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COUNCIL DIRECTIVE
of 16 February 1987
fixing guidelines for the assessment of additives in animal nutrition

(87/153/EEC)

(OJ L 64, 7.3.1987, p. 19)

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COUNCIL DIRECTIVE

of 16 February 1987

fixing guidelines for the assessment of additives in animal nutrition

(87/153/EEC)

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.

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(1) OJ No L 270, 14. 12. 1970, p. 1.
(2) OJ No L 310, 5. 11. 1986, p. 19.

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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.

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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 Directive 70/524/EEC.

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. When the additive is intended for a category of animals belonging to a defined species, these 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.

It may not always be necessary to subject additives intended exclusively for pet food to as exhaustive a program of chronic toxicity, mutagenicity and carcinogenicity testing as that required for additives intended for feeding to livestock from which products for human consumption are 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 productive livestock and of the residues and their bio-availability 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 does 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, the reference number, the date of the beginning and the end 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. Each batch of the feedingstuffs used in the experimental studies on animals must be analysed for the concentration of the relevant active substances using an appropriate method and a report of the analytical results must be provided. The report must also mention the individual doses determined in each experimental study, the corresponding dates, the name, address and signature of the person responsible for the controls. Furthermore, a certificate drawn up by the laboratory or laboratories where the experiments were conducted to the effect that the tests were carried out in accordance with the principles of good laboratory practices pursuant to Council Directive 87/18/EEC, on the harmonization 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], must be attached to the report.

The determination of physico-chemical, toxicological and ecotoxicological 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 substances [2], as last amended by Commission Directive 93/105/

[1] OJ No L 15, 17. 1. 1987, p. 29.
[2] OJ No L 196, 16. 8. 1967, p. 1.

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EC [1], 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, microorganisms 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. An active substance may be a chemically specified substance, a microorganism or an enzyme preparation.

In these guidelines the expression 'Chemically specified substances', means chemical substances for which a chemical name is agreed according to IUPAC nomenclature.

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 microorganisms, the genetic characteristics of which have been modified or which arise as natural mutants.

CONTENTS

[1] OJ No L 294, 30. 11. 1993, p. 21.

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SECTION I

SUMMARY OF THE DATA IN THE DOSSIER

SECTION II

IDENTITY, CHARACTERIZATION AND CONDITIONS OF USE OF THE ADDITIVE

METHODS OF CONTROL

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 of which is clearly definable, the main components must be described separately and the proportions in the mixture given.

2. **Specifications concerning the active substance**

2.1. For chemically specified substances: generic name, chemical name according to Iupac nomenclature, other generic international names and abbreviations. Chemical Abstracts Service Number (CAS).

For microorganisms: name and taxonomic description according to the internatinal Codes of Nomenclature. Other internationally recognized manuals of classification can also be used [1].

For enzyme preparations: name according to main enzymatic 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 microorganisms: name and place of culture collection, if possible one in an EC 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) and other genetic data for identification. Number of colony forming units (CFU) per gram.

For enzyme preparations: biological origin (in case of microbial origin: name and place of culture collection, if possible one in an EC collection, where the strain is deposited and depositing number, genetic modification and all relevant properties for its identification including genetic data), the activities towards relevant chemically pure model substrates and other physico-chemical characteristics.

2.3. *Degree of purity*

Qualitative and quantitative composition of the impurities.

For microorganisms: genetic stability and purity of strains cultivated.

For enzyme preparations:

— purity, by checking the level of contaminating microorganisms, heavy metals, absence of toxins derived from the source organism (e.g. mycotoxins), as shown by a suitable method;

— absence of antimicrobial activity at feed concentration level as determined by a suitable method;

— composition of the non-enzymatic components (particularly Total Organic Solids TOS [2]).

[1] Such as *Bergey's Manual of Systematic Bacteriology, The Yeasts, a taxonomic study* by Lodder and Kreger van Rij, *Ainsworth and Bisby's Dictionary of the Fungi* by Hawksworth, Sutton and Ainsworth or *The Genus Aspergillus* by Raper and Fennell.

[2] TOS (%) = 100 − (% ash + water + % diluents and/or additives and ingredients).

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2.4. *Relevant properties*

For chemically specified substances: 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 microorganisms: properties relevant to identification and proposed use (e.g. vegetative or sporulated form, CFU per g)

For enzyme preparations: optimal PH (value(s), optimal temperature(s) 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 microorganisms: loss of biological activity, e.g. viability) on exposure to environmental conditions such as light, temperature, pH, moisture and oxygen. Shelf life.

3.2. Stability (for microorganisms: 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 microorganisms: loss of biological activity, e.g. viability) during the storage of premixtures and feedingstuffs under defined conditions. Shelf life.

For enzyme preparations: Details of the presence of unexpected reaction products formed by either enzymatic or chemical reactions of the enzyme preparation with feed constituents or by degradation of the enzyme preparation during storage of the feedingstuff.

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 microorganisms 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 approved 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 premixtures and feedingstuffs expressed as:

— a percentage of the active substance by weight for premixtures and as mg/kg for feedingstuffs, in case of chemically specified substances,

— appropriate units of biological activity such as CFU per gram of product for microorganisms or relevant activity units for enzyme preparations.

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 contraindications.

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, sensitivity, limits

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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 microorganisms state methods of detection, enumeration, identification and relevant markets.

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, gelling agents etc., which are intended to improve or stabilize the characteristics of premixtures and feedingstuffs. Some microorganisms and/or enzyme preparations could also be considered as technological additives, if they improve relevant feed characterictics.

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, premixes 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 active 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 effciency, animal growth and marketable quantity and quality of the animal produce may be added.

2.2. For other zootechnical additives (including if appropriate microorgasnisms and/or enzyme preparations) information should be provided on the effects on: nutritional efficiency, animal growth, animal-product 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 (and, where it is the case, substances used for comparative purposes) in the feedingstuffs established by a control analysis, using the appropriate recognized method. 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 microorganisms through cross-contamination of feed by the microorganism).

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

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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, hygienic and technological qualities of produce from 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 from risks inhalation, other mucosal, eye or cutaneous contact for persons likely to handle the additive as such or as incorporated into premixtures or 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 persistence in the enviroment from the additive itself or by products derived from the additive and excreted by animals,

— possible 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.

As a general principle microorganisms and/or enzyme preparations must be, or be derived from microorganisms that are non-pathogenic and non-toxicogenic for target species and humans under the expected conditons of use.

In the case of microorganisms and/or enzyme preparations appropriate safety tests must be performed, unless other satisfactory documentation for safety of use is provided. For microorganisms at least a tolerance test on target species must be performed.

In principle toxicological tests for enzymes derived from edible parts of animals or plants are not required; where such products are not generally considered a normal part of the diet used, some toxicological testing may be required.

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 persistence in the enviroment.

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 and possesses antimicrobial activity at feed concentration level, studies of the antibacterial 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 must be provided.

1.2.2. Studies on the cross-resistance to therapeutic antibiotics by determination of the MIL, in mutants produced in vitro which exhibit chromosomal resistance to the additive. In the case of microorganisms which are resistant to therapeutic antibiotics, the genetic basis of this resistance should be shown.

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,

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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 colonization of the digestive tract, if it is a microorganism or a mixture of several strains of microorganisms,

— on the shedding or excretion of pathogenic microorganisms, if the active substance is chemically specified and has antimicrobial activity.

1.2.5. In cases where 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 (one month) the use of the additive.

1.2.6. If the active substance is a microorganism, it should be determined if it is resistant to antibiotics.

1.2.7. If the active substance (e.g. enzyme preparations) is produced by a microorganism the level of the respective viable organism should be determined.

1.2.8. If the additive contains or consists of a genetically modified organism within the meaning of Article 2 (1) and (2) of Council Directive 90/220/EEC the following information must be provided:

— A copy of any written consent or consents of the competent authorities to the deliberate release of the genetically modified organisms for research and development purposes pursuant to Article 6 (4) of Directive 90/220/EEC and the summary of the notification as referred in Article 9 of Directive 90/220/EEC according to the model set up in Council Decision 91/596/EEC [1];

— the complete technical file with the information required in Annex II of Directive 90/220/EEC, extended as necessary to take into account the diversity of sites of use of the additive, including information on data and results obtained from research and developmental releases concerning the ecosystems which could be affected by the use of the additive and an assessment of any risks for human health, animal health and the environment related to the GMO(s) contained in the product including information obtained from the research and development stage on the impact of the release on human health and the environment;

— the conditions for the placing on the market of the additive, including specific conditions of use and handling and a proposal for labelling and packaging which should comprise at least the requirements laid down in Annex III of Directive 90/220/EEC.

If on the basis of any release notified under Part B of Directive 90/220/EEC, or on substantive, reasoned scientific grounds, the person responsible for the dossier considers that the placing on the market or the use of the additive does not pose a risk to human health, animal health and the environment, he may propose not to comply with one or more of the requirements of Annex III B.

Information on data or results from releases of the same GMO or the same combination of GMOs previously or currently notified and/or carried out by the person responsible for the dossier either inside or outside of Community, shall be included.

Other data or results from notifications previously submitted by other persons may also be referred, provided that the latter have given their agreement in writing.

1.3. *Studies of the metabolism and residues* [2] [3] *(If 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,

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[1] OJ No L 322, 23. 11. 1991, p. 1.

[2] 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 utilized should be justified. The labelling should be suitable for the purpose intended.

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

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— 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 purposal use): persistence of the active substance and the main metabolites in the various organs and tissues after withdrawal of the supplemented feedingstuff.

1.3.4. Study of the bio-availability of the residues in animal food products. (see 3.7.).

1.3.5. Methods of monitoring: qualitative and quantitative method 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 (where 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 uptake). 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 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 (not relevant for microorganisms)*

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

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two weeks after ingestion. These studies are not relevant for enzyme preparations.

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*

3.2.1. If the active substance is chemically specified

In order to identify active substances or their metabolites or products 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 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 aberrations *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 fo 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.

3.2.2. In the case of enzyme preparations derived from microorganisms the following tests are normally required:

(a) a test for gene mutations in bacteria,

(b) a test for chromosomal aberrations (preferably *in vitro)*.

The toxicological tests shall, where possible, be performed on a batch from the final purified fermentation product, before addition of carriers, diluents or other substances. They should, as a general rule, be performed in accordance with established guidelines [1] from recognized international Institutions although, because of the effects exerted at the cellular level by the proteinaceous nature and/or enzymatic activities of certain enzyme preparations, some modifications of the standard test protocols, particularly in the case of in vitro tests, may be necessary. Such deviations will be acceptable if accompanied by adequate supporting arguments.

The test system is designed to uncover unspecified toxic reactions and to reveal genotoxic effects. The combined information from the general specifications and this test battery make it possible to evaluate the product for the presence of both specific, well known toxins and unknown toxic compounds.

The toxicological report shall contain satisfactory documentation that the tests have been performed on the material which forms the basis of the commercial product as described in the technological dossier.

3.3. *Pharmacokinetics 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.

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[1] Such as:
— Presentation of an application for assessment of a food additive prior to its authorization. 1989. (ISBN 92-826-0135-B)
— Report of the Scientific Committee for Food on guidelines for the safety assessment of food additives. 1980. 10th report series, (EUR 6892).

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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 may be desirable to establish the variation in sensitivity of different animal species to the test substance.

These studies are not relevant for microorganisms. In the case of enzyme preparations derived from microorganisms a 90-day oral toxicity test in a rodent species may be sufficient.

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 microorganisms and enzyme preparations.

3.6. *Reproductive toxicity (where the active substance is chemically specified)*

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 (where the active substance is chemically specified)*

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 on 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. bio-availability, neurotoxicity or immunotoxicity).

SECTION V

FORM OF MONOGRAPH

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. *Possible specific processing*

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

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2. **Specifications concerning the active substance**

2.1. For chemically specified substances: generic name, chemical name according to IUPAC nomenclature, other generic international names and abbreviations. Chemical Abstracts Service Number (CAS).

For microorganisms: name and taxonomic description according to the international Codes of Nomenclature. Other internationally recognized manuals of classification can also be used [1].

For enzyme preparations: name according to main enzymatic 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 microorganisms: name and place of culture collection, if possible one in an EC collection, where the strain is deposited and depositing number, genetic modification and all relevant properties for its identification.

For enzyme preparations: the biological origin (in case of microbial origin: name and place of culture collection, if possible one in an EC collection, where the strain is deposited and depositing number, genetic modification and all relevant properties for its identification), the activities towards relevant chemically pure model substrates and other physico-chemical characteristics.

2.3. *Degree of purity*

Qualitative and quantitative composition of the impurities

For microorganisms: genetic stability and purity of strains cultivated.

For enzyme preparations:

— purity [by checking the level of contaminating microorganisms, heavy metals, absence of toxins (e.g. mycotoxins) derived from the source organism as shown by a suitable method];

— absence of antimicrobial activity at feed concentration level as determined by a suitable method;

— composition of the non-enzymatic components (particularly Total Organic Solids T.O.S.)

2.4. *Relevant properties*

For chemically specified substances: 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 microorganisms: properties relevant to identification and proposed use (e.g. vegetative or sporulated form, CFU per g).

For enzyme preparations: optimal pH value(s), optimal temperature(s) and other appropriate properties.

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

3.1. Stability (for microorganisms: loss of biological activity, e.g. viability) on exposure to environmental conditions such as light, temperature, pH, moisture and oxygen. Shelf life.

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

3.3. Stability (for microorganisms: loss of biological activity, e.g. viability) during the storage of premixtures and feedingstuffs under defined conditions. Shelf life.

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 microorganisms 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*.

[1] Such as *Bergey's Manual of Systematic Bacteriology, The Yeasts, a taxonomic study by* Lodder and Kreger van Rij, *Ainsworth and Bisby's Dictionary of the Fungi* by Hawksworth, Sutton and Ainsworth or *The Genus Aspergillus* by Raper and Fennell.

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

4. **Control methods**

4.1. Description of the methods used for the determination of the criteria listed under items 1.3., 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 the literature references may suffice and the corresponding reprints should be given.

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 if appropriate microorganisms and/or enzyme preparations: particulars of the effects on nutritional efficiency, animal growth, animal product 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.**