# GUIDELINES FOR THE ASSESSMENT OF ADDITIVES IN FEEDINGSTUFFS

## GENERAL ASPECTS

This document is intended as a guide for establishing dossiers on substances being submitted for authorization as additives in feedingstuffs. These dossiers should enable an assessment of the additives based on present state of knowledge and should ensure their compliance with the fundamental principles laid down for their admission, which are the subject of the provisions of Article 6(2) of Council Directive 70/524/EEC, of 23 November 1970, on additives in feedingstuffs (*).

For this purpose all the studies outlined in this document and schematized below (cf. Presentation of studies), may be required and, if necessary, additional information may be requested. However, no study which is obviously superfluous or inappropriate for an additive will be required.

As a general rule, all the information to establish the identity, physico-chemical properties, presentation, conditions of use, methods of determination and effectiveness of the additive, its balance, tolerance, biological and toxicological effects on target species must be provided, together with data on the effects on human health and the environment, which may result directly or indirectly from its use. The studies necessary for the evaluation of risks will depend essentially on the nature of the product and the circumstances of its use. In this respect, no strict rule is applicable. It is understood that additives intended exclusively for pet foods will not be subject to the same requirements as additives intended for feedingstuffs for productive livestock whose products are consumed by man or whose excrements could constitute a significant source of pollution for the environment.

A knowledge of the metabolism and fate of the additive in productive livestock is essential. In particular it will permit the determination of the extent of pharmacological and toxicological studies to be performed on laboratory animals in order to assess the risks for the consumer. However, this evaluation cannot be based solely on studies confined to determining the direct effects of the additives on laboratory animals. The latter do not provide specific information on the toxicity and bioavailability of residues resulting from the metabolism in the species for which the additive is intended.

These guidelines are applicable generally but require that the necessary documentation be adapted to each case. This documentation should include detailed reports, presented in the order and with the numbering proposed in these guidelines, and should be accompanied by a summary. The omission of any studies proposed in these guidelines should be justified. The publications quoted as references should be attached.

(*) OJ No L 270, 14.12.1970, p. 1

OBSERVATIONS

The term "additive", as used in this document, refers to the active substances in the state in which they will be incorporated in premixes and feedingstuffs. The evaluation of these additives will also take into consideration the preparations to be used in feedingstuffs and the possible use of the relevant active substances in human nutrition and human and veterinary therapy.

The guidelines will be up-dated as new scientific knowledge develops.

PRESENTATION OF STUDIES

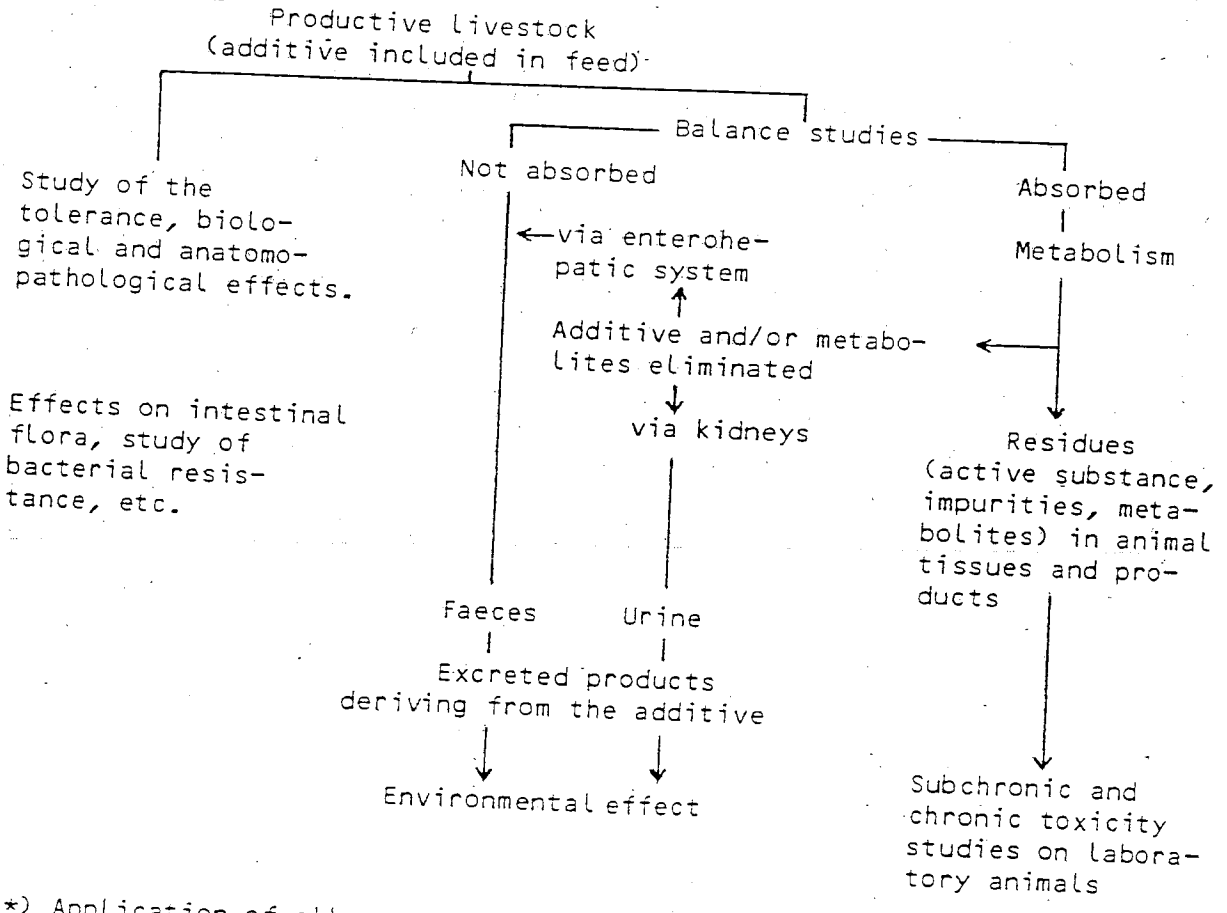
I. Identity, physico-chemical properties, presentation and conditions of use, methods of determination of the additive.

II. Studies concerning the effectiveness of the additive for the intended use.

III. Studies concerning the biological consequences of the use of the additive in feedingstuffs (for productive livestock, cf. scheme below).

IV. Other relevant studies.

Study scheme (*)




(*) Application of all or part of the scheme may be required, depending on the nature of the additive and the circumstances of its use.

SECTION I : IDENTITY, PHYSICO-CHEMICAL PROPERTIES, PRESENTATION AND CONDITIONS OF USE, METHODS OF DETERMINATION OF THE ADDITIVE.

1. IDENTITY

1.1. Chemical name of the additive, existing synonyms and abbreviations;

1.2. Molecular and structural formulae. If the active substance cannot be chemically defined or is a fermentation product, indicate empirical formula;

1.3. Degree of purity of the additive. Qualitative and quantitative data on any impurities present;

1.4. Manufacturing and purification processes, consistency of composition of the product in the course of production, methods used to check the consistency.

N.B. If the active substance is a mixture of two or more compounds, describe each compound separately and give their proportion in the mixture.

2. PHYSICO-CHEMICAL PROPERTIES

2.1. Physical properties of the additive (physical state, particle size, electrostatic properties, melting point, boiling point, decomposition temperature, density, vapour pressure, solubility in various solvents, UV and IR spectra, etc.);

2.2. Stability of the additive on exposure to atmospheric agents (light, temperature, moisture, etc.);

2.3. Stability of the additive in premixes and feedingstuffs during manufacture and storage. Incompatabilities with other components and possible decomposition products. Ability to obtain homogeneous mixtures at the proposed levels of incorporation in feedingstuffs.

3. PRESENTATION AND CONDITIONS OF USE

3.1. Proposed trade names for marketing the additive;

3.2. Proposed preparation for marketing the additive. Qualitative and quantitative composition of the premixes and their content of additive;

3.3. Intended use of the product in animal feed;

3.4. Proposed concentrations of the additive in feedingstuffs;

3.5. Other uses of the active substance in foodstuffs, human and veterinary therapy and the dosages to be applied. In each case list the trade names and indications for these uses.

4. METHODS OF DETERMINATION

4.1. Methods of analysis to determine the degree of purity of the additive, the nature and amount of impurities;

4.2. Qualitative and quantitative methods for the determination of the additive in premixes and feedingstuffs;

4.3. Qualitative and quantitative methods of analysis used to establish the balance sheet and the metabolism of the additive in livestock and its fate in the environment (determination of the active substance, its impurities and metabolites in organs and tissues, eggs, milk etc; determination of the nature of the excreted products, derived from the additive,

in excreta, manure, soil and water);

4.4. Qualitative and quantitative methods for the determination of residues in food (these methods should be specific and sensitive).

N.B. All the methods specified should be accompanied by information as to percentage recovery, specificity, sensitivity, possible interferences by other additives, limits of detection, margin of error. Reference standards of the additive, of the pure active substance and, if possible, of the main metabolites should be available.

SECTION II : STUDIES CONCERNING THE EFFECTIVENESS OF THE ADDITIVE FOR THE INTENDED USE

1. TECHNOLOGICAL AND ANIMAL PERFORMANCE STUDIES

1.1. Evidence of the effectiveness of the additive under the intended conditions of use

1.1.1. in technology, in comparison with reference feedingstuffs and, possibly, feedingstuffs containing additives of known effectiveness;

1.1.2. in animal production, in comparison with animals in control groups, and possibly, animals in groups receiving feedingstuffs containing additives of known effectiveness (effect on growth rate, feed conversion rate, morbidity, mortality, etc.).

1.2. Effect of the additive on nutritive, technological and organoleptic quality of carcasses, meat, offal, eggs, milk, etc.

2. EXPERIMENTAL CONDITIONS IN THE STUDIES ON ANIMAL PERFORMANCE

Give a detailed description of the tests performed and provide the following data :

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

2.2. Number of test and control groups; number of animals in each group (the number should be large enough for statistical analysis using suitable statistical parameters);

2.3. Dose-levels of the additive, percentage of the ingredients of the ration and detailed analysis thereof;

2.4. Location of each experiment, physiological and animal health conditions, rearing conditions (these should reflect those used in practice in the Community);

2.5. Date and exact duration of testing, date of examinations performed;

2.6. Adverse effects which occurred during the experiment and time of their appearance.

SECTION III : STUDIES CONCERNING THE BIOLOGICAL CONSEQUENCES OF THE USE OF THE ADDITIVE IN FEEDINGSTUFFS

The studies outlined in this section are intended to permit assessment of the safety in use of the additive in all the target species, and of the risks for man and the environment which could result directly or indirectly from this use. The data required may vary with the nature of the additive and the animal species concerned. A knowledge of the balance and fate of the additive will always be essential for determining the need for and the extent of the information required.

As a general rule, all the additives intended for feedingstuffs for productive livestock should be investigated to determine the nature of the excreted products, the fate of these products in manures, slurries, soils and water, and their effects on soil biology and aquatic life. For additives intended for feedingstuffs for livestock whose products are consumed by man, detailed studies are required on their metabolism (as indicated in the scheme outlined under "Presentation of studies") and on the residues in animal products (composition, persistence, pharmacological effects, subchronic and chronic toxic effects) in all cases where the balance studies indicate intestinal absorption. These studies should be performed on all animal species concerned under the practical conditions of use proposed for each additive.

1. STUDIES ON TARGET SPECIES

1.1. Study of the tolerance of the additive and of its biological, toxicological and anatomo-pathological effects. Determination of the safety coefficient (margin between the proposed maximum dose-level and that resulting in adverse effects);

1.2. Study of the balance of the additive (level of absorption, level of elimination in faeces, urine, etc.);

1.3. Study of the metabolism and pharmacokinetics of the additive (fate in the organism, absorption, distribution and elimination, etc.);

1.4. Study of the effects of the additive on microorganisms, in particular the microorganisms of the intestinal flora, and of the phenomena of bacterial resistance relating to the selection of bacteria with induced chromosomal parallel resistance to chemotherapeutics and R-plasmid carrying bacteria. Study of the effect of the additive on the colonization of pathogens in the intestinal tract.

2. STUDY OF THE RESIDUES IN ANIMAL PRODUCTS

2.1. Nature and concentration of the residues (active substance, impurities, metabolites) in tissues and organs, particularly in edible products (muscle, skin, liver, milk, eggs, etc.) after withdrawal of the supplemented feedingstuff;

2.2. Persistence, half-life value and kinetics of elimination of these residues. In some cases, studies on the effects of storage and cooking may be required.

3. STUDY OF EXCRETED PRODUCTS DERIVED FROM THE ADDITIVE

3.1. Nature and concentration of the excreted products, derived from the additive (active substance, metabolites), in urine and faeces;

3.2. Persistence, half-life value and kinetics of elimination of these products in manure, slurry, soil and water;

3.3. Their effects on soil biology, in particular on nitrifying bacteria, on plant growth and aquatic life;

3.4. Their effects on methane production.

4. PHARMACOLOGICAL AND TOXICOLOGICAL STUDIES ON LABORATORY ANIMALS

These studies may be carried out on the additive, on its residues and derivatives of these residues in edible animal products, which are likely to be toxicologically significant. As far as possible, attempts should be made to select laboratory animals showing metabolic similarities to the target species for which the additive is intended.
The test substance should be administered daily and continuously in the diet in all oral subchronic and chronic tests.

4.1. Acute toxicity

Acute toxicity studies should be carried out in at least two animal species, one of which being a rodent (preferably the rat). The test substance should be administered orally, at several dose-levels, preferably in logarithmic progression, in order to establish the LD 50. Detailed observations should be given of the biological effects during a period up to two weeks after ingestion.

4.2. Mutagenicity tests

Investigations of potential mutagenicity, including *in vitro* screening tests using metabolic activation systems may contribute to the evaluation of the toxicity.

4.3. Pharmacological studies

Appropriate investigations should be made to reveal any pharmacological activities.

4.4. Subchronic toxicity (at least 90 days)

In general, these studies should be carried out on two animal species, one of which being a rodent. The test substance should be administered orally at a minimum of three dose-levels. These should be chosen so as to determine a no-effect level and also a level permitting the establishment of a dose-response relationship. The experimental groups should contain an adequate number of animals of each sex. A control group should always be included.

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.

All relevant biological data should be recorded at appropriate intervals, particularly data on growth rate, feed consumption, haematology, urine analysis, biochemical parameters, mortality, organ weights, gross pathology and histopathology of major organs and tissues. If there is any evidence of specific toxic effects, these should be investigated to elucidate their origin and mechanism.

The results should be presented in detail and, as far as possible, should include statistical assessment.

4.5. Chronic toxicity

In general, chronic toxicity studies should be carried out on two species of rodents. The substance should be administered orally in at least three dose-levels. Experiments should extend for a minimum of two years in the rat and 80 weeks in mice. The experimental groups should contain an adequate number of animals of each sex. A control group should always be included. The experiment, if continued beyond the minimum period, should be terminated when survival in any but the highest dose-level group has fallen to 20 %.

The biological examinations mentioned above (cf. item 4.4) should be carried out at appropriate intervals throughout the experiment and on the surviving animals at the end of the experiment. For assessing carcinogenicity, particular attention should be paid to the time of appearance, the histological types of any observed tumours and their incidence. In certain cases, it may be necessary to investigate the possibility of transplacental carcinogenicity.

Reproduction studies should extend over at least two filial generations and may be combined with embryotoxicity including teratogenicity studies. Particular attention should be paid to fertility, fecundity and observation on post-natal development of litters.

The results should be presented in detail and, as far as possible, should include statistical assessment.

4.6. Relay toxicity

In certain circumstances - in particular, where it is not possible to isolate or identify the metabolites of the additive, or in order to obtain indications on the bio-availability of the residues - a relay toxicity study of at least 90 days may be desirable in addition to the studies required as indicated under items 4.1 to 4.5.

In this test, rats or other laboratory animals should be fed the edible products of target species which have received the additive in their ration. The experimental animals should then be examined as indicated in 4.4 and 4.5.

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

4.7. Experimental conditions

For all pharmacological and toxicological studies give a detailed description of the tests performed and provide the following data :

4.7.1. Species, breed, strain and sex of animals;

4.7.2. Number of test and control groups; number of animals in each group (the number should be large enough for statistical analysis using suitable statistical parameters);

4.7.3. Dose-levels of the test substance, percentage of the ingredients of the ration and detailed analysis thereof;

4.7.4. General rearing conditions throughout the period of testing;

4.7.5. Date and exact duration of testing; date of examinations performed;

4.7.6. Rate and timing of deaths for the various lots;

4.7.7. Pathological incidents which occured during the experiment and time of their appearance.

SECTION IV : OTHER RELEVANT STUDIES

Depending on the nature and the conditions of use of the additive, data on allergic effects, on irritation of the skin and mucous membranes of the eye, respiratory or digestive tract, and also tests on inhalation and percutaneous toxicity, using single and repeated doses, may be required to assess possible risks in handling premixes or supplemented feedingstuffs. If necessary the preventive measures and the means of protection to be applied should be indicated.