain homogeneous mixtures in premixtures and feedingstuffs, dust-forming properties, and for micro-organisms and/or enzyme preparations, assessment of resistance to degradation or loss of biological activity in the digestive tract or by systems of simulation *in vitro.*

3.5. Physico-chemical or biological incompatibilities or interactions (e.g. with feedingstuffs, other additives or with medicinal products).

4. Control methods

4.1. Description of the methods used for the determination of the criteria listed under items 1.2., 2.3., 2.4., 3.1., 3.2., 3.3. and 3.4. of this Section.

4.2. Description of the qualitative and quantitative analytical methods for determining, residues of additives in animal produce.

4.3. If the said methods have been published, literature references may suffice, in this case reprints should be provided.

5. Biological properties of the additive

5.1. Particulars of the prophylactic effects for coccidiostats and other medicinal substances (e.g. morbidity, oocyst count and lesion score).

5.2. For zootechnical additives other than those listed in 5.1.(including micro-organisms and/or enzyme preparations. Particulars of the effects on feed efficiency, animal growth, animal products characteristics and yield, animal welfare and other parameters having a positive influence on animal production.

5.3. Any contra-indications or warnings, including biological incompatibilities, with particulars of their justification.

6. Details of the quantitative and qualitative residues, if any, found in animal produce following envisaged use of the additive.

7. Other characteristics suitable for identification of the additive.