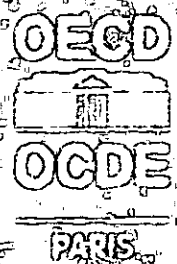
SAFETY EVALUATION OF FOODS DERIVED BY MODERN BIOTECHNOLOGY

CONCEPTS AND PRINCIPLES



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ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

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CONCEPTS ET PRINCIPES

FOREWORD

Safety Evaluation of Foods Derived by Modern Biotechnology: Concepts and Principles has been prepared by the OECD Environment Directorate, in collaboration with the Directorate for Science, Technology and Industry. It is the product of work undertaken by the Group of National Experts on Safety in Biotechnology. As such, it is related to another report recently published by the OECD, *Safety Considerations for Biotechnology 1992.*

This report is intended for the use of those involved in carrying out safety evaluations of new foods or food components derived by means of modern biotechnology. It elaborates scientific principles to be considered in making such evaluations, based on a comparison with traditional foods that have a safe history of use.

This report is published under the responsibility of the Secretary General.

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PREFACE

In 1983 the Committee for Scientific and Technology Policy created the Group of National Experts on Safety in Biotechnology (GNE). The work of the GNE led to the Recommendation of the OECD Council concerning Safety Considerations for Applications of Recombinant DNA Organisms in Industry, Agriculture and the Environment. This Council Act called, *inter alia*, for further research to improve the prediction, evaluation and monitoring of the outcome of applications of recombinant DNA organisms. *Recombinant DNA Safety Considerations*, which includes the Council Recommendations, published by the OECD in 1986, contained general safety guidelines for the use of genetically modified organisms in industry, agriculture and the environment.

In 1990, the GNE agreed that "work on food safety, with particular attention given to the elaboration of scientific principles for assessing the safety of new foods or food components produced by means of biotechnology, was of high priority and should be initiated as soon as possible". A Working Group was therefore established on food safety as related to modern biotechnology. Dr. Frank Young of the United States was elected chairman.

The Working Group participants identified a number of concepts underlying their work, issues that needed to be addressed, and approaches or processes that could be used to respond to the need expressed by the GNE. The terms of reference of the Working Group (see Annex I) were endorsed by the GNE.

Several points regarding the scope and objectives of the Working Group, as set out in the terms of reference, should be noted:

- the Working Group was not to address the safety assessment of food additives, contaminants, processing aids and packaging materials;
- it was not to address issues relating to the *environmental* safety of new foods or food components, as these issues were already addressed in OECD documents and by other working parties of the GNE; and
- the principles elaborated should focus initially on the safe use of new foods or food components of terrestrial microbial, plant or animal origin. (Organisms of aquatic origin were to be addressed in future work of the Working Group.)

Scientific principles to be considered in evaluating the safety of new foods and food components, as elaborated by the Working Group, are set out in Chapter II. As background for the discussions of the Working Group, a number of documents and publications available in OECD countries relating to the assessment of food safety were examined (see Annex II).

This report is based on material developed at several conferences and intergovernmental consultations on the subject of food safety and biotechnology. A number of

scientific meetings that addressed issues regarding the various traits, chemical composition, and properties of organisms used as food or as a source of food have also been relevant.

The terms of reference of the Working Group called for models or examples of new foods or food components to be identified, and for existing information related to their safety assessment to be collected and used to assist in developing and/or demonstrating the applicability of the proposed scientific principles and associated methods. The Working Group selected a number of novel foods or food components as examples. The case studies presented in Chapter III illustrate the application of the concepts and principles set out in Chapter II. However, they *cannot be regarded as actual evaluations or safety judgements* on the part of either the Working Group, the Group of National Experts on Safety in Biotechnology, the OECD, or any of its Member countries.

This report is intended for use by those involved in carrying out safety evaluations of new foods or food components derived by modern biotechnology. The scientific approach to such evaluations elaborated by the Working Group is based on a comparison with traditional foods that have a safe history of use. This approach is based in turn on the concept of *substantial equivalence*, which articulates procedures used in the past, albeit intuitively, for accepting new foods. The Working Group believed such an approach could also be used for the safety assessment of new foods and food components derived by other technologies.

The Working Group considered substantial equivalence to be the most practical way to address the issue of food safety at this time. This is not to imply, however, that the report is applicable to any other aspect of biotechnology safety, including environmental safety. Other OECD documents address such issues.

Chapter 1

Background

Recent years have seen tremendous advances in food biotechnology, including improvements in industrial process technology and control systems, improvements in farming systems for growing and harvesting food, genetic improvements to organisms used in the food supply, and improvements in techniques to monitor food safety and nutritional quality. It is thus expected that progress in biotechnology will play an increasingly important role in food supply.

Micro-organisms

Examples of traditional food biotechnology include the use of yeasts in the brewing and baking industries, and the use of bacteria and moulds and their components in the dairy industry for making cheese and yoghurt. Moulds and bacteria are also used for the fermentation of plants or plant products (for example, miso). Purified enzymes from micro-organisms are used extensively in making products such as high-fructose corn syrup and certain types of hydrolysed or predigested protein products.

In many such products, the micro-organisms function in the production process and the food product does not contain viable cells. In others, such as yoghurt, microbial cultures remain viable and are consumed. Such traditional applications have a long history of safe use, and many have formally been affirmed as safe by various national and international food safety evaluations. Key considerations have included non-pathogenicity and non-toxicity of the organism and its products.

Modern biotechnologies are being used increasingly to improve food micro-organisms for the enhanced production of essential components or products, as well as the improvement of nutritional value, flavour, texture, and the shelf life of fermented foods.

Plants

Plants are consumed directly as whole food, or are processed into many types of foods. Many plants have a long history of use as foods. Undoubtedly, the plants selected were the ones that appeared healthy, grew vigorously, and gave higher yields. Edible portions had desirable taste, smell and appearance. Selection might have included an evaluation of safety, although it was not formally recognised. In any case, there is little historical record or documentation of the process by which the safety of food plants was

maintained, or of involvement of national food authorities. Now that new biotechnology has vastly increased the variety of new traits that can be introduced into plants, the impact of plant biotechnology on food safety is receiving attention.

Early farmers selected and preserved plant variants that had desirable food or agronomic attributes, such as larger fruit or uniform dormancy and maturation times for seeds. Such properties are deleterious to wild plants and so would not have been developed without the efforts of early "breeders". Practices of early farmers led eventually to the development of desirable clones, land races, and varieties of major food crops, with predictable reproducible agronomic characteristics yielding foods with uniform properties. As the agronomic properties of individual crops were made more uniform, production methods could be designed to obtain optimum yields.

With the relatively recent advent of directed plant breeding for improvement of agricultural crops, the objectives of plant breeders became: *i)* to increase yield, *ii)* to improve quality, and *iii)* to reduce production costs by, for example, identifying traits which could increase resistance to pests and diseases.

Although it may not have been a major objective, plant breeders have been effective in conserving the nutritional quality of plants developed for food. Routinely, they have selected plants with desirable qualities and rejected undesirable plants by destroying them in the breeding plots.

Preferences of the humans consuming the crop have contributed to the food characteristics of plant varieties ultimately developed. For example, varieties of potatoes and beans are quite different in different areas of South America, where their selection has been influenced by the taste preferences of native peoples. As another example, the milling and baking quality of flour is checked during the variety development process since wheat is often developed for particular baking products.

In the case of certain crops, breeders have deliberately attempted to improve nutritional value. Often, as for example in the case of high-lysine corn or high-vitamin C tomato, other factors have prevented these varieties from becoming widely accepted. The best-tasting, most nutritious variety will not succeed as a commercial crop unless it also gives high yield. Difficulty in processing, susceptibility to pests or diseases, an undesirable flavour or colour, or simply difficulty in getting the plants to market will also limit the adoption of a new variety.

Public acceptance of a high-nutrient variety is not based on nutrient content alone. Carrot and sweet potato varieties that have a bright orange colour are more acceptable to humans than those that do not. They also have a higher content of the pigment that supplies vitamin A precursor in the human diet. The ascorbic acid (vitamin C) content of tomatoes has been extensively examined, and varieties with higher content developed. However, since the fruit of these tomatoes is more yellow-orange than red, they have not been as acceptable to consumers.

The nutritional value of fruit or vegetable crops can be quite variable, and may be difficult to assess definitively. The composition of plant foods, particularly fruits and vegetables, is transient because the edible portion undergoes rapid biochemical changes during the ripening process. For example, in red tomatoes the content of ascorbic acid is low in green fruit, increases rapidly as the fruit ripens, and then drops off with time. Ascorbic acid content also varies in ripe tomatoes with their position on the vine, since higher light intensity increases the amount. Moreover, field-grown tomato plants produce fruit of higher vitamin C content than those grown in glasshouses. In view of these

considerations, the significance of a genetically induced change in the level of a nutrient, such as ascorbic acid in modified tomatoes, would be difficult to assess. The significance of a genetically induced change in the level of a particular nutrient would also depend on the position of the food in the total diet.

Many plants are known to produce compounds toxic to other species. Acutely toxic poisonous plants, such as some fungi and ornamental plants, are not consumed. A number of plants consumed by humans are acutely toxic in the raw state, but are accepted as food because processing methods alter or eliminate their toxicity. For example, the cassava root is quite toxic, but proper processing converts it into a nutritious and widely consumed food. Soybeans and lima beans, among other crops, also require proper processing. Thus the mere presence of a toxicant in a plant variety does not necessarily eliminate its use.

In other plants that contain toxicants affecting humans, such as potato and tomato, plant breeders have succeeded in reducing the level of these toxicants in food varieties. Over time, there have been few reported examples of plant breeding inadvertently leading to increases in toxicants. Varieties with an increased toxicant level have been quickly removed from agricultural use. In some countries new varieties have been monitored for levels of a particular toxicant, but systematic food safety assessment has not generally been conducted. The impact of plant biotechnology on food safety is now receiving wider attention. At the same time, there is increasing general recognition of the relevance of plant breeding's historical record.

Toxicant levels might become important, particularly when traits are introduced for resistance to pests and diseases, simply because a compound inducing resistance to another organism might possibly affect humans. The molecular basis for the resistance mechanisms is just beginning to be understood by plant scientists, and may be a target for biotechnology approaches to enhance resistance. Some mechanisms appear to be quite general, while others have adverse effects on a specific pest or pathogen. Knowledge of the mechanisms should, in the future, provide a valuable tool for the plant breeder and should facilitate evaluations of safety.

Animals

The development of new strains of domestic mammals and birds for food has had a long history, and extensive procedures are in place to improve yield and assure the health of these animals. In general, foods from new strains of mammals and birds that appear to be in good health have proven to be as safe as the animal breeds from which they were derived. No endogenously produced toxicants are known to come from such domestic animals.

In recent years, breeding technologies have been developed that permit increased numbers of desirable individuals through techniques such as embryo splitting. In addition, improved knowledge of the genetic control of hormonal levels has permitted the alteration of carcass quality, for example of fat to lean ratios, which has resulted in consumer-desired lean meats. Increased hormone levels have also enhanced the rate of growth, as well as milk production. There is no evidence of adverse effects to humans from the use of such technologies.

Chapter II

Food Safety and Biotechnology: Concepts and Principles

The consideration of the safety of foods and food components derived from biotechnology involves several *continua:* from older to newer biotechnology; from traditional techniques to the latest techniques based on molecular and cellular biology; from simple to complex products; from a well-known history of exposure and safety of use to areas of less knowledge of the trait in different organisms; from whole organisms to specific chemical compounds or substances; and from simple to complex assessment approaches. For a rational and practical approach to ensuring safe use, these *continua* can be separated into manageable pieces, facilitating the description of the concepts or principles of safety. Accordingly, scientific principles and procedures should be applied in a flexible fashion, taking into account the knowledge of: the characteristics of the newly introduced trait(s); potential dietary exposure; the preparation and processing of the foods or food components; nutritional considerations; and toxicological aspects.

Concepts of food safety

The safety of food for human consumption is based on the concept that there should be a reasonable certainty that no harm will result from intended uses under the anticipated conditions of consumption. Historically, foods prepared and used in traditional ways have been considered to be safe on the basis of long-term experience, even though they may have contained natural toxicants or anti-nutritional substances. In principle, food has been presumed to be safe unless a significant hazard was identified.

Modern biotechnology broadens the scope of the genetic changes that can be made in food organisms, and broadens the scope of possible sources of foods. This does not inherently lead to foods that are less safe than those developed by conventional techniques. Therefore, evaluation of foods and food components obtained from organisms developed by the application of the newer techniques does not necessitate a fundamental change in established principles, nor does it require a different standard of safety.

Moreover, the precision inherent in the use of certain molecular techniques for developing organisms for use as food should enable direct and focused assessment of safety where such assessment is desired. Knowledge obtained using these methods might also be used to approach safety assessment of new foods or food components from organisms developed by traditional methods.

Safety considerations and substantial equivalence

For foods and food components from organisms developed by the application of modern biotechnology, the most practical approach to the determination of safety is to consider whether they are *substantially equivalent* to analogous conventional food product(s), if such exist. Account should be taken of the processing that the food may undergo, as well as the intended use and the exposure. *Exposure* includes such parameters as the amount of food or food component(s) in the diet, the pattern of dietary consumption, and the characteristics of the consuming population(s). This approach provides a basis for an evaluation of food safety and nutritional quality.

The concept of substantial equivalence embodies the idea that existing organisms used as food, or as a source of food, can be used as the basis for comparison when assessing the safety of human consumption of a food or food component that has been modified or is new.

If one considers a modified traditional food about which there is extensive knowledge on the range of possible toxicants, critical nutrients or other relevant characteristics, the new product can be compared with the old in simple ways. These ways can include, *inter alia*, appropriate traditionally performed analytical measurements (for example, alkaloid levels in potatoes, cucurbatin in vegetable squash cultivars, and psoralens in celery) or crop-specific markers, for comparative purposes. The situation becomes more complex as the origins/composition/exposure experience decreases, or if the new products lack similarity to old established products or, in fact, have no conventional counterpart.

A demonstration of substantial equivalence takes into consideration a number of factors, such as:

- knowledge of the composition and characteristics of the traditional or parental product or organism;
- knowledge of the characteristics of the new component(s) or trait(s) derived, as appropriate, from information concerning: the component(s) or trait(s) as expressed in the precursor(s) or parental organism(s); transformation techniques (as related to understanding the characteristics of the product) including the vector(s) and any marker genes used; possible secondary effects of the modification; and the characterisation of the component(s) or trait(s) as expressed in the new organism; and
- knowledge of the new product/organism with the new component(s) or trait(s), including the characteristics and composition [*i.e.* the amount of the component(s) or the range(s) of expression(s) of the new trait(s)] as compared with the conventional counterpart(s) (*i.e.* the existing food or food component).

Based on a consideration of the factors in the paragraph above, knowledge that a new food or food component(s) was derived from organism(s) whose newly introduced traits have been well-characterised, together with a conclusion that there is reasonable certainty of no harm as compared with its conventional or traditional counterpart, means that a new food or food component(s) can be considered substantially equivalent.

Set out below are the *principles for the application of substantial equivalence* to the assessment of foods from organisms developed by the application of biotechnology:

- If the new or modified food or food component is determined to be substantially equivalent to an existing food, then further safety or nutritional concerns are expected to be insignificant;
- Such foods, once substantial equivalence has been established, are treated in the same manner as their analogous conventional counterparts;
- Where new foods or classes of new foods or food components are less well-known, the concept of substantial equivalence is more difficult to apply; such new foods or food components are evaluated taking into account the experience gained in the evaluations of similar materials (for example, whole foods or food components such as proteins, fats or carbohydrates);
- Where a product is determined not to be substantially equivalent, the identified differences should be the focus of further evaluations;
- Where there is no basis for comparison of a new food or food component, that is, where no counterpart or similar materials have been previously consumed as food, then the new food or food component should be evaluated on the basis of its own composition and properties.

As an example of the application of substantial equivalence, potatoes have long been part of the human diet. The presence of viral coat proteins in the potato are due to natural viral infections; consequently, these proteins have a long history of human consumption. Coat proteins have never been associated with a toxicity problem and are not considered a food safety issue. Consequently, a potato in which the coat protein of one of these viruses is expressed after the gene has been introduced would be considered substantially equivalent to the infected potatoes that have a long history of safe use and consumption provided the amounts expressed were not grossly different from those occurring following natural infection. This analogy applies only to viral coat proteins in the portions of the plant traditionally consumed, taking into account the characteristics of the new trait and possible untoward effects of the modification on alkaloid levels and key nutrient starches, as well as the extent of consumption.

Some specific examples of additional considerations which it may be necessary to take into account when applying the concept of substantial equivalence are indicated in the following paragraphs.

The intended use(s) and degree of exposure must also be considered in assessing safety. This includes the effect(s) of the level of the food or food component in the diet, the pattern of dietary consumption, and the characteristics of the consuming populations (*i.e.* infants, the elderly, the immunocompromised, etc.).

The consideration of safety may include the need to evaluate possible effects occurring through cooking or other processing. For example, trypsin inhibitors from certain leguminous plants, such as the cowpea trypsin inhibitor, have a long history of safe consumption when properly cooked. However, if the cowpea trypsin inhibitor is expressed in other plants, the safety question relates to whether the normal use of these plants as food involves cooking sufficient for its inactivation.

In special cases, depending on the product consumed, the consideration of safety may also include the need to evaluate the potential for, and human health implications of, transfer of the new genetic material. For example, the use of some antibiotic resistance markers in micro-organisms should be carefully considered since transfer to the microflora of the human gut could, if demonstrated, possibly have human health implications.

Another consideration is the influence of the newly introduced modification(s) on the nutritional value of the food or food component(s). For the majority of modifications being carried out, such changes are unlikely. Nonetheless, when modifications are directed at metabolic pathways of key macro or micro nutrients, the possibility of an impact on nutritional value is increased. Such impacts are of potential significance in cases where the modified food or food component may become a major dietary source of the nutrient affected.

Conclusions

The main conclusion of this report is as follows: if a new food or food component is found to be substantially equivalent to an existing food or food component, it can be treated in the same manner with respect to safety. No additional safety concerns would be expected.

Where substantial equivalence is more difficult to establish because the food or food component is either less well-known or totally new, then the identified differences, or the new characteristics, should be the focus of further safety considerations.

Chapter III contains a number of case studies that illustrate the practical application of the concepts and principles for safety evaluation of new foods or food components, in particular the concept of substantial equivalence. In addition, the examples are representative of the range of new products produced by means of biotechnology. Given the wide applicability of substantial equivalence, experts on the Working Group were of the view that many new foods will be found to be substantially equivalent to existing products.

In the case of those products for which substantial equivalence cannot be established, or for which there is no traditional counterpart, further work will be helpful to increase our understanding of the appropriate information which may be needed and the methods to be used for safety evaluation.

Chapter III

Case Studies Illustrating the Application of Substantial Equivalence

The case studies in this chapter were chosen mainly to illustrate the application of the concept of substantial equivalence for the safety evaluation of new foods or food components produced by means of modern biotechnology. They are not evaluations or regulatory reviews, nor should they be seen as a commentary on the safety of the foods or food components selected.

These case studies were prepared by the experts indicated. Although the Working Group on Food Safety and Biotechnology discussed each case study, there was no attempt to reach consensus on the conclusions they contain.

The concepts and principles illustrated in the case studies relate only to food safety. Environmental issues were not included within the remit of the Working Group on Food Safety and Biotechnology. These issues were therefore not discussed by the Working Group.

The case studies were prepared following the general outline shown below:

1. Conceptual points to consider

a) Concept of continua

For example, the extension of the use of LEAR oil to infant formula from traditional uses of vegetable oils (margarine, shortening, and salad and vegetable oils).

b) Temporal considerations

For example, higher erucic acid content of traditional rape or LEAR oil in the 1970s and 1980s as compared with lower values in traditionally bred strains of rapeseed today.

c) Concept of "reasonable certainty" of no harm resulting from:

- intended uses; and
- expected conditions of consumption.

For example, there was a "reasonable certainty", based upon the evidence evaluated, that LEAR oil would behave as other vegetable oils for traditional uses, stated above, under the highest expected conditions of consumption (i.e. *by males aged 20-30 years). This was not the case for its use as infant formula.*

d) Concept of substantial equivalence

For example, LEAR oil was compared with traditional rapeseed oil and other commonly consumed vegetable oils and was shown to be composed of the same basic components, except for a lower level of erucic acid, the component of concern.

e) Concept of variability

For example, the concentration of the alkaloid tomatine is much higher in green tomatoes than in ripe ones.

f) Concept of sequential review (*i.e.* establishment of substantial equivalence *followed* by evaluation procedures).

g) The evaluation of marker genes in a substantial equivalence determination

For example, the use of kanamycin resistance derived from Tn5 is not effective against kanamycins used currently for medicinal purposes.

2. Organism/product

What is the organism/product that will be eaten by the consumer?

3. Traditional product evaluation

Approaches/considerations/results:

What kind of evaluation does this organism/product undergo traditionally? For example, tomato may be evaluated by the plant breeder when a new variety is being developed, whereas myco-protein may not have a traditional procedure for evaluation. When tomato is evaluated, or if there is some concern, the toxic compound tomatine may be considered. The result of this evaluation may be that the level of tomatine is not a problem normally, but that in some cases it is a problem (state circumstance).

4. Database available for traditional evaluation

Is there a database available in your country/department containing information useful for evaluation of this product? [For example, the Database of Contaminants in Food Products (COBA) developed in Denmark by the State Institute for Quality Control of Agricultural Products.]

5. Novel component(s)/product (including traits and sources)

Why is this product considered a novel food? For example, potato may contain a gene for insect resistance that has never been consumed as food before or the myco-protein may never before have been considered as food.

6. Additional evaluation procedures

Are additional evaluation procedures carried out, or are normal evaluation procedures sufficient in the case of the novel food?

7. Rationale for evaluation procedures

A short statement of the reason for the evaluation procedures.

Chymosin derived from *Escherichia coli* K-12 and *Bacillus stearothermophilus* alpha-amylase derived from *Bacillus subtilis*

Dr. Eric Flamm
Office of Biotechnology
United States Food and Drug Administration

Case No. 1 Chymosin derived from *Escherichia coli* K-12

1. Conceptual points to consider

a) Concept of continua

Different enzyme preparations may be similar in some attributes and dissimilar in others. The relative similarity or equivalence of different enzyme preparations can be determined by comparing characteristics of the enzymes themselves, the organisms from which they are produced, and the methods and materials used in the manufacture of the preparation. The importance of any differences will depend on how they affect the safety and utility of the preparations.

There is a good deal of scientific consensus on how to assess the safety of an enzyme preparation. However, there is less consensus regarding the criteria by which one decides at what point an enzyme preparation is different enough from an accepted one that formal review is required to establish safety. For example, at what point do manufacturing changes or strain modifications become significant enough to warrant review? At what point is the substantial equivalence of two enzyme preparations no longer self-evident? This is as much a regulatory question as a scientific one.

Two different batches of the same enzyme purified by the same methods from the same strain of production organism grown under the same conditions may be considered potentially different if a small change in activity is significant for its intended use. Alternatively, two different enzymes with similar functions, but produced by different methods from different species of organisms grown under different conditions, may be considered substantially equivalent if the differences do not significantly affect the safety and utility of the preparations. The point at which an enzyme preparation differs from its accepted counterpart enough to be considered different, and to warrant evaluation, is again as much a regulatory question as a scientific one.

In the case of the microbial chymosin preparation discussed in the *first case study*, the preparation's functional activity is identical to that of its traditional counterpart, animal rennet. However, it is produced by a completely different manufacturing method and consequently has completely different impurities. The United States Food and Drug

Administration (FDA) found that these differences were significant enough to warrant formal review in order to determine whether the new preparation was substantially equivalent to the traditional one.

In contrast to the chymosin preparation, the alpha-amylase preparation discussed in the *second case study below* was derived from the same organism as that traditionally used as a source of alpha-amylase, *Bacillus subtilis*, albeit from a new strain. The enzyme itself, *B. stearothermophilus* alpha-amylase, was independently reviewed and determined to be safe for use in food when derived from its native host. Additionally, it is functionally similar to the traditional enzyme, differing principally in its ability to perform at higher temperatures. Thus, in content and activity, the new preparation is very close to its traditional counterpart. Whether they are close enough that formal review should not be needed to determine substantial equivalence is a regulatory question.

b) Temporal considerations

Food-use microbial enzyme preparations derived from recombinant organisms are only newly being developed. At this early stage they may be considered more novel, or worthy of greater scrutiny, than they will be after a number of such products have been introduced. It is possible, for example, that the preparation of *B. stearothermophilus* alpha-amalyse derived from *B. subtilis* would not have been treated as a new preparation warranting review had it been introduced at some future time after a number of similar products had been reviewed.

c) Safety as defined as a "reasonable certainty" of no harm resulting from intended uses under expected conditions of consumption

It is not feasible to answer all possible questions pertaining to the safety of a new (or traditional, for that matter) food product. The standard of safety generally considered acceptable is that there is a reasonable certainty that no harm will result from the intended use of the product under the expected conditions of consumption.

The intended use of a food-grade enzyme preparation is usually to process food or food ingredients in a particular way. The enzyme is generally present in the final food product, if at all, at very low levels.

Commercial food-use enzyme preparations, even when purified, are typically quite impure and may comprise more cell debris than enzyme. Therefore, in assessing the safety of an enzyme preparation it is at least as important to review information concerning the production strain, and the methods and materials used in growing it and purifying the enzyme, as it is to review the characteristics of the enzyme itself.

In general, when assessing the safety of the enzyme itself one determines the relationship of that enzyme to other enzymes used in food or food processing. If it is of a type commonly used in food or food processing and has no unusual properties that warrant concern, then the enzyme itself may be considered substantially equivalent to other accepted food-use enzymes. Since food-use enzymes are in (and of) themselves safe, a determination of substantial equivalence generally constitutes a finding of safety. If the enzyme has unusual properties or is of a type not previously used in food, then information will be required to show that the enzyme will be safe for its intended use.

In assessing the safety of the production organism, one generally focuses on whether it is pathogenic or produces toxins. The species of production organism should be shown

to have a history of safe food use, or otherwise be shown by scientific information to be safe for such use. The particular strain used should also be shown to be safe, *i.e.* to have no new properties that would affect it as a source of enzyme preparation safe for use in food.

In assessing the safety of recombinant production organisms, one typically first determines if the parent organism is acceptably safe for the intended use. If so, one then reviews all steps in strain construction to ensure that all vectors used are safe and that the inserted DNA does not encode toxic or otherwise undesirable proteins. The entire segment of cloned DNA, including sequences flanking the target gene, should be analysed. If the donor organism produces toxins or other undesirable compounds, data should be provided demonstrating that DNA encoding these substances was not inadvertently cloned along with the target DNA.

If the safety of the parent organism for use in food processing has not been established, there would probably have to be substantial information, including results of toxicology tests, to demonstrate that the modified strain was acceptable for food use.

As discussed below, the microbial chymosin and alpha-amylase preparations were found to be safe after evaluation of the production organisms, the enzymes, and the manufacturing processes. The manufacturing method destroys the production organism and removes the bulk of the cell debris, and this was an added factor in assuring the safety of the preparation.

d) Concept of substantial equivalence

Microbial enzyme preparations can be considered substantially equivalent to each other if three conditions are met: the enzymes themselves are substantially equivalent, for example having similar intended uses and functional properties; the microbes from which they are derived are substantially equivalent, for example being safe strains of species with a safe history of use as sources of food-use enzymes; and the manufacturing and purification processes are substantially equivalent. However, there are as yet no agreed-upon criteria by which substantial equivalence is determined for each of these parameters.

A new enzyme preparation may be substantially equivalent to an accepted preparation even if the production organisms and manufacturing methods are not, so long as the differences do not affect the safe use of the final preparation. The more the new production organisms or manufacturing methods differ from traditional ones, the more information will be necessary to determine whether the new preparation is substantially equivalent to the old.

The concept of substantial equivalence can be applied broadly or narrowly. For example, all enzymes of any type used for food processing might be considered substantially equivalent; or all carbohydrates might be considered substantially equivalent; or all amylases; or all alpha-amylases; or all alpha-amylases that have the same functional activities under the same conditions and are intended for use in the same foods. The *preparations* of substantially equivalent enzymes might then be considered substantially equivalent enzymes if they are produced by a safe strain of any microbial species with a safe history of use in food; or only if they are produced by the same microbial species; or only if they are native to and produced by the same microbial species. Additionally, the manufacturing processes might have to meet certain criteria to assure that the final product meets acceptable specifications before the enzyme preparations would be considered substantially equivalent.

In the safety evaluation of the two enzyme preparations described below, the term "substantial equivalence" was nowhere used by the evaluators. However, though not articulated as such, the safety of the preparations was determined essentially by establishing that each was substantially equivalent to an accepted preparation.

In the case of chymosin derived from *E. coli* K-12 it is obtained from a completely different source organism and by a completely different method than is its traditional counterpart, animal rennet. Thus the types of potential impurities differ, and significant characteristics of the preparations may differ. To determine if the preparations were substantially equivalent, the FDA compared the enzymatic activities of the preparations and evaluated whether the impurities in the microbial preparation affected its safe use. As described in Section 3 below, FDA determined that the enzymes themselves and the functional activity of the enzyme preparations were substantially equivalent, and that the impurities in the microbial preparation did not affect its safe use. Thus, while the two preparations are clearly different and have different names, they are substantially equivalent in safety and function.

In the case of *B. stearothermophilus* alpha-amylase obtained from *B. subtilis*, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated the production organism and determined that the genetic modifications were well-characterised and did not cause it to produce toxins or other undesirable substances. It could therefore be considered substantially equivalent to other food-use strains of *B. subtilis*. JECFA evaluated the enzyme and found that it was the same as that produced by *B. stearothermophilus*. JECFA evaluated the manufacturing method and found it met acceptable standards for producing microbial enzyme preparations.

Thus, by determining that the enzyme, the production organism, and the manufacturing method were substantially equivalent to accepted counterparts, JECFA determined that the new enzyme preparation was safe for its intended use. Depending on the interpretation of substantial equivalence, one could also conclude that the new enzyme preparation is substantially equivalent to the traditional *B. subtilis* preparation, despite the fact that the stearothermophilus enzyme will likely be used with different substrates because of its ability to digest starches at higher temperatures.

e) Concept of variability

Inapplicable.

f) Concept of sequential review

The first step in evaluating a new enzyme preparation is to compare characteristics of the enzyme itself, the production organism, and the manufacturing method with those of the closest accepted counterpart. One can then focus on those characteristics that differ between the new and the old preparations to determine whether the differences affect the safe use of the new product.

Where the enzyme, the production organism, and the manufacturing method are determined to be substantially equivalent to those of accepted enzyme preparations, and any new combinations do not affect the safe use of the product, the new preparation can be accepted as safe. When there are no accepted counterparts, or where the differences between the accepted and the new are too large to allow meaningful comparison, additional information is necessary to establish the safety of the preparation.

g) Evaluation of marker genes in a substantial equivalence determination

Recombinant organisms frequently contain marker genes, some of which may encode resistance to therapeutically useful antibiotics. Whether the presence of a marker gene in a production organism affects its substantial equivalence to an accepted safe preparation will depend on a number of considerations. For example, does the marker gene encode a protein product? If so, at what levels would it be expected to be in the food, what is its function, and are there any concerns about its safety in food at the predicted levels?

For antibiotic resistance marker genes, does the marker gene encode resistance to a clinically useful form of an antibiotic? If so, does ingestion of the product at the time of therapeutic use of the antibiotic interfere with the clinical effectiveness of the antibiotic? In general, this would not be expected to be a concern for enzyme preparations. The preparations are present in very low levels in the food. Thus, the levels in the food of any constituent of the preparation active against the antibiotic would almost always be biologically insignificant

Finally, what is the likely level of horizontal transfer of resistance genes to pathogens in the food or in the intestinal tract of the consumer? For an enzyme preparation derived from an antibiotic-resistant microbe to be substantially equivalent to one derived from an antibiotic-sensitive microbe, the likely level of transfer must be biologically insignificant.

In the case of chymosin derived from *E. coli* K-12, the level of transfer of the antibiotic resistance marker was found to be insignificant because the purification method destroyed the production organism and degraded its DNA to fragments smaller than that of the gene encoding resistance. In the case of the particular alpha-amylase preparation described here, there was no intact antibiotic resistance gene in the production strain.

2. Organism/product: chymosin derived from *E. coli* K-12

Chymosin, also known as rennin, is the principal milk-clotting enzyme present in rennet. Rennet is derived from the stomach of a variety of animals, most commonly unweaned calves but also kids and lambs. It has been used for millennia to make cheese. Chymosin is a protease that hydrolyses one bond in the kappa-casein protein of milk, cleaving it into two peptides. Kappa-casein normally stabilises micelles in milk. When kappa-casein is cleaved, the micelles precipitate into curds. After removal of the liquid whey, the curds may be processed into cheese or other dairy products such as frozen dairy desserts.

3. Traditional product evaluation

As discussed in 1.*c)* above, a new enzyme preparation is evaluated to determine if it is safe for its intended use. Such an evaluation focuses on characteristics and properties of the enzyme, the production organism, and the materials and methods used in the manufacturing process. *E. coli*-derived chymosin preparation is manufactured by a completely different method than is rennet. Therefore, it was important to determine whether the change in manufacturing method affected the safety of the enzyme preparation.

The safety of chymosin derived from *E. coli* K-12 was established from the following information. First, the enzyme was shown to be structurally and functionally identical to that of the chymosin in rennet, and was therefore considered safe as a replacement for the chymosin in rennet. Data was provided documenting that the prochymosin gene had been cloned and that it was properly expressed in its microbial hosts to produce functional chymosin.

Three lines of evidence were used to show that the correct gene had been cloned. The cloned DNA was digested with restriction enzymes, and the resulting fragments were found to be the sizes predicted by the DNA sequence of the prochymosin gene. The cloned DNA, and RNA synthesised from it, were found to hybridise appropriately with the calf prochymosin gene. Finally, the sequence of the cloned DNA was found to correspond to the amino acid sequence of the prochymosin protein.

The cloned prochymosin gene produced chymosin of the expected size and biological activity. Cloned chymosin was shown to have the same molecular weight as chymosin derived from calf rennet, as demonstrated by SDS polyacrylamide electrophoresis. Cloned chymosin was also shown to have the same functional activity as chymosin derived from calf-rennet, as demonstrated by milk clotting assays performed under various conditions of temperature, salt concentration and pH.

Second, the production organism, *E. coli* K-12, was found to be safe as a source of chymosin, based primarily on published evidence demonstrating that *E. coli* K-12 is non-pathogenic and non-toxigenic. Such evidence includes published studies showing that *E. coli* K-12 does not colonise the gut of man or other animals after being fed at high concentrations (10^9 to 10^{10} viable organisms per ingestion), that the K-12 strain has been widely used as a laboratory organism for 30 years with no reported incidents of illness, that it does not produce toxins that cause illness upon ingestion, and that it is deficient in virtually all characteristics necessary for pathogenesis. Additionally, non-pathogenic strains of *E. coli* are a part of the normal flora of the gastrointestinal tract of man, where they are found at 10^6 to 10^9 organisms per gram of intestinal contents.

Third, the fermentation and purification methods were shown not to introduce any unsafe substances into the preparation and to remove the bulk of the cellular materials from it. All the chemicals used in the fermentation and purification are approved for use in food. By removing the bulk of the microbial material from the final product, the purification process yielded a preparation having acceptably low levels of endotoxin. Endotoxin is a component of the cell wall of *E. coli* of potential concern for people with certain intestinal tract disorders. The endotoxin levels in the chymosin preparation are comparable to those in US drinking water.

The purification method was also shown to destroy the *E. coli* and degrade its DNA, thereby adding another level of safety assurance and eliminating the possibility that the antibiotic resistance gene present in the vector could be transferred at a biologically significant level to pathogens in the consumer or on food in contact with the enzyme preparation. Data were provided demonstrating that the preparation did not contain sufficient DNA of a quality capable of transforming transformation-competent cells to permit detectable transformation of such cells. In addition, no DNA fragments larger than 200 bases were detected when assayed by radiolabelled hybridisation after gel electrophoresis. For comparison, the coding sequence of the antibiotic resistance gene carried by the production strain is 858 bases long.

As corroborative evidence of safety, two short-term feeding studies were conducted with the enzyme preparation: a five-day feeding study in dogs and a one-month gavage study in rats. No adverse results were observed in these studies at any dose tested.

Based on the information described above and the fact that consumers would be exposed to it at relatively low levels, the US FDA concluded that the chymosin preparation is safe for its intended use as replacement for rennet.

4. Database available for traditional evaluation

None.

5. Novel component(s)/product

Microbial chymosin differs from its traditional counterpart, rennet, in its impurities because it is obtained from a different source organism and by different manufacturing methods. In all other aspects, such as activity, function, use, and active component, the two preparations are substantially equivalent, in fact are identical.

6. Additional evaluation procedures

The chymosin enzyme preparation was subjected to safety evaluation because it is manufactured by a completely different method from that of its traditional counterpart, animal rennet. It was not subjected to review simply because it is derived from a recombinant organism. The parts of the review that could be considered specific for a recombinant organism were the review of the antibiotic resistance marker and the review of the strain construction, including information concerning vectors and intermediate strains. Non-recombinant micro-organisms used to produce enzymes for food use have not had antibiotic markers and have not been subject to extensive strain construction.

7. Rationale for additional evaluation procedures

Chymosin preparation is obtained from a different source organism and by a different manufacturing process than is rennet. Any time there are significant changes in the source and manufacturing method of a product, there are likely to be changes in types of impurities. Therefore, specifications written for one manufacturing method may not be appropriate for a different manufacturing method. It is also important to determine whether any significant characteristics affecting the use of the product are changed, that is, whether in fact the new product is substantially equivalent to the traditional product.

Case No. 2 Bacillus stearothermophilus alpha-amylase derived from *Bacillus subtilis*

1. Conceptual points to consider

(see Case No. 1 above).

2. Organism/product: alpha-amylase of *B. stearothermophilus* expressed in *B. subtilis*

Amylases have been extensively used by the food industry to hydrolyse starch. Alpha-amylase catalyses the hydrolysis of 1.4 alpha-glucosidic linkages in common polysaccharides. Bacterial alpha-amylase derived from *B. subtilis* has been in common use to control the viscosity of chocolate syrup since 1929 and in the brewing industry since 1936. The enzyme preparation derived from these various *B. subtilis* strains is usually added directly to the food to be processed and then removed from the final product by filtration.

3. Traditional product evaluation

As discussed in 1.*c)* above, a new enzyme preparation is evaluated to determine if it is safe for its intended use. Such an evaluation focuses on characteristics and properties of the enzyme, the production organism, and the materials and methods used in the manufacturing process. Whether the evaluation performed on the alpha-amylase preparation is "traditional" or "additional" depends upon whether or not the enzyme is considered to be a new one. As discussed above in 1.*a)*, 1.*b)*, and 1.*d)*, this is essentially a regulatory question.

If the amylase preparation were considered to be simply another example of a *B. subtilis* alpha-amylase preparation, the traditional product evaluation would be done by the manufacturer to determine that the new example had no unusual properties that would affect its safe use. At least in the past, there would have been no formal review by a regulatory body.

The safety evaluation focused on: the structural and functional properties of the enzyme; the safety of the donor, recipient and intermediate organisms, particularly on whether the genetic modifications of the recipient introduced any properties that would adversely affect its safety for its intended use; the safety of the vectors used in the strain construction; and the material and methods used in fermentation and enzyme purification.

JECFA found that the production strain is not antibiotic-resistant, that the donor (*B. stearothermophilus*), intermediate (*E. coli*), and recipient strains (*B. subtilis*) are non-pathogenic and non-toxigenic, and that the vectors used in strain construction (pBR327, used in *E. coli*, and pUB110, used in *B. subtilis*) are well-characterised and do not encode toxins. The production strain does not express Shiga-like toxin, as shown by Vero cell

assay, and does not express staphylococcal enterotoxins A, B, C or D, as shown by antibody tests.

The *B. stearothermophilus* alpha-amylase derived from *B. subtilis* was shown to possess the same enzyme-specific activity, molecular weight, peptide maps, and reactivity towards antibody raised against alpha-amylase from *B. stearothermophilus* as the *B. stearothermophilus* alpha-amylase derived from *B. stearothermophilus*. The enzyme preparation produced no significant toxicological effects in a 13-week feeding study in dogs, nor in a one-generation reproduction study in rats.

Based on the information described above, and on the levels of the enzyme preparation needed to achieve its intended effect, JECFA concluded that the enzyme preparation is safe for its intended use and does not require a numerically specified acceptable daily intake.

4. Database available for traditional evaluation

None.

5. Novel component(s)/product

The *B. stearothermophilus* enzyme is expressed from a *B. subtilis* strain. The cloning might have affected either the enzyme itself or the production strain. Whether this is considered novel or simply another example of a *B. subtilis* preparation is a regulatory question, as discussed in 1.*a)*, 1.*b)* and 1.*d)* above.

6. Additional evaluation procedures

As discussed above, whether the evaluation procedures are considered "additional" or "traditional" depends on whether or not the enzyme preparation is considered new.

7. Rationale for additional evaluation procedures

The rationale for the evaluation procedures, whether deemed additional or traditional, was that both the enzyme and the production strain might have been altered by the genetic manipulations such that the enzyme preparation would no longer be safe for its intended use.

References

US Federal Register (1990). Vol. 55, pp. 10932-10936, 23 March, and references therein. Also see: Flamm, E. (1991). *Bio/Technology*, 9:349-351.

Thirty-seventh Report of the Joint FAO/WHO Expert Committee on Food Additives, WHO Technical Report Series No. 806, and references therein.

Lactic acid bacteria

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1. Conceptual points to consider

a) Concept of continua

Traditionally, the use of lactic acid bacteria is not considered a food safety issue. This is covered in Section 3 below.

b) Concept of temporal considerations

The use of genetically modified lactic acid bacteria is concurrent with the use of some novel compounds used in dairy practice, *e.g.* chymosin obtained through novel biotechnology, added egg-white lysozyme. This is covered in Sections 5 and 6.

c) Concept of reasonable certainty of no harm

Covered in Sections 5 and 6.

d) Concept of substantial equivalence

Covered in Sections 5 and 6.

e) Concept of variability

Not applicable.

f) Concept of sequential review

Covered in Sections 5 and 6.