Guide to the constitution of dossiers for the use of enzyme preparations in food

26 September 2003

Area of application

This guide applies to enzyme preparations intended for use in the manufacture of foods for human consumption. Its purpose is to specify the data required for the use of these enzyme preparations in conditions which guarantee consumer safety. It is designed to define an assessment process which reflects the different situations encountered and is based, in the context of current scientific knowledge, on the guidelines produced by the SCF (Reports of the Scientific Committee for Food "Guidelines for the presentation of data on food enzymes") Opinion expressed 11 April 1991, EUR 14181 EN, 1992)), currently followed for the preparation of dossiers.

It presents the information required for a scientific assessment when an application is being submitted for placing an enzyme preparation on the market. It also details the scientific procedure to be followed in situations relating to a marketing declaration, without prejudice to the data to be produced for the DGCCRF[1] pursuant to the Order of 5 September 1989 on the use of enzyme preparations in the manufacture of foodstuffs and beverages intended for human consumption. If, for a specific enzyme preparation, some of the data prove to be unnecessary or irrelevant, they may be omitted as long as the applicant presents evidence in support of the omission. This guide is subject to change and may be revised as required to reflect developments in scientific knowledge.

[1] DGCCRF: Direction générale de la concurrence, de la consommation et de la répression des fraudes [General Directorate for Fair Trading, Consumer Affairs and Fraud Control]

Validation of the document

The presentation and content of a dossier as described below, requesting the assessment of enzyme preparations for food use, were proposed by the "Biotechnology" Expert Committee of the Agence française de sécurité sanitaire des aliments[2]. This committee is tasked with assessing the risks to human and animal health from the use of enzyme preparations and novel foods containing or produced from Genetically Modified Organisms (GMO).

National and Community regulatory context

There are no European regulations on the use of enzymes as processing aids in the food industry. However, dossiers submitted by manufacturers and assessed by the scientific authorities are prepared based on the guidelines established by the SCF[3], issued in 1992.

In France, the use of enzymes in the food industry, whether as food additives or as processing aids, is covered by regulations based on the principle of a positive list. This means that enzyme preparations which have undergone expert assessment and are expressly permitted may be used in certain conditions. This variable list is therefore subject to updating and the addition of new enzymes, new preparations or new areas of application.

The Order concerning the use of enzyme preparations in the manufacture of foods and beverages intended for human consumption of 5 September 1989 defines the general framework for their use and provides a list of permitted enzymes.

Other legislation concerns the use of enzymes in food:
- the Order of 2 October 1997 on the additives which can be employed in the manufacture of foods intended for human consumption,
- the Order of 20 June 1985 permitting the use of lactase for lactose hydrolysis,
- the Order of 21 December 1988 giving a list of plant, animal and microbial proteases permitted for the preparation of protein hydrolysates intended for particular nutritional uses,
- the Order of 24 March 1993 on the use of beta–cyclodextrins and permitting cyclodextrin-glycosyltransferase from *Bacillus macerans* and *Bacillus circulans,*
- a series of Orders, dated 16 June 1993, 27 August 1993, 1 February 1994, 18 August 1994, 29 May 1997, 12 January 1998, 28 April 1998, 28 July 1999 and 14 November 2001, supplementing the Order of 5 September 1989 by permitting new enzymes.

It should be noted that, at present:
- enzymes of animal origin (pepsin, rennet…) used in cheesemaking, are the subject of a specific regulation in France: Circular of 20 January 1981 concerning rennet, bovine pepsin and acid proteases used in cheesemaking.
- the enzymes used in winemaking are covered by a European regulation: Commission Regulation (EC) No. 3220-90 of 7 November 1990 laying down conditions for the use of certain oenological practices provided for in Council Regulation (EEC) No. 822-87 (replaced by Regulation 1493/1999 of 17 May 1999).
- Directive 93/77/EC permits the use of pectolytic, proteolytic and amylolytic enzymes in fruit juice production.

Document application

This document states the information concerning enzyme preparations which is required for the constitution of dossiers for authorization of use requests, extension of use requests and marketing declarations, and defines the criteria for the consumer risk assessment.

Dossiers concerning enzyme preparations must include all the points listed in the section headed "required data". The responses must be scientifically substantiated. The data required for the dossier must be adapted to changes in technology. If some data prove to be unnecessary or irrelevant, they

[2] French Food Safety Agency
[3] Guidelines for the presentation of data on food enzymes, 1991. Reports of the Scientific Committee for Food, EUR 14181 EN, Commission of the European Communities , Brussels, Luxembourg

may be omitted as long as the applicant provides evidence to support their omission from the dossier. Conversely, if a doubt remains in the safety of use assessment, Afssa may require additional tests. Finally, if special or exceptional circumstances so require, the option remains of calling the applicant, at the either party's request – the applicant or the expert committee charged with the scientific assessment – to clarify some scientific or technical aspects.

Presentation of dossiers

This document proposes three types of dossier in which the requirements are tailored on a case-by-case basis for each one (cf. decision tree).

In the case of enzymes obtained using GMO, the dossier must be accompanied by the opinion on classification from the Commission de Génie Génétique (CGG)[4]. The sequences to be exploited (genetic construction) must also be supplied as a computer file.

Justification of the guide

The regulations define an "enzyme preparation" as an extract, purified to a greater or lesser extent, obtained from an animal, plant or micro-organism.

An industrial enzyme preparation therefore contains:

- a large mass of proteins, of which only some are defined, and some of which have an enzymatic action, in particular the one(s) being claimed,
- substances from fermentation in the case of microbial enzymes,
- substances added for standardisation and stabilisation purposes.

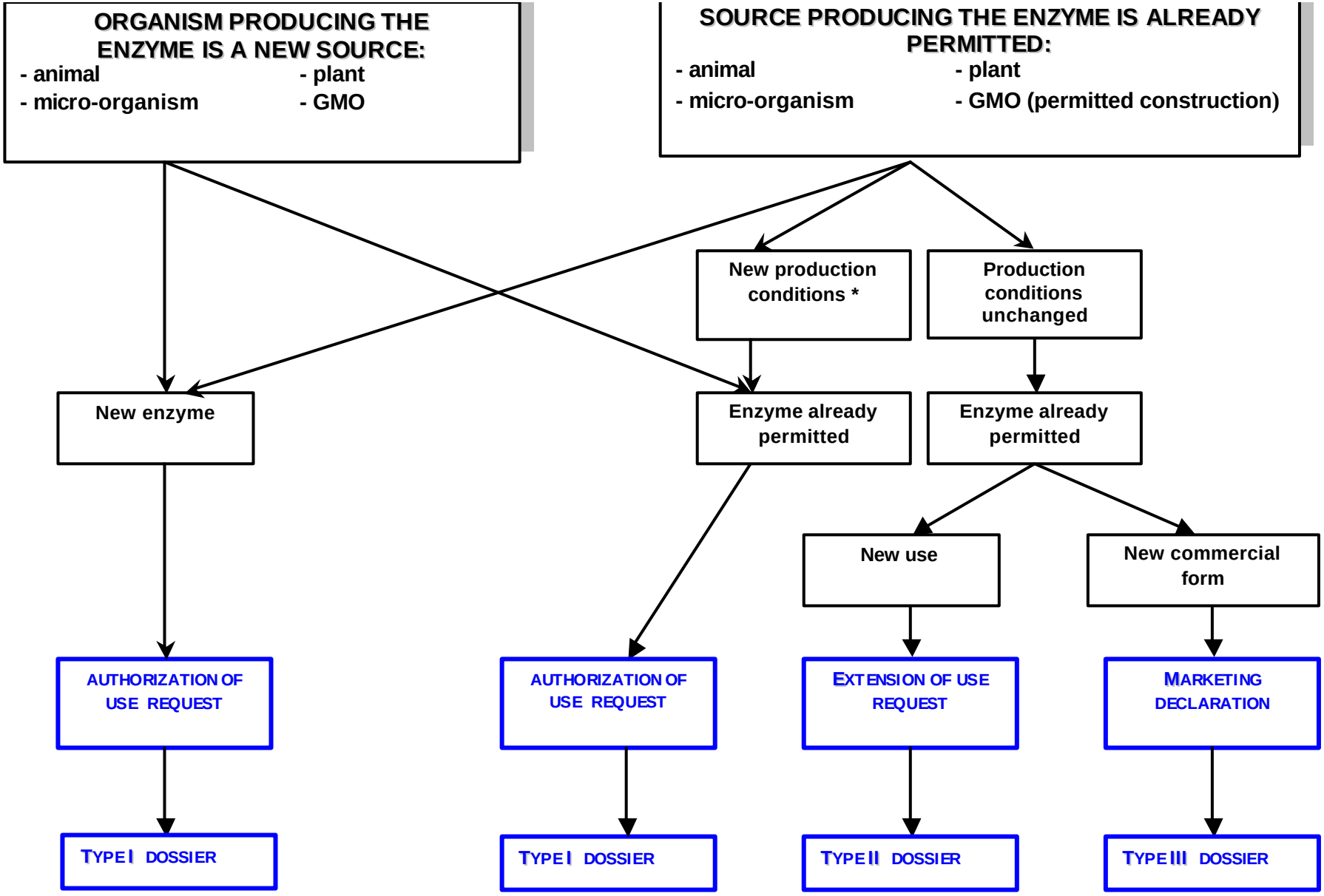
In addition, industrial preparations are standardised in terms of one or more enzymatic actions being claimed.

Hence the need to:

1- define precisely the enzymatic action claimed, including the specific action,
2- indicate the subsidiary enzymatic actions,
3- demonstrate the safety of use, generally through toxicological testing, given the heterogeneity of the preparation composition,
4- define the fate, during the industrial process, of the enzyme preparation in the presence of the food matrix: principal activity, subsidiary activity, reaction products; and provide evidence of the absence of any harmful effects on human health,
5- ensure source stability and reproducibility of processes used and the characteristics of the enzyme preparation, including quality assurance checks.

[4] French government committee on Genetic Engineering

DÉTERMINING THE TYPE OF DOSSIER TO BE CONSTITUTED



* This concerns any significant modification of the fermentation process (for microbial enzymes) or the extraction, purification and standardisation phases.

DATA TO BE SUPPLIED FOR THE 3 TYPES OF DOSSIER
(cf. decision tree)

Type I dossier[5]**: authorization of use request:** new enzyme preparation from a new or already permitted source.

Type II dossier[5] **: extension of use request:** an enzyme preparation which is already permitted, from a source organism which is already permitted.

Type III dossier[6]**: marketing declaration:** the enzyme preparation is already permitted for the same use.

Any "marketing declaration" for a given enzyme preparation, already permitted for a defined use, should concern a preparation obtained using the protocol involved in the authorization (source organism[7], process, etc.) and presenting exactly the same properties.

Data	Elements to be supplied	Type I dossier	Type II dossier	Type III dossier
Administrative data	- Name of applicant	X	X	X
	- Manufacturer	X	X	X
	- Person responsible for the dossier	X	X	X
Technical data	I. active components of the enzymE preparation	X	X*	X*
	II. THE SOURCE ORGANISM	X	X*	X*
	III. PRODUCTION process	X	X*	
	IV. carriers and other additives and ingredients	X	X*	X*
	V. Usage of the enzyme	X	X	X*
	VI. Stability and fate of the enzyme preparation in the foodstuff	X	X	
General requirements and specifications	I. Hygiene	X		
	II. Contaminants	X	X*	X
Data on safety in use	I. toxicological data required	X	X **	
	II. Exemptions from the required toxicological data	X (if relevant)		

* reference to the current authorisation and summarised data
** calculation of the new safety factor

[5] This type of dossier is assessed by Afssa
[6] Declaration to the DGCCRF pursuant to the Order of 5 September 1989 on the use of enzyme preparations in the manufacture of foods and beverages for human consumption
[7] Indexed strain for example

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PRESENTATION OF "STANDARD" DOSSIER

A. ADMINISTRATIVE DATA

- Name of applicant
- Manufacturer
- Person responsible for the dossier

B. TECHNICAL DATA

I. ACTIVE COMPONENTS OF THE ENZYME PREPARATION

I.1. PRINCIPAL ENZYMATIC ACTIVITY

Classification of the enzyme according to the international nomenclature when it is listed there and its reference characteristics.

I.2. ACTIVITY OF THE ENZYME PREPARATION

As complete a characterisation as possible based on current scientific data on the enzyme preparation: mode of function of the enzyme with a reference substrate and in the conditions of use being claimed, influence of environmental conditions, pH and temperature.

Measurement of the enzymatic activity shall be determined if possible based on a reference method using a reference substrate. When the industrial substrate is complex, the applicant must present a defined, reproducible and validated protocol. The activities should, if possible, be expressed as International Units (IU); otherwise, and where technically possible, the equivalents in IU shall be supplied.

I.3. SUBSIDIARY ENZYMATIC ACTIVITIES

The applicant must list the subsidiary activities, specifying those likely to cause a health risk, for example, protease and phospholipase activities (due to their action on the mucous membranes) and those intervening from a technological point of view.

The subsidiary activities must be quantified if possible and their proportion must be reproducible. They must be subject to regular checks.

A 2D electrophoretic analysis of the enzyme preparation, after the purification process and before the addition of adjuvants, should be available: this analysis enables visualisation of the complexity level of the enzyme preparation in the presence of molecular weight markers.

II. THE SOURCE ORGANISM

II.1. SOURCE

II.1.1. ANIMAL

The animal and/or the part of the animal used must be identified. Animal tissue used for the preparation of enzymes must comply with human consumption and its use must be in accordance with good hygienic practice. It must be free of all risk of infectivity (for example: bovine spongiform encephalopathy (BSE) agent and in conformity with the regulations on preventing transmissible spongiform encephalopathies (TSE) in France.

II.1.2. PLANT

The plant and part of the plant used to obtain the enzyme preparation must be precisely identified and any toxicity must be discussed.

II.1.3. MICRO-ORGANISM

Strains of micro-organisms used to obtain the enzyme preparation can be indexed strains or variants or derived from indexed strains or variants by the process of successive serial culture or by genetic modification (they are then considered as new strains). These source strains or those deriving from them must be neither pathogenic nor toxigenic. They must be stable, characterised and their origin must be established (description available in a register kept by the manufacturer). The number of the source strain must be supplied.

Conditions for storage of the strains, their industrial preculture and culture must guarantee the absence of strain drift and ensure reproducibility between the different batches of enzyme preparation. These procedures must ensure the absence of any toxin production by the source organism and prevent the introduction of contaminant micro-organisms which could be the source of undesirable substances in the final enzyme products.

II.2. TAXONOMY

Applicants are recommended to refer to the OECD document [8] (draft).

II.3. GENEALOGY AND GENETIC MODIFICATION

Most, if not all, of the enzyme preparations produced by recombinant organisms and on which an Afssa opinion is required, are derived from micro-organisms classified in Group 1: Genetically Modified Micro-organisms unlikely to cause human, animal or plant disease or to have negative effects on the environment.

II.3.1. GMO COVERED BY THE AREA OF APPLICATION OF DIRECTIVE 90/219 [9]

The dossier must provide precise information on the host organism, the vector system (for example: plasmid) and the DNA sequence incorporated in the vector used as an expression system or in the chromosome. For a plant, an animal or a micro-organism, the donor organism must also be identified.

It is important to have detailed knowledge of the genetic structures involved, so that any undesirable interaction between the original genetic material of the host and the new genetic material to be inserted can be anticipated.

Data on:

[8] OECD - draft Guidance Document on the use of Taxonomy in Risk Assessment of Micro-Organisms: Bacteria

[9] Council Directive 90/219/EEC of 23 April 1990 on the contanined use of genetically modified micro-organisms

- presence of extra DNA (plasmids or foreign DNA incorporated in the host chromosome),
- specific genetic characteristics ("markers"),
- genetic stability (mutation rate and factors influencing the mutation rate, intra and intermolecular recombinations, restriction barriers),
- gene transfer (mobilization/conjugation ability) and
- resistances (to antibiotics, heavy metals)

will be used to predict undesirable effects on human health, animals, plants and ecological behaviour.

Precise knowledge of the identity and properties of the vectors forms the basis for evaluating whether their introduction increases or reduces the safety level of the host micro-organism. A vector should be characterized at DNA level (size, restriction map and full sequence) and notably genetically with respect to genes that could be used as markers, as well as functional elements, which can be presented on disk. A vector must be free of sequences which could lead to the synthesis of harmful elements as well as non-conjugative and non-mobilizable.

A complete sequence of the DNA inserted in the host organism and a molecular characterization must be supplied: number of inserted genes, chromosome location, type of regulation (promotor properties), functional gene products. Characterization of the sequences at the insertion borders is required. If the DNA sequences come from a micro-organism, a plant or an animal, the exact origin of the inserted DNA and the characteristics of the genetic construct and any modifications must be given to enable a safety assessment to be carried out.

Each recombinant product is to be evaluated on a case-by-case basis, considering the host, the vector and the insert, as the potential hazard from the final recombined product may be greater than the sum of the hazard from each of these events taken individually.

Biologically active DNA[10]:

Enzyme preparations are subject to toxicological testing.

1) As long as these tests do not show any toxic effect, the presence of DNA from the genetically modified organism in the enzyme preparation presents no more risk than that of the DNA of the non-modified organism itself. There is therefore no need to take particular precautions in this case.

2) If these tests show a toxic effect or if the micro-organism is not classified “C1 L1”[11], a specific study on the quantity of residual DNA from this micro-organism must be carried out and its possible risks assessed.

[10] DNA of a size and structure such that it could lead to the expression of a protein in an organism" - GMO glossary from http://www.finances.gouv.fr/ogm/ website

[11] Cf. the French « Commission du génie génétique » classification

II.3.2. SELF-CLONED MICRO-ORGANISMS

Self-cloned micro-organisms, recognised as such by the CGG, are not covered by directive 90/219. They are deemed to be new strains.

II.4. STRAIN MONITORING

Strain monitoring must be guaranteed at several levels:

1. presenting a regular strain characterisation, a strain number corresponding to a reference strain, and monitoring to ensure absence of strain drift,
2. providing evidence of internal quality control within the company with strain monitoring: register recording all modifications to the strain (laboratory notebook).

II.5. SAFETY OF THE SOURCE ORGANISM

Afssa's Recommendations of November 2002 on the presentation of data for assessing the safety of micro-organisms used in the feed/food sector may be taken as the reference.

With reference to the Opinion[12] of the Scientific Committee on Animal Nutrition (SCAN) of February 2000, certain source micro-organisms, notably *Bacillus* sp., may require testing for toxins.

III. PRODUCTION PROCESS

Equipment, description of the different stages in the process, compliance with good manufacturing practice, description of inspection methods and their frequency, selection of raw materials, monitoring of parameters (temperature, pH, etc.), etc.

The manufacturer is responsible for ensuring that any adaptation or modification of the production and purification processes does not in any way alter the properties and the qualities of the product and that it remains identical. Otherwise, the enzyme preparation must be considered to be new.

III.1. INFORMATION ON THE METHOD OF PRODUCTION OR FERMENTATION

In the case of animal or plant enzyme sources, details must be given on the selection of organs and tissue and the extraction process. In the case of microbial sources, it is essential that full information is supplied on fermentation conditions: composition of media, culture parameters. All components used must be of food grade quality.

III.2. PURIFICATION PROCEDURE

The applicant must supply full information (equipment, aids used, etc.) on purification procedures.

[12] Opinion of the Scientific Committee on Animal Nutrition on the safety of use of Bacillus species in animal nutrition (expressed on 17 February 2000) – European Commisssion – Health & Consumer Protection Directorate-General

IV. CARRIERS AND OTHER ADDITIVES AND INGREDIENTS

IV.1. CARRIERS, DILUENTS, STABILISERS AND OTHER ADDITIVES AND INGREDIENTS

Article 5 of the Order of 5 September 1989 contains a positive list of carriers and preservatives which may be used. The use of any new substance belonging to these categories must be the subject of a specific licence application. Information must be supplied on the carriers, diluents, stabilisers and other additives, as well as on the processing aids and ingredients used in production, formulation and packaging, depending on the use of the enzyme preparations. These substances must be compatible with the intended food use of the enzyme preparations concerned or be completely eliminated from the food product after processing.

IV.2. IMMOBILIZED ENZYMES

For immobilized enzymes, the carriers and immobilization agents used must be in conformity with the regulations[13] on the relevant use. When new materials are being considered, they should be tested to prove no harmful residues might leak out into the food.

Reference may be made to the specific provisions in Council Directive 89/109/EEC of 21 December 1988 on the approximation of the laws of the Member States relating to materials and articles intended to come into contact with foodstuffs[14]. Appropriate tests should ensure any leakage of immobilised agents is controlled and remains within safe limits.

IV.3. TOTAL ORGANIC SOLIDS (TOS)

TOS (Total Organic Solids) are defined as the sum of the organic components excluding diluents and ingredients found in the final preparation.

% TOS = 100 - (% ash + % water + % diluents and/or other additives and ingredients).

TOS enables comparison of the percentage of active elements from one preparation to another.

V. USAGE OF THE ENZYME

V.1. TECHNOLOGICAL FUNCTION OF THE ENZYME

Enzyme usefulness.
Where appropriate, advantages compared with other processes already in use.

V.2. TYPES OF FOODSTUFFS IN WHICH THE ENZYME WILL BE USED

V.3. RECOMMENDED AND MAXIMUM QUANTITIES TO BE USED IN EACH FOODSTUFF

VI. STABILITY AND FATE OF THE ENZYME PREPARATION IN THE FOODSTUFF

VI.1. INACTIVATION OF THE ENZYME PREPARATION IN THE FINAL PRODUCT

[13] Order of 5 September 1989 on the use of enzyme preparations in the manufacture of foodstuffs and beverages intended for human consumption.
[14] Council Directive 89/109/EEC of 21 December 1988 on the approximation of the laws of the Member States relating to materials and articles intended to come into contact with foodstuffs - Official Journal No. L 040 of 11/02/1989 p. 0038 - 0044

VI.1.1. INACTIVATION

Scientific data on the stability of the enzyme and its inactivation conditions. This will enable inactivation during the process to be assessed if necessary.

VI.1.2. FATE OF THE ENZYME PREPARATION IN THE PROCESSED PRODUCT (CF. I.1. AND I.2.)

The applicant must supply data on the behaviour of the enzyme preparation in the presence of the food substrate and in the conditions of the defined industrial process for obtaining the final product. If, for safety reasons, certain enzymes have to be inactivated (proteases, phospholipases, transglutaminases, peroxydases, etc.) experimental studies must be carried out to demonstrate the inactivation of the preparation in terms of both its principal and subsidiary activities in the final consumer product.

VI.2. REACTION PRODUCTS

The dossier must present elements guaranteeing the safety of the main reaction products and any possible reaction products not considered normal constituents of the diet.

VI.3. POSSIBLE EFFECTS ON OTHER COMPONENTS OF THE FOOD, PARTICULARLY NUTRIENTS

VI.4. ALLERGENICITY

The protein, even when inactivated, remains in the final product and may retain any allergenic potential. Thermal denaturation may maintain allergenic potential or unmask allergenic epitopes.

There is currently no recognised animal experimentation method available enabling evaluation of the power of a biochemical substance to cause allergic and/or intolerance reactions in sensitive individuals following oral exposure. All the information available permitting the allergic or intolerance risk from the enzyme preparation to be assessed must be supplied (for example analogy study of the chemical structure with a known allergen…).

C. GENERAL REQUIREMENTS AND SPECIFICATIONS

I. HYGIENE

I.1. GOOD MANUFACTURING PRACTICE

Enzyme preparations must be produced in conformity with good manufacturing practice. Stocks of micro-organism cultures used as sources for enzyme preparations must be tested regularly to ensure their purity.

I.2. GOOD PRACTICE IN USE

The method of use of the enzyme preparation must not cause any alteration in the processed product or microbial contamination.

II. CONTAMINANTS

These specifications must be supplied experimentally by means of tests on several manufactured batches.

II.1. HEAVY METALS

Enzyme preparations must not contain toxicologically significant amounts of heavy metals such as lead, cadmium, arsenic and mercury. Levels of heavy metals must be below the thresholds fixed by the Order of 5 September 1989, stated in Annex I.

II.2. MICROBIOLOGICAL CONTAMINANTS

Levels of microbiological contaminants must be below the thresholds fixed by the Order of 5 September 1989, stated in Annex I.

II.3. ABSENCE OF SOURCE MICRO-ORGANISM

Tests must be performed to ensure that viable cells from the source micro-organism are not present in the final enzyme preparation.

II.4. ABSENCE OF ANTIBIOTIC ACTIVITY

Enzyme preparations must not contain any antibiotic activity. The test method used must be stated.

II.5. ABSENCE OF SECONDARY TOXIC METABOLITES

Enzyme preparations must not contain detectable levels of toxic secondary metabolites pursuant to the Order of 5 September 1989 (cf. Annex I). When a given source is known to be likely to produce toxins, the absence of these metabolites must be demonstrated using a suitable method (refer to recommendations by the JECFA[15] and SCAN[16]).

[15] General considerations and specifications for enzyme proparations from genetically modified microorganisms. Joint FAO/WHO Expert Committee on Food Additives (JECFA) FAO Food and Nutrition Paper 52, Addendum 6, pages 215-218; Joint FAO/WHO Expert Committee on Food Additives, 51st session, Geneva, 9-18 June 1998.

[16] Opinion of the Scientific Committee on Animal Nutrition on the safety of use of Bacillus species in animal nutrition (expressed on 17 February 2000) – European Commisssion – Health & Consumer Protection Directorate-General

D. DATA ON SAFETY IN USE

As a preamble, mention may be made, when this information is available, of the GRAS (Generally Recognized As Safe) status of enzyme preparations granted by the FDA, and/or the QPS (Qualified Presumption of Safety) status of strains granted by the EC.

I. TOXICOLOGICAL DATA REQUIRED

I.1. ENZYMES DERIVED FROM PLANTS OR ANIMALS

Toxicological tests are not normally required. Data on non-infectivity must be supplied based on the classification of the tissues in terms of their infectious titer in natural diseases established by the WHO[17] (1992). When parts of plants or animals which are not usually considered as being normally part of the diet are used, toxicological tests may be required unless satisfactory documentation on safety in use is supplied.

I.2. ENZYMES DERIVED FROM MICRO-ORGANISMS

Toxicological tests must, where possible, be performed on a batch from the final purified fermentation product, before any addition (carriers, diluents, etc.). They must be performed in accordance with the established guidelines (EC/OECD[18].). Because of the effects exerted at cellular level by the proteinaceous nature of certain enzyme preparations, some modifications of the standard test protocols, especially in the case of *in vitro* tests, may be necessary, in view of the nature or the activity of certain enzyme preparations. Such amendments will be acceptable if accompanied by relevant supporting evidence.

I.2.1. ORAL TOXICITY TEST

The single dose toxicity test is not justified in terms of the information provided. A 13-week sub-chronic oral toxicity test is recommended. It must be performed in young rodents.

I.2.2. GENOTOXICITY TEST

I.2.2.1. Mutagenesis test

It could be considered that in most cases, a direct mutagenic effect from the macromolecules is unlikely. However, one cannot exclude the effect of their degradation products or of impurities in the enzyme preparation. This makes a mutagenicity test necessary, such as an Ames test.

I.2.2.2. Clastogenicity test

A chromosomal abnormality test on human lymphocytes *in vitro* and/or the micronucleus *in vivo*.

I.2.3. CONSUMPTION SAFETY FACTOR

Its calculation must include:
- the no effect dose established from the results of the subchronic toxicity test used and presented in the dossier,
- determination of the residual quantities of enzyme (or enzyme preparation) in the foodstuffs, based on the maximum dose considered,
- the consumption levels of the final products. For this, the uses already permitted in the same conditions must be taken into account when calculating the safety factor.

[17] Report of the WHO consultation on public health issues related to animal and human spongiform encephalopathies - Geneva. 12-14 November 1991 (WHO/CDS/VPH/92.104).
[18] Organisation for Economic Co-operation and Development 1981. Guidelines for the testing of chemicals

II. EXEMPTIONS FROM THE REQUIRED TOXICOLOGICAL DATA

It is important that toxicological tests are performed on every specific enzyme preparation produced from a microbial source. Special circumstances may enable the applicant to dispense with the full battery of tests. These situations must be examined and decided on a case-by-case basis.

Moreover, there may be circumstances in which additional tests over and above the basic requirements will be required to resolve questions arising in particular situations.

E. LIST OF ANNEXES

REFERENCES

1. Circulaire du 20 janvier 1981 relative à la présure, pepsine bovine et protéases acides utilisées en fromagerie.

2. Organisation for Economic Co-operation and Development 1981. OECD guidelines for the testing of chemicals.

3. Arrêté du 20 juin 1985 relatif à l'emploi de lactose hydrolysé dans certaines denrées alimentaires. JORF, 13.07.85 modifié par l'arrêté du 14.10.91 (JO du 10.12.91).

4. Arrêté du 21 décembre 1988 relatif aux hydrolysats de protéines dans la fabrication des aliments destinés à une alimentation particulière. JORF du 07.01.89.

5. Council Directive 89/109/EEC of 21 December 1988 on the approximation of the laws of the Member States relating to materials and articles intended to come into contact with foodstuffs - Official Journal No. L 040 of 11/02/1989 p. 0038 - – 0044.

6. Arrêté du 5 septembre 1989 relatif à l'emploi des préparations enzymatiques dans la fabrication des denrées et boissons destinées à l'alimentation humaine. JORF, 01.10.89 modifié par les arrêtés du 15.06.93 (JO du 10.07.93), du 27.08.93 (JO du 04.09.93), du 01.02.94 (JO du 25.02.94), du 18.08.94 (JO du 11.09.94), du 29.05.97 (JO du 03.06.97), du 12.01.98 (JO du 08.11.97), du 12.01.98 (JO du 04.02.98), du 28.04.98 (JO du 19.05.98), du 28.07.99 (JO du 22.08.99), du 14.11.01 (JO du 07.12.01).

7. Council Directive 90/219 EEC on the confined use of genetically modified micro-organisms

8. Guidelines for the presentation of data on food enzymes, 1991. Reports of the Scientific Committee for Food, EUR 14181 EN, Commission des Communautés Européennes, Bruxelles, Luxembourg.

9. Report of the WHO consultation on public health issues related to animal and human spongiform encephalopathies - Geneva. 12-14 November 1991 (WHO/CDS/VPH/92.104).

10. Arrêté du 24 mars 1993 relatif à l'emploi de beta–cyclodextrine comme auxiliaire technologique. JORF, 27.03.93, modifié par l'arrêté du 18 avril 1994 (JO du 26.04.94).

11. V. Larreta-Garde, Enzymes en agroalimentaires, Tec & Doc 1997.

12. Arrêté du 2 octobre 1997 relatif aux additifs pouvant être employés dans la fabrication des denrées destinées à l'alimentation humaine. (JO n°260 du 08.11.97).

13. General considerations and specifications for enzyme preparations from genetically modified micro-organisms. Joint FAO/WHO Expert Committee of Food Additives (JECFA) FAO Food and Nutrition Paper 52, Addendum 6, pages 215-218; Joint FAO/WHO Expert Committee on Food Additives, 51st session, Geneva, 9-18 June 1998.

14. Opinion of the Scientific Committee on Animal Nutrition on the safety of use of Bacillus species in animal nutrition (expressed on 17 February 2000) – European Commission – Health & Consumer Protection Directorate-General.

15. Reports on tasks for scientific co-operation. Study of the enzymes used in foodstuffs and collation of data on their safety. Directorate-General for Health and Consumer Protection. December 2000.

16. EC Regulation No. 1622/2000 as regards the use of lysozyme in wine products, amended by commission regulation (EC) No. 2066/2001 of 22 October 2001.

17. Recommandations de l'Afssa relatives à la présentation des données permettant l'évaluation de l'innocuité des micro-organismes utilisés dans le secteur agro-alimentaire - novembre 2002.

18. OECD – 2003- draft Guidance Document on the use of Taxonomy in Risk Assessment of Micro-Organisms: Bacteria.

19. On a generic approach to the safety assessment of micro-organisms used in feed/food production. European Commission 2003. Working paper open for comment.

ANNEX I:

Specifications in the Order of 5 September 1989 on the use of enzyme preparations in the manufacture of foodstuffs and beverages intended for human consumption

Specifications regarding heavy metals:

Cadmium	< 0.5 mg/kg
Mercury	< 0.5 mg/kg
Arsenic	< 3 mg/kg
Lead	< 10 mg/kg

Specifications regarding microbiological contaminants:

Total viable mesophilic aerobic bacteria	< 50 000 / g
Salmonella	none in 25 g product
Coliforms	< 30 / g product
Anaerobic sulfito-reducing bacteria	< 30 / g product
Staphylococcus aureus	none in 1 g product
Mycotoxin and other toxic metabolites	no detectable amount