参考資料１－４

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# food science and techniques

## REPORTS OF THE SCIENTIFIC COMMITTEE FOR FOOD

**(Twenty-seventh series)**

Foods intended for weight control diets
*(Opinion expressed on 19 October 1990)*

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*(Opinion expressed on 11 April 1991)*

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*(Opinion expressed on 21 June 1991)*

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# Guidelines for the presentation of data on food enzymes

*(opinion expressed 11 April 1991)*

## Introduction

These guidelines cover enzyme preparations intended for use in the preparation of food. Some enzyme preparations are used as processing aids and others as true food additives. Whilst this distinction may be of administrative importance, from a toxicological point of view it is not pertinent to distinguish between these two categories since, in both cases, the enzyme preparations may remain in the food.

These guidelines should be considered as setting out the minimum requirements for information to be supplied to the Scientific Committee for Food (SCF) in connection with any request for an evaluation of the safety in use of an enzyme preparation. If, for a specific enzyme preparation, some of the requirements are considered irrelevant, they may be omitted provided satisfactory supporting arguments are presented.

The **safety issues** involved in the use of enzyme preparations can roughly be split into the following categories:

1. **Toxicological properties of the enzyme preparation** (i.e. the active enzyme as such and any byproducts and contaminants). Care should be taken to ensure that toxic contaminants are not present in the enzyme preparation (e.g. mycotoxins and antibiotics in enzymes of microbial origin).

   In order to ensure a consistent and safe product there is a need:

   a) for process specifications including appropriate quality assurance checks;
      and
   b) to ensure that the characteristics of the source material or organism do not change with time.

2. **Quantity of enzyme consumed.** This in turn depends on the amount added to food, the concentration in the food when consumed, the number of different foods in which it may be used and the frequency with which these foods are consumed.

3. **Allergies and irritations caused by enzyme activity in the final product.** This is primarily considered to be an occupational health problem when workers are handling concentrated enzyme preparations. There are no confirmed cases of allergies induced in consumers through intake of enzyme treated food.

4. **Unintended reaction products in the food caused by enzymatic reactions in the final foodstuffs** (e.g. transformation of histidine to histamine). The possibility of any adverse health effects from this cause should be addressed in the submission.

5. **Safety of the source organism (microbials).** The use of pathogenic microorganisms is primarily of occupational concern as viable cells from the source organism should not be present in the final commercial enzyme, but as a general principle pathogenic microorganisms should not be used in the production of food enzymes

With regard to the **toxicological properties** of enzyme preparations, enzymes which are derived from edible parts of plants and animals are generally considered as posing no health problems. No special documentation for safety need be supplied provided that the potential consumption following normal use does not lead to an intake of any components which is larger than can be expected from normal consumption of the source as such, and provided that satisfactory chemical and microbiological specifications can be established.

For enzymes derived from microorganisms, it is very important to ensure that the source does not produce toxic compounds which can remain in the final product. It is important to carry out toxicological test programmes on all the individual strains used in the production of specific enzyme preparations since:

1. Different strains belonging to the same species can behave differently. For many microorganisms it is known that some of the strains in one species are harmless, while others belonging to the same species are toxic.
2. For some fungal genera, especially *Penicillium* and *Aspergillus*, there have been many misidentifications of fungal isolates. As a consequence of this, there is a risk of misclassification of fungal strains. For example in some cases it has been difficult to distinguish *A. oryzae* from *A. flavus* which has the ability to produce aflatoxin. As long as there is a risk of misidentification of microbial isolates, it is very important that the microorganism used is correctly identified and, in case of doubt, the identity should be verified by an independent, recognized laboratory.
3. The ability of a microorganism to produce toxins depends – qualitatively and quantitatively – on environmental factors such as the composition of fermentation media, pH, temperature and fermentation period. Therefore there is a risk that a microorganism which does not produce toxins under some conditions will turn out to be toxin-producing under other conditions.
4. The continuous selection processes applied to source microorganisms in order to maximize and optimize enzyme production may result in spontaneous mutations which give rise to the possibility of changing a non-toxic strain to a toxic strain.
5. There is a considerable potential to apply new techniques of genetic modification in the production of food enzymes. Along with the introduction of desirable traits, there is also the potential for introducing toxin production and therefore there is a need

explicitly to characterize and evaluate the genetic construct as to host, vector and insert (see section 2.4 under "Information to be supplied").

# Information to be supplied for the evaluation of an enzyme preparation to be used in foodstuffs

## Administrative data

Name of applicant, manufacturer(s) of enzyme and person responsible for the dossier.

## Technical data

### *1. Active components*

**1.1** **The principal enzyme activities** are to be characterized by their systematic names and Enzyme Commission numbers.[1]

**1.2** **The activity of the enzyme preparations** should be measured according to the reaction catalysed by individual enzymes and should usually be expressed in activity units per unit weight or volume of preparation as appropriate. In commercial practice the activity of the product is sometimes also given as the quantity of the enzyme preparation to be added to a given quantity of food in order to achieve the desired effect.

**1.3** **A list of subsidiary enzymatic activities**, whether they perform a useful function or not.

### *2. Source materials*

If any specific source is likely to contain substances which may be harmful to health, the absence of such substances in the enzyme preparation shall be shown (see section 8.6).

**2.1** **Animal sources.** The animal and the part of the animal used in the preparation must be identified. Animal tissues used for the preparation of enzymes must comply with meat inspection requirements and be handled in accordance with good hygienic practice.

**2.2** **Plant sources.** The plant and the part of the plant used in the preparation must be identified.

**2.3** **Microbial sources** used in the production of enzyme preparation can be native strains or variants of microorganisms, or be derived from native strains or variants by the process of selective serial culture or genetic modification. They must be discrete and stable strains or variants which are sufficiently well characterized according to well accepted identification keys, to enable them to be assigned unique identities as the

sources of the enzyme preparations which are the subject of individual specifications (see point 2 relating to enzymes derived from microorganisms in "Introduction").

The type cultures of the production microorganisms must be maintained under conditions which ensure the absence of strain drift and when prepared for use in the production of enzyme preparations they must be subjected to methods and culture conditions which ensure consistency and reproducibility from batch to batch. These procedures must ensure the absence of toxin production by the source organism and prevent the introduction of foreign microorganisms which could be the source of toxic materials and other undesirable substances in the final enzyme products.

2.4 **Genetically modified organisms.** The specification shall contain information about the host organism, the vector (plasmid) and the DNA-sequence incorporated in the vector or in the chromosome. Whether plant, animal or microorganism, the donor organism should 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 genetic structure such as information on presence of extra DNA (plasmids or foreign DNA incorporated in the host chromosome), specific genetic characteristics ("markers"), presence of dormant genes (which can be expressed by mutations), genetic stability (mutation rate and factors influencing the mutation rate, inter- and intramolecular recombinations, restriction barriers), gene transfer (mobilization/conjugation ability) and resistances (antibiotics, heavy metals) will assist in the prediction of undesirable effects on human health, animals, plants and ecological behaviour.

Exact knowledge of the identity and the biology of the vectors forms the basis for the evaluation of whether the introduction of the vector increases or reduces the safety level of the host microorganism. A vector should be characterized at the DNA level (size, restriction map and possibly full DNA sequence) and genetically with respect to genes found on the vector and which could be used as marker genes. A vector must be free of harmful sequences as well as non-conjugative and non-mobilizable.

The DNA sequence(s) to be inserted in the host organism has (have) to be fully characterized both at the molecular level and in terms of the number of inserted genes, type of regulation (promotor activity) and actual gene product(s). Whether the DNA sequence originates from a microorganism, plant or animal, the exact origin and pedigree of the genetic construct has to be given in order to enable a proper safety evaluation 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 and taking into account that the potential hazard from the final product might be more than simply the sum of the single elements.

## 3. *Manufacturing process*

3.1 Adequate information on the **method of manufacture**. For microbial sources information on fermentation media and conditions are considered essential. All components used must be of food grade quality.

3.2 Adequate information on the **purification procedure** shall be given.

If changes occur in the manufacturing process or in the purification of the enzyme preparation it will be considered as new unless it can be demonstrated that the final product can be considered the same as that prepared by the original procedures.

## 4. *Carriers and other additives and ingredients*

4.1 Informations on **carriers, diluents, excipients, supports and other additives and ingredients** (including processing aids) used in the production, distribution, and applications of enzyme preparations must be given. They must be substances that are acceptable for the relevant food-uses of the enzyme preparations concerned, or substances which are insoluble in food and removed from the food material after processing and before consumption.

4.2 In the case of **immobilized enzyme preparations**, the carriers and immobilization agents used should be acceptable for the relevant use. When new materials are being considered, they should be tested to prove that no harmful residues will leak out into the food. Tests should be performed showing that any leakage of immobilization agents or enzymes is kept within acceptable limits as specified in the individual specifications.

4.3 In order to distinguish the proportion of the enzyme preparation derived from the source material from that contributed by diluents and other additives and ingredients, individual specifications may require a statement of percentage Total Organic Solids (T.O.S.) which is defined as follows:

$$\% \text{ T.O.S.} = 100 - (A+W+D)$$

where A = % ash, W = % water and D = % diluents and/or other additives and ingredients.

The T.O.S. may be expressed as a ratio to the pure active ingredient (i.e.the enzyme content). Depending on the product in question the ratio may be very close to 1.

## 5. *Usage*

Information should be given on:

5.1 Technological function of the enzyme.

5.2 Types of foodstuffs in which the enzyme is intended to be used.

5.3 Maximum amount of enzyme preparation to be used in each foodstuff.

### 6. *Stability and fate in the food*

Information should be given on:

**6.1** Amount of enzyme preparation (i.e. active enzyme as well as other constituents) in the final food preparation.

**6.2** Main reaction products and possible reaction products not considered normal constituents of the diet, formed during the production and storage of enzyme treated food (see point 4 of the general safety issues in the Introduction).

**6.3** Possible effects on nutrients.

## General requirements and specifications

### 7. *Hygiene*

**7.1** Enzyme preparations are to be produced in accordance with good food manufacturing practice. The stock cultures of microorganisms used as the sources of enzyme preparations should be periodically tested to ensure their purity (see section 2.3).

**7.2** Addition of the enzyme preparation to a foodstuff must not cause any increase in the total microbial count in the foodstuff.

### 8. *Contaminants*

**8.1** **Heavy metals:** Preparations should not contain toxicologically significant amounts of heavy metals such as lead, cadmium, arsenic and mercury. The actual levels of heavy metals should be stated for each preparation.

**8.2** **Microbiological contaminants**

- No pathogenic micro-organisms (eg. *Salmonella, Shigella, Escherichia coli, Listeria, Campylobacter, Clostridium perfringens*) should be detectable using appropriate techniques.[2]

- Coliforms not more than 30 per gram as determined by a suitable method (eg. ISO 4832).[3]

- Total viable count not more than $10^2 - 10^4$ per gram as determined by a suitable method.[4]

**8.3** Tests shall be performed to ensure that viable cells from the microbial source organism are not present in the final product.

**8.4** Enzyme preparations may not contain any antibiotic activity as determined by a suitable method.[5]

**8.5** Enzyme preparations may not contain detectable amounts of toxins. When a given source is known to be able to produce toxins the absence of those toxins relevant to the organism shall be shown by a suitable method.

## Documentation for safety in use

### *9. Basic toxicological requirements*

**9.1** For enzymes derived from edible parts of animals or plants no toxicological tests are normally required. Where parts which are not generally considered as a normal part of the diet are used, some toxicological testing may be required unless other satisfactory documentation for safety in use is provided.

**9.2** For enzyme preparations derived from microorganisms the following tests are normally required:

(a) 90-day oral toxicity test in a rodent species;

(b) Two short-term tests:

1. a test for gene-mutations in bacteria,
2. 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, etc. They should, as a general rule, be performed in accordance with established guidelines (EC/OECD; see also references [8] and [11]) 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.

## *10. Exemptions from the basic toxicological requirements*

From a toxicological point of view it is important to perform a toxicological testing procedure on each specific enzyme preparation produced from a microbiological source.

**10.1** If, however, one enzyme preparation from a specific strain has been thoroughly tested and the manufacturing process does not differ significantly for other enzymes from the same strain, the full testing battery may be waived for these enzymes. This will be decided on a case-by-case basis.

**10.2** If the microorganism used in the production

- has a long history of safety in food use, and
- belongs to a **species** about which it has been documented that no toxins are produced, and
- the actual **strain** used is of well documented origin,

the acceptance of an enzyme preparation from this organism without specific toxicological testing may be justified. In this case a correct and confirmed identification of the organism is of extra importance.

Presently the Committee can give only one example of such an organism which is *Saccharomyces cerevisiae*.

Enzyme preparations from such sources still have to comply with the general specifications.

**10.3** When a mutant strain is substituted for the original strain of microorganism used in the production of an enzyme preparation previously tested and approved, a modified, less comprehensive test procedure may be appropriate. Justification for such a reduced procedure must be provided on a case-by case basis.

**10.4** In connection with immobilized enzyme preparations, where immobilization techniques are evaluated and approved on the basis of adequate toxicity testing, they may be combined with previously evaluated and approved enzyme preparations without the need for additional toxicity testing on the combined product if analytical data are provided to indicate that the leakage of components of the combined product is within acceptable limits (see section 4.2).

**10.5** With the introduction of well specified, non-toxin-producing genetically engineered source organisms for the production of food enzyme preparations, it may in future be possible to produce enzymes of very high purity and specificity. For products where it is possible to demonstrate such high purity and specificity, the full toxicity testing may not be needed.

Notwithstanding the circumstances listed above where testing procedures may be acceptable, there may be circumstances where additional testing over and above the basic requirements is necessary to resolve questions that arise in any of the basic studies.

## Evaluation of safety in use

On the basis of technological and toxicological data submitted, the Committee will ascertain the safety in use of the enzyme preparation. This may be done either by defining the acceptable conditions of use or, when appropriate, by allocating an acceptable daily intake for a specified enzyme preparation based on the no-observed-effect-level in the sub-chronic rodent study with the application of a suitable safety factor.

The evaluation will be confined to the product described in the submission and cannot automatically be considered to cover other preparations of the same enzyme prepared from other sources or by other processes.

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