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 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, the 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 for 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 would not be justified to subject additives intended exclusively for pet food to as exhaustive a programme of toxicity testing in laboratory animals as that required for additives intended for feeding to livestock, where meat or other products are consumed by man.

Knowledge of the metabolism of the additive in productive livestock, of the residues and their bioavailability is essential. In particular it must enable the extent of toxicological studies to be performed on

---

(1) OJ No L 270 of 14.12.1970, p. 1 and No L 319 of 08.12.1984, p. 13

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 speicific information on the actual effects of residues resulting from the metabolism in the species for which the additive is intented.

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 omission in the dossier of any data prescribed in the 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.

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 timė Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances (1) or with methods internationaly recognized by scientific bodies. The use of other methods should be justified.

Each dossier shall be accompanied by an adequate summary. The dossiers relating to antibiotics, coccidiostats and other medicinal substances, and growth promoters shall necessarily be accompanied by a monograph enabling the additive concerned to be identified and

---

(1) OJ No L 251 of 19.09.1984, p. 1

characterised in accordance with article 8(1) of Council Directive 70/524/EEC, of 23 November 1970, concerning additives in feeding-stuffs.

OBSERVATIONS

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.

Any modification to the manufacturing process or the composition of an additive, in its field of application or its conditions of use will require notification and could necessitate the submission of documentation suitable for a new assessment. These requirements will be especially necessary for products derived from micro-organisms whose genetic characteristics have been modified or which arise as natural mutants.

CONTENTS

SECTION I : IDENTITY, CHARACTERISATION AND CONDITIONS OF USE OF THE ADDITIVE, METHODS OF CONTROL

1. Identity of the additive

1.1. Proposed proprietary names.

1.2. Type of product 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. Possible specific processing.

2. Specifications concerning the active substance

2.1. 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 spectra and any other appropriate physical property.

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

N.B. : If the active substance is a mixture, each 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).

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 preservation 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, dustforming properties.

3.5. Incompatibilities with feedingstuffs, other additives or with medicines.

4. Conditions of use of the additive

4.1. Intended use in animal nutrition (animal species, period of administration, withdrawal period).

4.2. Contra-indications

4.3. Proposed concentrations in premixtures and feedingstuffs (expressed as percentage of active substance by weight for premixtures; as mg/kg for feedingstuffs).

4.4. Other possible uses of the active substance or the preparation (in foodstuffs, human or veterinary therapy, agriculture, etc.). For each use the proprietary names, indications and contra-indications must be given.

4.5. If necessary, measures for the prevention of risks and means of protection for the users.

5. Methods of determination

5.1. Description of the methods used for establishing 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 the control of the additive in premixtures and feedingstuffs.

N.B. : 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 should be available.

SECTION II : STUDIES CONCERNING THE EFFECTIVENESS OF THE ADDITIVE

1. Technological studies

These studies concern essentially technological additives such as 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 conditions of temperature and humidity, 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. Animal performance studies

These studies concern essentially additives such as antibiotics, growth promoters, coccidiostats and other medicinal substances,

etc. which have effects on the 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 antibiotics and growth promoters, study of the effects on nutritional efficiency, growth of the animal, yield and market quality of animal produce. Determination of the dose/response relationship.

2.2. For coccidiostats and other medicinal substances, in addition to the studies mentioned under 2.1., study of the pharmacological and prophylactic effects (morbidity, oocyst counts, lesion scores, etc.). Determination of the optimum prophylactic dose-level for each species of pathogenic agent controlled by the use of the additive.

2.3. Experimental conditions :

A detailed description of all the tests performed must be given. 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 test design should enable an adequate statistical analysis to be made.

2.3.3. Concentration of the active substance in the feedingstuffs. Reference number of the batches. Formulation and chemical composition of the daily ration.

2.3.4. Location of each experiment, physiological and animal health conditions, feeding and rearing conditions (these should reflect those used in 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.

SECTION III : 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 for 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 products 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 and anatomohistopathological effects. Determination of the safety factor (margin between the maximum proposed dose-level and the level resulting in adverse effect).

1.2. Microbiological studies of the additive

Study of the effects of the additive on microorganisms, in particular, those of the intestinal flora : colonization of the intestinal tract by pathogenic or non pathogenic strains, possible development of chromosomal or plasmid mediated resistance in bacteria to prophylactic or therapeutic substances.

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; remaining part in the organism.

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 products 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 determination : qualitative and quantitative

---

(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 could be extended to related substances derived from the production process.

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 sensitive and permit the detection of residues at levels which are toxicologically negligible.

1.4. Study of the quality of edible animal produce

Study of the organoleptic, nutritional, hygienic and technological qualities of edible produce of animals fed with feedingstuffs containing the additive.

2. Study on excreted products

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

2.2. Persistence (half-life value) and kinetics of elimination of these products 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, etc.). These studies should be carried out under controlled and under 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. Acute toxicity in non-vertebrates (e.g. Daphnia magna)

2.8.3. Acute toxicity in fish (at least two species selected among wild species in the Community territory).

3. Studies on laboratory animals.

These studies shall 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 metabolise the additive in a similar way to man. Full detailed descriptions shall 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 shall 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, shall be presented in detail.

3.1. Acute toxicity

3.1.1. Acute oral toxicity studies shall be carried out on two animal species (preferably the rat should be one). The maximum dosage should not be higher than 2000 mg/kg b.w. 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 shall 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, shall be carried out. Tests shall be performed in the presence and absence of a 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* or *in vivo*.

The battery of tests suggested above does not imply, however, that other tests are inappropriate or that other tests would not be acceptable as alternatives. Tests for DNA integrity, metabolism and repair capacity would be useful optional additions to the proposed minimal package.

In all cases reasons for the choice of tests shall be given. Tests should 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 shall be performed using suitable labelled molecules and cover both single and multiple dose administration of the active substance over appropriate periods. Metabolism studies should also include investigation of the pharmacokinetics of the active substance and of the major metabolites. Consideration shall be given to species differences in the metabolism of the active substance in order to select the most relevant species for subsequent toxicological investigations.

3.4. Subchronic toxicity

These studies shall 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 shall be administered orally and a dose-response relationship shall be established. The duration in rodents shall 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 toxicity/carcinogenicity

Chronic toxicity studies shall be carried out on one species (preferably the rat), carcinogenicity studies preferably on two species of rodent. The substance shall be administered orally at several dose levels. A combined chronic toxicity-carcinogenicity study with in utero exposure is also acceptable. Experiments shall extend for a minimum of two years in rats and 80 weeks in mice. If continued beyond the minimum period, the test shall be terminated when survival in any but the highest dose level groups has fallen to 20 %. Full clinico-chemical, haematological and urine examinations shall be carried out at appropriate intervals throughout the experiment. Full macroscopic and histopathological examinations should 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 shall be carried out preferably on the rat. They shall extend over at least two filial generations and may be combined with embryotoxicity including teratogenicity studies. All relevant fertility, gestation, parturition, peri- and postnatal parameters shall be carefully observed and reported. Specific teratogenicity studies shall be carried out in at least two species, one being the rabbit.

3.7. Relay toxicity

In certain circumstances, particularly when all the components of the residues cannot be isolated or identified easily or if interactions between some components are

suspected, a relay toxicity study of at least 90 days may be performed in addition to the abovementioned toxicity studies.

3.8. 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 administered to laboratory animals.

3.9. Other relevant studies

Any further study providing additional information useful for the assessment of the test substance should be made available.