COUNCIL DIRECTIVE
of 18 April 1983
on the fixing of guidelines for the assessment of certain products used in animal nutrition

(83/228/EEC)

THE COUNCIL OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community,

Having regard to Council Directive 82/471/EEC of 30 June 1982 concerning certain products used in animal nutrition (1), and in particular Article 7 thereof,

Having regard to the proposal from the Commission,

Whereas Directive 82/471/EEC provides that the products belonging to certain groups must be examined on the basis of a dossier forwarded officially to the Member States and the Commission;

Whereas such dossiers must make it possible to verify that the products in question comply with the general principles laid down in the Directive in respect of the inclusion of new products in the Annex;

Whereas it has been found necessary to provide for the dossiers to be compiled in accordance with common guidelines defining, for each principle, the scientific data which make it possible to identify and characterize the products concerned and the studies necessary in order to evaluate their nutritional properties and biological effects; whereas these guidelines must be applicable on the date on which Directive 82/471/EEC itself enters into force;

Whereas the guidelines are intended primarily as a general guide; whereas, depending on the nature of the product or its conditions of use, the extent of the studies necessary in order to evaluate its properties or its effects may vary;

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 on the products listed in points 1.1 and 1.2 of the Annex to Directive 82/471/EEC are to be compiled in accordance with the guidelines set out in the Annex hereto.

Article 2

Member States shall bring into force the laws, regulations or administrative provisions necessary in order to comply with this Directive by 13 July 1984 at the latest. They shall forthwith inform the Commission thereof.

Article 3

This Directive is addressed to the Member States.

Done at Luxembourg, 18 April 1983.

For the Council

The President

I. KIECHLE

(1) OJ No L 213, 21. 7. 1982, p. 8.

ANNEX

GUIDELINES FOR THE ASSESSMENT OF CERTAIN PRODUCTS USED IN ANIMAL NUTRITION

General aspects

These 'guidelines' constitute a guide intended to establish dossiers on products listed in items 1.1 and 1.2 of the Annex to Directive 82/471/EEC, which have been obtained from culturing micro-organisms and which are likely to be admitted as a new source of proteins in animal nutrition. These dossiers should enable an assessment of such products based on the present state of knowledge and should ensure their compliance with the fundamental principles laid down for permitting their use, which are the subject of Article 6 (2) of the abovementioned Directive.

All the studies outlined in this document may be required and, if necessary, additional information may be requested. As a general rule, all the information necessary to establish the identity of the micro-organism and the composition of the culture medium, and also the manufacturing process, characteristics, presentation, conditions of use, methods of determination and nutritional properties of the product must be provided. The same applies to the information necessary to assess the tolerance of the product by the target species and the risks for man and the environment, which could result directly or indirectly from the use of the product. The toxicological studies required for this purpose will depend on the nature of the product, the animal species concerned and the metabolism of the product in laboratory animals.

The documentation to be provided 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 proposed studies should be justified. The publications quoted as references should be attached.

Observations

The term 'product', as used in these guidelines, refers to any proteinaceous product in the state in which it will be presented as feedingstuff or component of a feedingstuff.

Any modification in the manufacturing process or in the conditions of use of a product will require notification and, if necessary, additional documentation for a new assessment.

Presentation of studies

I. Micro-organism, culture medium and manufacturing process, characteristics of product, presentation and conditions of use, methods of determination

II. Studies on the nutritional properties of the product

III. Studies on the biological consequences of the use of the product in animal nutrition

IV. Other relevant studies

SECTION I

MICRO-ORGANISM CULTURE MEDIUM AND MANUFACTURING PROCESS, CHARACTERISTICS OF PRODUCT, PRESENTATION AND CONDITIONS OF USE, METHODS OF DETERMINATION

1. MICRO-ORGANISM

1.1. Classification, provenance, morphology, biological properties, any genetic manipulation.

1.2. Innocuity, possible survival outside the fermenter and any environmental consequences.

1.3. Constancy and purity of strains cultivated. Methods used to check these criteria.

2. CULTURE MEDIUM AND MANUFACTURING PROCESS

2.1. Composition of substrate, added substances, etc.

2.2. Manufacturing, dessication and purification processes. Devitalizing process for micro-organisms. Methods used to check the constancy of composition of the culture product and the detection of any chemical, physical and biological contamination during production.

2.3. Technical processes of preparation for use.

3. CHARACTERISTICS OF PRODUCT

3.1. Physical and physico-chemical properties: macro- and micro-morphology, particle size, density, specific weight, hygroscopicity, solubility, electrostatic properties, etc.

3.2. Chemical composition and characteristics.

3.2.1. Content of moisture, crude protein, crude fat, crude cellulose, crude ash, carbohydrates. Limits of variation of these contents.

3.2.2. Content of total ammonium, amide, nitrate and nitrite nitrogen, nucleic acids, protein. Qualitative and quantitative composition of total and free amino acids, and purine and pyrimidine bases.

3.2.3. Qualitative and quantitative composition of total lipids: fatty acids, non-saponifiable matter, lipid soluble pigments, phospholipids.

3.2.4. Composition of the carbohydrate fraction.

3.2.5. Qualitative and quantitative composition of inorganic components.

3.2.6. Qualitative and quantitative composition of vitamins.

3.2.7. Qualitative and quantitative composition of the other constituents: additives, residues of substrate and solvents, other potentially harmful residues of the metabolism of the substrate, of the culture medium, of the manufacturing process.

3.3. Microbiological contamination of the product.

3.4. Behaviour and stability of the product, as such and when mixed with feedingstuffs in current use, during storage.

4. PRESENTATION AND CONDITIONS OF USE

4.1. Proposed names of marketing the product.

4.2. Proposed formulations for marketing the product.

4.3. Intended use of the product in animal nutrition. Intended concentrations in the complete feedingstuffs and in the intended quantities in the daily rations for the animal species concerned.

5. METHODS OF DETERMINATION

Qualitative and quantitative methods for determination of the product in complete and complementary feedingstuffs.

NB: Description of these methods should be accompanied by information as to specificity, sensitivity, limits of detection, margin of error, possible interferences by other substances. Samples of the product in its various proposed presentations should be available.

SECTION II

STUDIES ON THE NUTRITIONAL PROPERTIES OF THE PRODUCT

1. ASSESSMENT OF PROTEIN VALUE

1.1. Chemical, biochemical and microbiological studies.

1.2. Studies on laboratory animals, compared with reference proteins.

2. STUDIES ON TARGET SPECIES

The following studies should be performed on each target species in comparison with a control group receiving, under the same conditions of nutritional balance, a diet in current use containing equivalent amounts of protein nitrogen, for ruminants of total nitrogen.

2.1. Protein and energy supplementation value of the product in the rations under the proposed conditions of use at various physiological stages of the animals (e.g. growing period, pregnancy, laying).

2.2. Influence of the product under the proposed conditions of use on growth rate, feed conversion rate, morbidity, mortality.

2.3. Optimum nutritional levels of incorporation of the product in the rations.

2.4. Effect of the product under the proposed conditions of use on the technological, organoleptic or other qualities of edible products of animal origin.

3. EXPERIMENTAL CONDITIONS IN THE STUDIES ON TARGET SPECIES

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

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

3.2. 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).

3.3. Levels of incorporation of the product, qualitative and quantitative composition of the ration and its analysis.

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

3.5. Exact duration of testing and date of the analyses performed.

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

SECTION III

STUDIES CONCERNING THE BIOLOGICAL CONSEQUENCES OF THE USE OF THE PRODUCT IN ANIMAL NUTRITION

The studies outlined in this section are intended to permit assessment of the safety in use of the product in the target species, and of the risks for man and the environment which could result directly or indirectly from this use. The toxicological studies required for this purpose will depend on the nature of the product, the animal species concerned and the metabolism of the product in laboratory animals.

1. STUDIES ON TARGET SPECIES

The following studies should be performed on each target species in comparison with a control group receiving, under the same conditions of nutritional balance, a diet in current use containing equivalent amounts of protein nitrogen, for ruminants of total nitrogen.

1.1. Maximum incorporation rates of the product in the daily ration without producing any adverse effect.

1.2. Possible effect of the product on fertility and reproduction, if appropriate.

1.3. Effects of ingestion of the product under the proposed conditions of use on micro-organisms of the flora of the alimentary tract and on colonization of pathogens in the alimentary tract.

1.4. Investigation under the proposed conditions of use of possible residues of the product (substrate, culture medium, solvents, contaminants) in edible products of animal origin.

1.5. Investigation under the proposed conditions of use of possible residues of the product (substrate, culture medium, solvents, contaminants) in excreta.

2. STUDIES ON LABORATORY ANIMALS

2.1. **Metabolism**

Fate of the product in the animal: absorption, accumulation, biotransformation, elimination.

2.2. **Mutagenicity**

Investigations of potential mutagenicity due to contaminants (in particular mycotoxins and bacteria) or residues of the product (substrate, culture medium, solvents) including *in vitro* screening tests using metabolic activation systems.

2.3. **Toxicological studies**

The following studies should be performed in comparison with control groups receiving, under the same conditions of nutritional balance, a diet in current use containing equivalent amounts of protein nitrogen. Toxic effects should be investigated to elucidate their cause and mechanisms and to ascertain that they do not result from nutritional imbalance or from an overdosage of the product in the diet.

2.3.1. *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 product should be administered in the daily ration in at least two levels of incorporation. These should be chosen so as to determine, if possible, a no-effect level and a level showing some adverse effect. The animal groups should contain an adequate number of subjects of each sex. A control group should always be included.

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

2.3.2. *Chronic toxicity*

In general, chronic toxicity studies should be carried out on two animal species, one of which being a rodent. The product should be admininstered in the daily ration in at least two levels of incorporation. Experiments should extend for a minimum of two years in the rat or 80 weeks in mice. The animal groups should contain an adequate number of subjects of each sex. A control group should always be included.

The biological examinations mentioned under point 2.3.1 should be carried out preferably on a small satellite group of animals (a group separated from and dependent upon the main group) at appropriate intervals throughout the experiment and on the surviving animals at the end of the experiment.

2.3.3. *Carcinogenicity*

For assessing carcinogenicity, particular attention should be paid to the time of appearance, the histological types of any observed tumours and their incidence. Any effect on the incidence of tumours and/or the incidence or progress of diseases should be assessed by reference to control groups, as indicated in paragraph 2.3. The results should be presented in detail and, as far as possible, should include statistical assessment.

2.4. **Other studies**

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. Any other method that is scientifically justifiable and likely to produce measurable results (e.g. relay toxicity) may be provided.

2.5. **Experimental conditions in the studies on laboratory animals**

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

2.5.1. Species, breed, strain and sex of animals.

2.5.2. 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).

2.5.3. Levels of incorporation of the product, qualitative and quantitative composition of the ration and its analysis.

2.5.4. General rearing conditions throughout the period of testing.

2.5.5. Exact duration of testing and date of examinations performed.

2.5.6. Rate and timing of deaths for the various test groups.

2.5.7. Clinical symptoms and pathological alterations which occurred during the experiment and time of their appearance.

3. STUDIES CONCERNING THE ENVIRONMENT

Depending on the nature of possible residues of the product (substrate, culture medium, solvents, contaminants) in excreta of target species, data on the fate of these residues in manure, soil and water and also their effects on soil biology, plant growth and aquatic life may be required.

SECTION IV

OTHER RELEVANT STUDIES

Depending on the nature and the conditions of use of the product, data on allergic effects, on irritation of the skin and mucus membranes of the eye, respiratory or digestive tract may be required to assess possible risks in handling the product and to prevent them.