Council Directive 87/153/EEC of 16 February 1987 fixing guidelines for the assessment of additives in animal nutrition
Official Journal L 064 , 07/03/1987 P. 0019 - 0028

THE COUNCIL OF THE EUROPEAN COMMUNITIES,
Having regard to the Treaty establishing the European Economic Community,
Having regard to Council Directive 70/524/EEC of 23 November 1970 concerning additives in feedingstuffs (1), as last amended by Commission Directive 86/525/EEC (2), and in particular Article 9 thereof,
Having regard to the proposal from the Commission,
Whereas Directive 70/524/EEC provides that the examination of additives must be performed on the basis of a dossier forwarded officially to the Member States and to the Commission;
Whereas such dossiers must make it possible to verify that additives comply, in respect of their proposed use, with the general principles laid down in the Directive for their inclusion in the Annexes thereto;
Whereas it has been found necessary to provide for the dossiers to be compiled in accordance with common guidelines defining the scientific data which make it possible to identify and characterize the products concerned and the studies necessary in order to evaluate, in particular, their efficacy and their safety for man, animals and the environment;
Whereas the guidelines are intended primarily as a general guide; whereas, depending on the nature of the additive or its conditions of use, the extent of the studies necessary in order to evaluate its properties or its effects may vary;
Whereas it is indispensable to apply the principles of good laboratory practice when developing additives intended for use in feedingstuffs to ensure that the results of laboratory tests are not disputed; whereas recourse to procedures involving the use of laboratory animals for experimental or other scientific purposes should be kept to a minimum;
Whereas the guidelines have been drawn up on the basis of present scientific and technical knowledge and they may be adapted if necessary to any developments in this sphere,
HAS ADOPTED THIS DIRECTIVE:
Article 1
Member States shall prescribe that the dossiers which must accompany every request for the inclusion of an additive or a new use of an additive in the Annexes to Directive 70/524/EEC are to be compiled in accordance with the guidelines set out in the Annex to this Directive.
Article 2
This Directive shall apply without prejudice to provisions on:
(a) good laboratory practice for the purposes of mutual acceptance of data for the evaluation of chemical products; and
(b) the protection of animals used for experimental or other scientific purposes.
Article 3
Member States shall bring into force the laws, regulations or administrative provisions necessary in order to comply with this Directive by 31 December 1987 at the latest. They shall forthwith inform the Commission thereof.
Article 4
This Directive is addressed to the Member States.
Done at Brussels, 16 February 1987.
For the Council
The President
L. TINDEMANS
(1) OJ No L 270, 14. 12. 1970, p. 1.
(2) OJ No L 310, 5. 11. 1986, p. 19.
ANNEX

GUIDELINES FOR THE ASSESSMENT OF ADDITIVES IN FEEDINGSTUFFS

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 (1).

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 will not always be necessary to subject additives intended exclusively for pet food to as exhaustive a programme of chronic toxicity, mutagenicity and carcinogenicity testing as that required for additives intended for feeding to livestock from which are derived produce for human consumption. 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 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 (2), 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 and growth promoters 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.

(1) OJ No L 270, 14. 12. 1970, p. 1 and
OJ No L 319, 8. 12. 1984, p. 13.

(2) OJ No L 251, 19. 9. 1984, p. 1.

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SECTION II

IDENTITY, CHARACTERIZATION AND CONDITIONS OF THE USE OF THE ADDITIVE METHODS OF CONTROL

1. Identity of the additive

1.1. Proposed proprietary names.

1.2. Type of additive according to its main function (e.g. antibiotic, coccidiostat, histomonostat, preservative, etc.).

1.3. Physical state, particle size.

1.4. Qualitative and quantitative composition (active substance, other components, impurities).

1.5. Manufacturing process including any specific processing procedures.

2. Specifications concerning the active substance

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

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

2.3. Degree of purity. Qualitative and quantitative composition of the impurities.

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

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

NB: If the active substance is a mixture of active components, each chemically definable, main component must be described separately and the proportions of the mixture given.

3. Physico-chemical and technological properties of the additive

3.1. Stability on exposure to atmospheric agents (light, temperature, moisture, oxygen, etc.).

3.2. Stability during the preparation of premixtures and feedingstuffs, in particular, stability to heat, pressure and moisture. Possible decomposition products.

3.3. Stability during the storage of premixtures and feedingstuffs (storage time).

3.4. Other appropriate physico-chemical and technological properties such as ability to obtain homogeneous mixtures in premixtures and feedingstuffs, dust-forming properties.

3.5. Physico-chemical interactions (incompatibility with feedingstuffs, other additives or with medicinal products, etc.).
4. Conditions of use of the additive
4.1. Proposed use in animal nutrition (species or category of animal, category of feedingstuff, period of administration, withdrawal period, etc.).
4.2. Contra-indications.
4.3. Proposed concentrations in premixtures and feedingstuffs (expressed as a percentage of the active substance by weight for premixtures; as mg/kg for feedingstuffs).
4.4. Other known uses of the active substance or the preparation (in foodstuffs, human or veterinary medicine, agriculture, etc.). 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 1.4, 2.3, 2.4, 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.
NB: The methods specified should be accompanied by information as to percentage recovery, specificity, sensitivity, possible interferences, limits of detection, reproducibility and to the sampling method used. Reference standards of the preparation and of the active substance must be available.
SECTION III
STUDIES CONCERNING THE EFFECTIVENESS OF THE ADDITIVE
1. Studies concerning improvements in the quality of feedingstuffs
These studies concern technological additives such antioxidants, preservatives, emulsifiers, gelling agents, etc., which are intended to improve the quality of premixtures and feedingstuffs or to prolong their preservation time.
Evidence of the effectiveness of the additive should be shown 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, premixes and feedingstuffs examined, the reference number of the batches, the concentration of the active substance in premixtures and feedingstuffs, the testing conditions (temperature, humidity etc.) and also the dates and duration of testing, the adverse effects and further negative effects which occurred during testing shall be specified for each experiment.
2. Studies concerning the effects of additives on animal produce
These studies concern zootechnical additives such as antibiotics, growth promoters, coccidiostats and other medicinal substances, etc. which have effects on animal produce. The following studies should be performed on each target species in comparison with negative control groups and, possibly, groups receiving feedingstuffs containing additives of known effectiveness.
2.1. For antibioitcs and growth promoters, study of the effects on nutritional efficiency, growth of the animal and yield of animal paroduce. Determination of the dose/response relationship.
2.2. For coccidiostats and other medicinal substances, importance should primarily be attached to evidence of specific effects and particularly to prophylactic properties (e.g. morbidity, oocyst counts, assessment of impairment etc.). Information on the effect on feed efficiency, animal growth and amount and marketable quality of the animal produce should be attached.
2.3. Experimental conditions:
The test performed must be described individually in detail. The test record should enable statistical analyses to be made. 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 test animals of both sexes selected must be sufficient for statistical purposes.
2.3.3. Concentration of the active substance in the feedingstuffs established by a control analysis. Reference number of the batches. Nutritional composition of the daily ration in terms of quality and quantity.
2.3.4. Location of each experiment, physiological and animal health conditions, feeding and rearing conditions in accordance with standard practice in the Community.
2.3.5. Date and exact duration of testing, date of examinations performed.
2.3.6. Adverse effects and further negative effects which occurred during the experiment and time of their appearance.
3. Studies concerning the quality of animal produce
Studies on the organoleptic, nutritional, hygienic and technological qualities of edible produce of animals fed with feedingstuffs containing the additive. 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 to the consumer which could result from the consumption of food containing residues of the additive,
- the risks from inhalation or cutaneous contact for persons likely to handle the additive as such or as incorporated into premixtures or feedingstuffs,
- the risks of pollution of the environment from products derived from the additive and excreted by animals.
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. Knowledge of the metabolism of the active substance 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 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 factor (margin between the maximum proposed dose-level and the level resulting in adverse effects). It may be sufficient to indicate a minimum or approximate value for this factor if it can be shown that the level resulting in adverse effects greatly exceeds the maximum proposed dose-level.
1.2. Microbiological investigation of the additive
1.2.1. Investigation of the microbiological spectrum of action of the additive by determination of the minimum inhibition concentration (MIC) in various pathogenic and non-pathogenic gram-negative and gram-positive species of bacteria.
1.2.2. Investigation into the cross-resistance to therapeutic antibiotics by determination of the MIC in mutants produced in vitro which exhibit chromosomal resistance to the additive.
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 normal intestinal flora and on the colonization of the intestinal tract and the execretion of pathogenic micro-organisms.

1.2.5. Field studies to determine the percentage of bacteria resistant to the additive. These are to be carried out at major intervals before and during the use of the additive (monitoring).
1.3. Studies of the metabolism and residues of the active substance (1) (2)
1.3.1. Study of metabolic balance: rate and extent of elimination of the active substance in urine and faeces and, possibly, by expiration; residues in the target species.
1.3.2. Study of metabolism: absorption, distribution, biotransformation and elimination. If appropriate, estimation of the extent of excretion through bile, existence of an enterohepatic cycle, influence of caecotrophy.
1.3.3. Analytical studies of the residues: qualitative and quantitative composition of the residues (active substance, metabolites) in the various organs and tissues of the animal and in edible produce originating from the animal, when metabolic equilibrium is reached and under practical conditions of use of the additive.
1.3.4. Pharmacokinetic 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.
1.3.5. Study of the bioavailability of the residues in tissues and produce of target species (see 3.8).
1.3.6. Methods of monitoring: qualitative and quantitative methods of determination used in the studies mentioned under items 1.3.1. to 1.3.5. 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
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 (decomposition of plant and animal residues, N transformation, etc.).
2.6. Effects on terrestrial plants (seed germination, plant growth, plant up-take etc.) 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
These studies must be carried out with the active substance and its major metabolites, 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 metabolize the additive in a similar way to man.
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 that possess mutagenic properties a selected combination of mutagenicity tests, based on different genetic endpoints, must be carried out. 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. Metabolic and pharmacokinetic aspects

Balance studies and identification of metabolites must be performed using suitable labelled molecules 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 in 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 dogs or other non-rodents may be desirable to establish the variation in sensitivity of different animal species to the test substance.

3.5. Chronic toxicicity/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 adminsitered 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 two years in rats and 80 weeks 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.

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. Bioavailability

Investigation on the fate of residues of the labelled active substance in tissue and produce of target species will require bioavailability studies including at least a balance study of the residues when adminsitered to laboratory animals.

3.8. 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.

3.9. Other relevant studies

Any further study providing additional information useful for the assessment of test substance may be made available, e.g. studies on relay toxicity. SECTION V

FORM OF MONOGRAPH

1. Identity of the additive

1.1. Type of additive according to its main function (antibiotic, coccidiostat, histomonostat, growth promoter, etc.).

1.2. Physical state, particle size.

1.3. Qualitative and quantitative composition (active substance, other components, impurities).

1.4. Possible specific processing.

2. Specifications concerning the active substance

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

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

2.3. Degree of purity. Qualitative and quantitative composition of the impurities.

2.4. Appropriate physical properties such as electrostatic properties, melting point, boiling point, decomposition temperature, density, vapour pressure, solubility in water and organic solvents, absorption spectrum, etc.

NB: If the active substance is a mixture of active components, each chemically definable main component must be described separately and the proportions of the mixture be given.

3. Physico-chemical and technological properties of the additive

3.1. Stability on exposure to atmospheric agents (e.g. light, temperature, moisture, oxygen).

3.2. Stability during the preparation of premixtures and feedingstuffs, in particular, on exposure to heat, pressure and moisture. Possible decomposition products.

3.3. Stability during the storage of premixtures and feedingstuffs (storage time).

3.4. Other appropriate physico-chemical and technological properties such as ability to obtain homogeneous mixtures in premixtures and feedingstuffs, dust-forming properties.

3.5. Physico-chemical interactions (incompatibility with feedingstuffs, other additives or with medicinal products, etc).

4. Control methods

4.1. Description of the methods used for the determination of the criteria listed under items 1.3, 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.

5. Biological properties of the additive

5.1. Particulars of the prophylactic effects for coccidiostats and other medicinal substances (morbidity, oocyst count, etc.).
5.2. Particulars of the effects on the feed efficiency, growth and quality of animal products for antibiotics and growth promoters.
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.
(1) The studies mentioned under 1.3.1., 1.3.2., 1.3.4. and 1.3.5. should be carried out preferably with labelled molecules. The labelling should be suitable for the purpose intended.
(2) If the active substance is a fermentation product, these studies should be extended to related substances derived from the production.