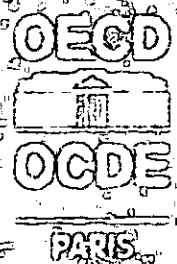
SAFETY EVALUATION OF FOODS DERIVED BY MODERN BIOTECHNOLOGY

CONCEPTS AND PRINCIPLES



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ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

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Publié en français sous le titre :

ÉVALUATION DE LA SÉCURITÉ DES DENRÉES ALIMENTAIRES
ISSUES DE LA BIOTECHNOLOGIE MODERNE
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.

ALSO AVAILABLE

Biotechnology, Agriculture and Food (1992) (93 92 03 1) ISBN 92-64-13725-4	FF185	£26.00	US$43.00	DM75
Safety Considerations for Biotechnology 1992 (1992) (93 91 05 1) ISBN 92-64-13641-X	FF70	£10.00	US$18.50	DM29

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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.

g) Evaluation of marker genes

Covered in Section 6.

2. Organism/product

"Lactic acid bacteria", a generic name which includes the bacterial genera *Lactobacillus, Leuconostoc, Lactococcus, Pediococcus* and *Streptococcus.*

3. Traditional product evaluation

Lactic acid bacteria may be considered as food constituents of dairy products, or they may be considered as food additives. They are among the oldest organisms used in classical biotechnology, comparable to yeast.

Originally, lactic acid bacteria found in the unfermented dairy product were used. This is still done on a larger scale in Dutch dairy farming. In industrial dairy production, so-called starter cultures are used in order to have better control of the fermentation. Starter cultures are derived from the lactic acid bacteria present in classical dairy products.

Traditionally, in most countries, the evaluation procedure for new varieties of lactic acid bacteria is not formally regulated except that the bacteria used should be harmless. Other criteria for evaluation are production of acid, flavour and extracellular polysaccharides. In countries where the use of industrial starter cultures is formally regulated, the lactic acid bacteria are normally considered non-toxigenic and non-pathogenic. In some cases (yoghurt) food legislation specifies a minimum number of lactic acid bacteria that should be present in the final product.

4. Database available for traditional evaluation

Not available in the Netherlands or Denmark.

5. Novel product

Two classes of products should be considered:

i) lactic acid bacteria with cloned homologous genes, or cloned genes derived from other lactic acid bacteria; *e.g.* genes encoding proteolytic enzymes, enzymes involved in sugar metabolism, nisin production, production of bacteriocins, and bacteriophage resistance; and

ii) lactic acid bacteria carrying heterologous genes; *e.g.* genes encoding fimbriae from other prokaryotic sources (bacteria), genes encoding egg-white lysozyme, prochymosin or genes encoding proteolytic enzymes from eukaryotic plant sources.

Class i) organisms would probably not be considered novel unless levels of expression were exceptionally high compared with traditional organisms.

Class ii) organisms are novel at least in the respect that these gene products have not been actively synthesised in dairy products, although they may be added to the product (egg-white lysozyme, chymosin). Addition of chymosin produced by transgenic *Escherichia coli* to dairy products is allowed (see case study).

6. Additional evaluation procedures

For traditional strains of lactic acid bacteria, no special evaluation would be required. In the safety evaluation of class *i)* organisms, the requirement should be met that the bacteria are harmless. Substantial equivalence can be claimed for these bacteria, depending on the degree of expression of the transgene(s).

The evaluation of class *ii)* organisms should as a general rule rely upon the concept that these organisms are considered novel. They should be evaluated on a case-by-case basis, taking into consideration *both* the gene product as a compound in relation to traditional dairy products *and* the effect of the new trait in relation to the function of the organism in its traditional habitat, as far as this effect relates to food safety issues. The evaluation of class *ii)* organisms can in special cases follow the paradigm of substantial equivalence.

Horizontal gene transfer is considered an additional safety issue for the use of genetically modified bacteria in food. As gene transfer by any one of the classical transfer mechanisms (conjugation, transduction, transformation, and the influence of transposition on any of these) is likely to occur, an additional risk assessment may be necessary to address the possibility of the emergence of novel micro-organisms, either in the alimentary tract or in waste water, that could have an adverse effect on the food chain.

7. Rationale for evaluation procedures

Cloning of genes from lactic acid bacteria into other lactic acid bacteria would in general not lead to production of novel compounds that have not been consumed before, if adverse effects from over-production or influencing of pathways can be excluded. In special cases (*e.g.* the transfer of bacteriocin genes) the population dynamic considerations might have to be included in the evaluation.

Expression of eukaryotic genes in prokaryotes will in general lead to the same gene product that is found in the eukaryotic cell, except for the absence of post-translational modification in the prokaryotic system. In the evaluation procedure, these bacteria should be considered as novel and reviewed on a case-by-case basis.

Marker genes will probably not be a problem if the same genes are also present in the traditional population of lactic acid bacteria.

Low erucic acid rapeseed oil (LEAR oil)

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Low erucic acid rapeseed oil (LEAR oil) provides an example of the evaluation of a "novel" food. Although this product was not produced as a result of biotechnological modification, nor would it be considered novel today, its affirmation as a Generally Recognised As Safe (GRAS) food ingredient illustrates the application of a number of the principles developed by the Working Group regarding the establishment of safety.

This summary provides an overview of the petition submitted with respect to the GRAS affirmation for LEAR oil[1] as well as the results of the evaluation.[2]

1. Conceptual points to consider

The LEAR oil case study is illustrative of a number of the conceptual points related to the evaluation of novel foods and food components. The concept of *continua* in food use – *i.e.* uses ranging from specific food applications to general food use – is demonstrated by the discussions regarding the proposed use of LEAR oil in infant formula as distinct from food applications generally. The evolution of products over time with attendant diminution in their degree of novelty (temporal considerations) is also noted.

The LEAR oil example also demonstrates the concept of a reasonable certainty of no harm, taking into account the *continua* of intended uses and expected conditions of consumption in place of "traditional" oils. This case study is also illustrative of the application of the concept of "substantial equivalence", in that the LEAR oil was shown to be very similar to and composed of the same basic components as traditional rapeseed and other commonly consumed vegetable oils except for the low level of erucic acid, the component of concern. The establishment of substantial equivalence also took into account the variability in the available database for the commodities in question, as well as in estimates of consumption.

2. Organism/product

Low erucic acid rapeseed oil (LEAR oil).

3. Traditional product evaluation

Rapeseed oil has had a long history of use as an edible oil source in a number of European countries as well as in China, India and Japan. Canada began growing rapeseed in the 1940s to supply the edible oil market at home and abroad. Oil prepared from rapeseed grown before 1971 contained high levels of a fatty acid known as erucic acid. The erucic acid level in this oil varied considerably, but was generally in the range of 30 to 60 per cent.

In response to potential safety concerns regarding effects associated with high levels of erucic acid (cardiac lesions in experimental animals), efforts were made in Canada in the 1960s to develop strains of *Brassica napus* and *B. campestris* with a low erucic acid content. By 1974, new varieties capable of producing oil containing less than 5 per cent erucic acid comprised almost the entire Canadian rapeseed crop. The level of erucic acid has continued to decline over the years through continued selective breeding practices. In the GRAS affirmation petition, LEAR oil was defined as rapeseed oil containing no more than 2 per cent erucic acid based on its total fatty acid content.

4. Database available for traditional evaluation

Databases relating to the composition of a number of edible fats and oils are available both nationally and internationally. Standards of identity and composition for fats and oils such as those of the Codex Alimentarius have also been elaborated. The Codex Standard for edible low erucic acid rapeseed oil (Codex Standard 123-1981) included oils in which the erucic acid content is as high as 5 per cent of the component fatty acids. In addition to erucic acid, the principle fatty acids in LEAR oil are palmitic acid (2.5 to 6 per cent), oleic acid (50 to 66 per cent), linoleic acid (18 to 30 per cent) and linolenic acid (6 to 14 per cent).

The evaluation of "traditional" fats and oils normally takes into account history of use together with information from studies in humans and experimental animals on safety aspects, nutritional properties and exposure, in addition to information on product characterisation and composition.

5. Novel component(s)/product

At the time of the Petition for GRAS affirmation, the "novel" aspect of this product related to its low erucic acid content when compared with the "traditional" counterpart. LEAR oil is currently the major rapeseed oil of commerce.

6. Additional evaluation procedures

Extensive information was obtained on the composition of the LEAR oil, proposed uses, dietary intake, nutritional data and toxicology, as noted below:

Product Information:

a) Composition

Samples of the crude and refined oil were characterised in terms of fatty acid composition. The erucic acid levels were consistently low. In 1982, for example, they were at an average of 1.2 per cent. Precise comparisons between LEAR oil and other vegetable oils could not be made because vegetable oils vary in composition depending upon the variety of plant and the growing conditions. The submitted data, however, showed that refined and deodorised LEAR oil was composed primarily of triglycerides (96.5 per cent). Except for the presence of the low level of erucic acid, the levels of individual fatty acids were comparable with those of other traditional oils, for example soy, corn, peanut, safflower, olive and sunflower.

Questions relating to the levels of pesticide residues and naturally occurring contaminants such as mycotoxins in LEAR oil were also examined in the course of the review. These were not considered to be of concern.

b) Dietary exposure

LEAR oil can be used by itself as a salad or vegetable oil; however, it is usually blended with other vegetable oils in the production of margarine, shortening, salad oil and vegetable oil. Different blends or formulations have differing physical properties that are specifically derived for different applications. In 1977, LEAR oil constituted 33 per cent of the fat used in margarine, 20 per cent of the fat used in shortening and 52 per cent of the fat used in salad oil.

Dietary intakes for total fats and oils were developed using both apparent per capita food consumption data and the results of the Nutrition Canada Food Consumption Survey (1971-72). These data, and information concerning the proportion of LEAR oil in such commodities as margarine, shortening and salad oil, were used as a basis for estimating both average and upper limit intakes for LEAR oil on a per capita basis. Further calculations in which it was assumed that all visible fat consisted of LEAR oil were developed to estimate intakes in the highest fat-consuming segment of the population (20- to 30-year-old males). Estimates of exposure to erucic acid were also developed.

c) Nutritional data

Feeding studies in laboratory animals including rats, dogs, monkeys and pigs were conducted to investigate the nutritional adequacy and digestibility of LEAR oil. Controlled, volunteer studies were also undertaken in man.

d) Toxicology

There was a large body of data provided on the effects of feeding rapeseed oil containing varying levels of erucic acid, as well as other vegetable oils, on laboratory

animals. The presence of cardiac lesions in certain strains of laboratory rats raised particular concern. This resulted in the presentation of a detailed rationale which concluded that the laboratory rat, in particular Sprague Dawley rats, may be unusually susceptible to cardiac lesions when fed vegetable oils. This rationale was developed through the consideration and comparison of the results of experiments conducted in a variety of animal species with oils from different sources. Studies with LEAR oil included monkeys, dogs and pigs and showed no significant increase in myocardial lesions when compared with animals fed a control diet. The results of these and other studies supported the conclusion that the observations in laboratory animals fed LEAR oil were no different from the response to other food oils. Other reported effects, including cold stress mortality and reduced energy utilisation, were observed only when the exposure was to erucic acid levels that greatly exceeded the anticipated human exposure to LEAR oil.

7. Rationale for evaluation procedures and commentary on the approach to the assessment of LEAR oil

a) Product characterisation

Product characterisation is a principal requirement in the evaluation of novel food entities. Data were provided to demonstrate the substantial equivalence of the "new" and "traditional" oils – *i.e.* LEAR oil was shown to be composed of the same basic components as the traditional rapeseed oil product, as well as other commonly consumed vegetable oils, apart from the presence of low levels of erucic acid.

The demonstration of substantial equivalence, which took into account the inherent variability of the compositional databases, was an important factor in the evaluation process and in the development of the rationale for GRAS affirmation.

b) Dietary exposure

In the evaluation of the safety of foods or food components, it is considered necessary to have exposure information available. Detailed estimates of the potential intakes of LEAR oil and erucic acid were developed based on actual areas of use in Canada, projections of maximum levels of use, and per capita consumption of oils and fats. The estimated upper level of exposure to erucic acid and the exposure from general food use of LEAR oil were not considered to pose a safety concern except in the case of infant formula (see "Overview and conclusion" below, second paragraph).

c) Nutritional acceptability/adequacy

The data considered in assessing the nutritional adequacy of the new oil paralleled those that would be considered for any new oil. The composition of the oil was determined and, apart from the presence of erucic acid, the levels of individual fatty acids were found to be comparable with commonly consumed vegetable oils. Insofar as the general population is concerned, no unique issues were raised, with respect to the digestibility or nutritional adequacy of food products containing LEAR oil in place of traditional vegetable oils, that would preclude such use.

d) Toxicology

Because of concerns with respect to cardiac effects of rapeseed oils in general, it was considered necessary to have available the results of toxicological studies in animals. The approach taken was to develop the supporting toxicological database, which included a large body of data published in the scientific literature, explain the observations in different animal species, and provide a rationale for the observed effects.

Overview and conclusion

The LEAR oil case study illustrates the evaluation of the safety for human consumption of a "novel" ingredient. It included a description of the history of food use of the "traditional" rapeseed oil counterpart and background information on the development of the "novel" varieties with low erucic acid levels. The composition of the novel oil was detailed, and a comparison made with respect to the similarities to both traditional rapeseed oil and other common vegetable oils, in order to illustrate the concept of substantial equivalence, taking into account factors such as exposure estimates.

This case study also illustrates the need to examine the *continua* of food uses and to have support data available with respect to specific applications. The original proposal for LEAR oil included both use in infant formula products and general use in food. However, because further data were considered necessary regarding the properties of a number of food oils used in infant formula, this specific use of LEAR oil was not reviewed nor was its GRAS status affirmed at the time.

Due to concerns regarding the safety of the erucic acid component in both "traditional" rapeseed and LEAR oil, the results of toxicological studies were considered to be an important and necessary component of the evaluation. An extensive toxicology database was reviewed, with particular reference to cardiac effects noted in laboratory animals. A scientific rationale, supported by the results of animal feeding studies in several species with a range of vegetable oils, was provided to demonstrate that "LEAR oil is safe for human consumption as a fat or oil in food when used in accordance with current good manufacturing practice". LEAR oil was affirmed as Generally Recognised As Safe (GRAS) in 1985.

Notes and References

1. *US Federal Register* (1982), "Agriculture Canada, Research Branch, Filing of Petition for Affirmation of GRAS Status", Vol. 47, No. 157, p. 35342, 18 August.
2. *US Federal Register* (1985), "Direct Food Substances Affirmed as Generally Recognized as Safe: Low Erucic Acid Rapeseed Oil", Vol. 50, No. 18, pp. 3745-3755, 28 January.

Myco-protein

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1. Conceptual points to consider

The concept of "reasonable certainty of no harm" takes into account:

- intended uses; and
- expected conditions of consumption.

Evidence to support this concept came from the extensive toxicological and nutritional testing carried out on myco-protein. Details are provided in Section 6 below.

In this study, the concept of a "continuum" demonstrates that myco-protein, being at the extreme end of the *continuum* of novelty, cannot be considered to be substantially equivalent and therefore requires safety testing to show reasonable certainty of no harm. This is reflected in Sections 5 and 6.

Once myco-protein produced using a specific process has been pronounced safe, the concept of "substantial equivalence" can be applied in the future to myco-protein produced by minor process changes. This is reflected in the "Addendum".

2. Organism/product

Myco-protein is a high-fibre, low-fat food derived from a non-pathogenic, naturally occurring strain of the filamentous fungus *Fusarium graminearum*. It has a protein content similar to that of whole egg and a texture resembling that of lean meat.

Myco-protein is produced through controlled continuous aseptic fermentation of the fungus in a carbohydrate medium. At harvest, the recovered mixture of mycelia and fermentation medium is subjected to thermal shock, to reduce the RNA content of the mycelia, and then filtered to remove the fermentation medium. After vegetable flavours and egg white have been added, the myco-protein is cooked and, depending on the type of product in which it is to be used, may be sliced, diced or shredded.

3. Traditional product evaluation

There is no traditional food equivalent to myco-protein. An entirely new procedure had to be developed to assess this product.

In the light of the evaluation and experiences gained, it should be possible to facilitate future evaluations of equally novel single-cell protein products (concept of a *continuum*).

4. Database available for traditional evaluation

As myco-protein was a novel food with no traditional equivalent, a database was not available for the evaluation of this type of product. Assessment was based on detailed compositional and safety information relating to the specific strain of *F. graminearum* used for myco-protein production.

5. Novel component(s)/product

Myco-protein was the first truly novel food to be evaluated in the United Kingdom for safety in use (concept of a *continuum*).

6. Additional evaluation procedures

The developers submitted extensive data for review, both on the manufacturing process and on the product.

a) Organism

Information on the taxonomy of *F. graminearum* was supplied, and the potential for myco-toxin formation by the strain used to produce myco-protein was investigated. No detectable myco-toxin formation was found under the fermentation conditions nor under test conditions where other strains of *F. graminearum* can be induced to produce myco-toxins.

b) Process

The culture medium is an aqueous solution of carbohydrate to which a number of micro-nutrients have been added. The carbohydrate may be obtained only from sources which have been approved as part of the final specification.

The inoculum cultures are maintained under aseptic conditions. Tests for contamination and for strain stability are carried out at all stages of the fermentation procedure.

The fermentation is carried out under aseptic conditions and is controlled through both on-line and off-line monitoring. A number of parameters are measured, including dissolved oxygen, pH and suspended solids. In addition, the fermentation is regularly monitored to ensure the absence of foreign organisms and mutants.

Following the World Health Organisation recommendations in 1972 that single-cell protein for adult consumption should not provide more than 2g of RNA per day, an effective method of RNA reduction in the harvested fungus was sought. Thermal shock was found to reduce the RNA content of the product by about 90 per cent.

The myco-protein, recovered by filtration after the thermal shock treatment from the mixture of culture medium and products of RNA degradation, contains about 30 per cent solids.

c) Product

Analytical data submitted on the composition of typical myco-protein included details of the following: nitrogenous material, amino acids, carbohydrates, fibre, lipids, minerals and vitamins. No unusual nitrogenous compounds (*e.g.* D isomers of amino acids) or fatty acids were found, and the carbohydrate consisted largely of chitin.

Safety assessment was designed to establish whether there were any toxic substances in the myco-protein. Since it was impossible to predict what these substances might be and hence what effect the extraction/concentration procedure might have upon them, a battery of toxicity tests was carried out on the whole product.

No dose-related adverse effects relevant to the safety evaluation for man were recorded in any of the studies carried out. However, certain problems arose from the formulation of test animal diets caused by the incorporation of high levels of protein.

Animal feeding trials were carried out on myco-protein to investigate protein quality, limiting amino acids, amino acid availability and metabolisable energy. The effect of chitin on the absorption of amino acids, vitamins and minerals was also evaluated.

No results were obtained that demonstrated an anti-nutritional effect from myco-protein. Indeed, the human nutritional studies indicated that myco-protein is a source of good quality protein.

No major toxic effects were identified from the ingestion of myco-protein by humans. Studies on allergenicity provided no conclusive evidence of allergic reaction in volunteers. However, standard antigens for skin tests were developed before the product was marketed to allow the identification of any clinical reaction to myco-protein.

From the properties of myco-protein, it was possible to identify potential markets and hence to assess intakes.

The results of the toxicological studies and the evaluation of the *Fusarium* strain for potential myco-toxin production indicated that there are no potential toxicological effects from the use of myco-protein as a human food, provided that the product that is sold complies with the same specification as the product that was tested.

The nutritional studies demonstrated that myco-protein can provide a source of good quality protein and that no anti-nutritional factors are present.

After evaluation of this data by the UK authorities, product and process specifications were agreed and approval was given to test market myco-protein in a limited geographical area. No adverse reactions were reported in marketing trials involving some 4 000 people. Following this, UK-wide marketing was authorised.

7. Rationale for evaluation procedures

Myco-protein had no history of consumption. Assessment of the safety of its use as a human food focused on potential toxicological and nutritional effects since the product complied with the microbiological specifications of the PAG (Protein Advisory Group) for single-cell proteins (see *PAG Bulletin*, 1970, Vol. 4, No. 3).

From the analytical data, no toxicological problems were anticipated from the major components of the myco-protein (protein, fat and carbohydrate). However, specific studies were designed to address areas of obvious concern – the potential for myco-toxin production and the nutritional value of the chitin present. In addition, a battery of tests designed to determine the presence/absence of unknown toxins was applied (concept of sequential review).

Addendum

After myco-protein had been evaluated and found safe for use as human food, a change in production process was needed to increase capacity to provide sufficient product for marketing.

Organism/product:

Myco-protein.

Traditional product evaluation:

A procedure had been established for the original product application.

Database available for traditional evaluation:

The information that had been submitted in the original product application formed a database.

Novel component(s)/product:

The UK authorities requested additional data to demonstrate that myco-protein produced in an airlift fermenter was sufficiently similar to that produced in a stirred tank fermenter to be safe for use as human food.

Genetically modified baker's yeast

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1. Conceptual points to consider

A "temporal consideration" is the long-term use of *Saccharomyces cerevisiae* to leaven bread, mentioned in Section 3 below.

The concept of "substantial equivalence" is reflected in Section 6, where the evaluation of the genetically modified baker's yeast is described and its characteristics compared with those of the unmodified strain. Section 7 also notes this concept.

The concept of "reasonable certainty" follows from the concept of "substantial equivalence" in this case, and is mentioned in Section 7.

2. Organism/product

Genetically modified baker's yeast, *S. cerevisiae*.

3. Traditional product evaluation

Traditionally, new strains of baker's yeast are not assessed for safety as the species is non-pathogenic and has been consumed in leavened bread for many centuries (temporal consideration).

4. Database available for traditional evaluation

No formal database on the composition of baker's yeast strains was available to aid the evaluation of this product, although many strains have been serotyped. However, the company which made the submission for the safety evaluation of the genetically modified yeast provided necessary comparative information relating to conventional strains.

5. Novel component(s)/product

A strain of baker's yeast traditionally used to leaven sweet doughs has been genetically modified to leaven both lean and sweet doughs. The modification has enhanced the secretion of the maltose fermenting enzymes, maltase and maltose permease, especially at the beginning of the leavening process. The modified baker's yeast has a higher metabolic rate and releases higher levels of carbon dioxide earlier in the leavening period than the unmodified parent strain. It has been found that this reduces the time needed for leavening.

6. Additional evaluation procedures

Since the novel yeast strain is obtained by genetic modification, it was assessed to establish that it presented no greater hazard to production and bakery workers, to the environment, or to consumers of food containing the yeast than did the unmodified strain (concept of substantial equivalence). The evaluation of the safety of the modified yeast to workers and to the environment is not described in this study.

The evaluation of the novel strain as a novel food took into account that consumption of both live and dead cells could occur. Specific aspects considered included:

a) Characteristics of the host and donor organisms

The host organism is a well-characterised strain of the non-pathogenic species *S. cerevisiae* that is used extensively in the industrial production of baker's yeast for leavening sweet dough. The natural inducible promoters for the maltase and maltose permease genes were removed and replaced by strong, constitutive promoters from the same strain of baker's yeast.

b) Genetic modification procedure

The donor DNA was taken entirely from *S. cerevisiae*, apart from small pieces of synthetic, non-coding DNA used as linker sequences. It consisted of genes coding for the enzymes maltase and maltose permease, together with two well-characterised, strong, constitutive promoters.

At each stage of the transformation procedure, the construct was cloned in *E. coli* and restriction enzyme digestion and/or sequence analysis were used to confirm that the sequences were as predicted.

A schematic presentation of the insert showing its location on the chromosome was available. This confirmed that no untoward effects were likely from the insertion.

The transformation procedure was designed to ensure that the construct was integrated into the chromosome and was devoid of any heterologous DNA. Antibiotic resistance markers used to facilitate the transformation procedure were removed, and no prokaryotic sequences remain in the genetically modified yeast.

c) Genetically modified organism

Hybridisation patterns of DNA from the genetically modified strain were unchanged after 100 generations of vegetative growth, indicating that the strain was as stable as conventional strains (concept of substantial equivalence).

All heterologous prokaryotic DNA had been eliminated during the transformation procedure, and the Southern-blot experiments had demonstrated the stability of the insert. Transfer of DNA from the genetically modified baker's yeast to other organisms is unlikely. It is known that on cell death in unmodified strains of *S. cerevisiae*, autolysis of the cell contents occurs before the cell wall is destroyed and no free DNA, which could be taken up by other organisms, is released. Mating or normal exchange of DNA does not occur between *S. cerevisiae* and any known fungal or bacterial pathogens, and no known DNA viruses are harboured by *S. cerevisiae* which might transfer DNA to other organisms. This indicates that the risk of DNA transfer from the genetically modified baker's yeast would be no different from that of its transfer from the unmodified parent strain (concept of substantial equivalence).

Production of toxic metabolites by the genetically modified strain is unlikely for several reasons: the host organism is non-pathogenic; the donor DNA was obtained from the same strain as the host; only homologous, constitutive promoters were rearranged; and the initial activities of the genes controlling maltase and maltose permease only are affected. The biochemical reactions occurring during the leavening process are the same in the genetically modified strain as in the unmodified strain since both produce maltase and maltose permease, though less efficiently in the unmodified strain (concept of substantial equivalence).

7. Rationale for evaluation procedures

In theory, the changes to the baker's yeast effected through the use of genetic modification techniques could have been made using traditional yeast breeding. Had traditional yeast breeding been used, the new strain of baker's yeast would not have been subjected to the detailed consideration described.

The data presented for the safety assessment demonstrated that the genetically modified baker's yeast is sufficiently similar to the unmodified strain, in respect of its stability, potential for genetic transfer and potential for toxin production, as to present no greater risk to the consumer than that presented by the unmodified strain (concept of substantial equivalence). Since unmodified strains are presumed safe, it follows that the modified strain, being substantially equivalent to an unmodified strain, is also safe (concept of reasonable certainty).

Tomato

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1. Conceptual points to consider

Although this study is not based on a specific case, there are some general points to consider when a genetically modified tomato is to be evaluated. These points will be among the major elements for establishing *substantial equivalency*. Also to be considered in this evaluation, of course, will be the new trait(s).

A description of the tomato today, specifying the content and variation of different substances, will contribute to the discussion of the *concept of variability*.

In order to make more specific comments on genetically modified tomatoes, some examples from the literature describing such tomatoes will also be discussed.

2. Organism/product

Organism: Lycopersicon esculentum, tomato.

Transgenic tomatoes used as examples in this text:

- Tomatoes that are *glyphosate-resistant* due to the insertion of a gene coding for 5-enolpyrovylshikimate-3-phosphate synthase (EPSPS). The EPSPS normally found in plants is inactivated by glyphosate (Roundup). The inserted gene is identical to the normal gene found in plants, except for a few changes (mutations) in the DNA sequence.
- Tomatoes that are *virus-resistant* due to the insertion of the gene coding for a virus coat protein (cp). This protein is normally found in the plant when infected by the virus and confers resistance to the same virus.
- Tomatoes with *prolonged fruit ripening* due to the insertion of a DNA coding for an antisense RNA sequence, which partially inactivates the "sense" gene coding for polygalacturonase (PG).

In all the above examples, there are also insertions of marker genes giving rise to, for example, kanamycin resistance in the plant.

Product eaten:

The raw mature fruit or immature green fruit for preserving (pickling). The mature fruit can also be skinned and canned for later use. When tomatoes have to be transported over longer distances, they are often harvested as immature green fruit. Green tomatoes are not as sensitive to knocks as mature ones. When mature, the shelf lives of green tomatoes are longer than those of tomatoes that were harvested red.

Other parts of the plant are not used as food.

3. Traditional product evaluation

Varieties of traditional tomatoes are frequently tested in Denmark through cultivar examination studies, especially when new cultivars reach the market. After harvesting, a quality assessment based on parameters such as yield, texture, taste and flavour is carried out on the individual cultivars. In addition, chemical constituents such as acids, sugars and various nutrients are analysed. The most recent study was carried out in 1988 (Willumsen *et al.*, 1990).

Since tomato is regarded in Denmark as an important food for the intake of certain nutrients, it is included in the Danish Food Monitoring System. As a part of this system, the level of important nutrients in tomatoes is analysed in selected cultivars every five years. Results from the first five-year period are reported in *Food Monitoring in Denmark* (LST, 1990*a*). The second cycle of the Food Monitoring System for fruits and vegetables was carried out in 1988 (LST, 1990*b*). In addition to these five-year studies, a special investigation of the nutrient content of different cultivars of tomato was carried out with the purpose of influencing the choice of varieties for commercial use. Nutrients regarded as important in tomatoes, and so included in the Food Monitoring System, are vitamin C, folacin, vitamin B1 and vitamin B6. However, due to available analytical methods, only vitamin C was included in the first cycle.

No great differences in nutrient content have been found in the individual studies, and there is a good agreement with the Danish food composition table (Moller, 1989). The following figures are examples of normal content as given in the food composition table:

Vitamin C:	11.3-23.1 mg/100g
Folacin:	3 mg/100g
Vitamin B1:	0.016-0.053 mg/100g
Vitamin B6:	0.0074-0.154 mg/100g

Until now, no natural toxins have been included either in cultivar examination studies or in the Food Monitoring System. This is partly due to the lack of an approved analytical method and partly because no real concern has been expressed that this was a problem. However, the need for studies in this area should be considered in the future in order to determine a "normal" level of alkaloids in traditional cultivars of tomato.

4. Database available for traditional evaluation

At the Institute of Toxicology in Denmark, a database is being built up containing information on the naturally occurring toxic, nutritional and flavouring substances in the 250 plants most normally used for human consumption. The intention is to use this database to provide benchmarks for the evaluation of foods in general.

5. Novel component(s)/product

To consider whether a tomato fruit developed by the application of biotechnology is *substantially equivalent* to analogous conventional tomato fruit requires knowledge of the parental organism. Measurement of every substance with potentially adverse effects, or every nutritionally valuable substance, in the plant is impossible. It is therefore important to focus on the levels of key substances, *i.e.* compounds which could influence the health aspects of the plant. Alpha-tomatine, the naturally occurring toxicant in the tomato, is such a substance.

The level of alpha-tomatine decreases through fruit maturation (0.87 mg tomatine per gram fresh weight in green fruit, 0.45 mg in yellow fruit, and 0.36 mg in red fruit) (Jadhav *et al.*, 1981). Ripe red fruit loses almost all its tomatine when left on the plant for two to three days. Alpha-tomatine is not mobile in the plant, and therefore the tomatine level is determined only by synthesis and degradation in the fruit (Eltayeb and Roddick, 1985).

Alpha-tomatine from cultivated tomatoes causes only minor inactivation of acetylcholinesterase compared with the glycoalkaloids from potato (solanine and chaconine), but its toxicity is about the same level as solanine and chaconine (Keeler *et al.*, 1991). When tomatine was administrated by gavage to hamsters, it caused severe gross changes in the gastric glandular mucosa and intestinal mucosa similar to changes induced by equimolar doses of solanine and chaconine (Baker *et al.*, 1991). Alpha-tomatine is not teratogenic (Keeler *et al.*, 1991).

Wild relatives of tomato often contain resistance genes attractive to the tomato breeder. But several wild relatives contain various glycoalkaloids in high levels. For instance, *Lycopersicon hirsutum glabratum* (insect-resistant) shows an exceedingly high content (3.39 mg per g fresh weight) in the mature green fruit (Van Gelder and De Ponti, 1987). From the genus *Solanum*, other glycoalkaloids than alpha-tomatine can be introduced into the tomato plant by wide crossings. The new biotechnique of somatic cell fusion may act in the same way as wide crossings. Genetic engineering allows the introgression of genes from virtually any organism into the tomato.

If the degradation of alpha-tomatine in developing tomato fruits is related to a substrate-specific enzyme, as indicated by Juvik (1977), the process could in theory easily be blocked, resulting in a change in alpha-tomatine levels in ripe fruits without any other effects on the tomato.

Benchmarks for substances with potentially adverse effects, and for nutritionally valuable substances, found in the tomato plant are an instrument for carrying out risk evaluation on a scientific basis – for example, through the establishment of a list of the highest (or lowest) acceptable levels of specific substances based on today's tomatoes. Such a list would be useful to plant breeders and authorities when evaluating new tomato

strains. Key substances could be selected from this list. The number of key substances depends on the specific case. For example, additional key substances might be needed for the evaluation of plants with elevated levels of pest resistance. The table below shows the known toxins and some other substances with potentially adverse effects found in tomato. From this list, only alpha-tomatine is expected to be a general key substance.

Toxic constituents in tomato

+ : found in the fruit, but quantifications are not available
– : the constituent has not been found in tomato fruit

Toxic constituent	
Alpha-tomatine	0-0.87 mg/g
Tomatidine aglycone of tomatine	+
Saponines	+
Coumarins	–
Lectins	+
Serotonine	–
Oxalic acid	0.012-0.015 mg/g
Protease inhibitor	+
Histamine	+ (tomato juice)

Glyphosate-resistant tomato:

The EPSPS enzyme giving rise to the resistance has been analysed. It has the same enzyme activity as the equivalent EPSPS enzyme normally found in plants. According to reports (Kishore and Shah, 1988 for review), the differences are confined to the affinity of the enzyme to glyphosate. If results from such analyses show no other differences, the new enzyme should be considered as substantially equivalent in relation to food safety.

Virus-resistant tomato:

In order to establish whether or not the coat protein in the transgenic tomato fruit should be considered substantially equivalent, further information is needed on the natural level of coat protein in tomato. A decision on substantial equivalence could be based on the following procedure: *1)* from information on the normal level and variation of coat protein in tomato fruit, as well as the exposure pattern, determine the maximum level (M level) of coat protein in the fruit that can be claimed to be safe, with a high degree of certainty, on a scientific basis (a higher level might well be safe, but we do not have the scientific evidence); and *2)* if the average amount of coat protein in green fruit, etc. in both control and transgenic tomatoes is higher than the M level, the tomato fruit should not be considered as substantially equivalent.

Tomato with prolonged fruit ripening:

Because there are no reports with respect to food safety on any special adverse effects from specific DNA or RNA molecules in plants, the antisense RNA should not be regarded as a new product and should be considered to be safe as such (i.e. information that no new protein is produced from this antisense RNA should be required). What is essential in this case is to focus on what the impact on the plant will be when the level of the enzyme PG is lowered, and to be aware of any other changes that might be found due to, for example, somaclonal variation (that is, to consider differences in key substances

using a benchmark). Because the fruit in the transgenic tomato will develop normally but more slowly, there is no expectation of any "secondary" changes.

Marker genes:

From a food safety point of view, marker genes should be dealt with in the same way as any other inserted genes. The fact that a good marker gene can be expected to be inserted into all transgenic plants should be taken into consideration when the evaluation takes place. If the gene cannot be accepted in all plants, an "audit" may be necessary when the first evaluation of the marker gene is made.

6. Additional evaluation procedures

Glyphosate-resistant tomato:

No further evaluation of the inserted gene coding for EPSPS.

Virus-resistant tomato:

If substantial equivalence has been rejected due to a high level of coat protein in the plant, the protein should be evaluated more thoroughly. Perhaps from the knowledge *1)* that it is a protein with a structural function to build up the coat of the virus; *2)* that the protein has no enzyme function; and *3)* that the protein has (in a smaller amount) a safe history from the eating of infected tomatoes, the protein can be regarded as safe for human consumption and for use in further breeding.

Tomato with prolonged fruit ripening:

No further evaluation of the new product – *i.e.* the antisense RNA.

7. Rationale for evaluation procedures

Factors leading to the rejection of substantial equivalence:

a) the level of one or more of the key substances is changed significantly in an unfavourable direction, seen from a food safety point of view;
b) the product from the inserted gene is at significantly higher levels than normally seen in the plants, or the products do not exist naturally in the plant.

Other remarks: The evaluation of new tomatoes should be based on the tomato being eaten as a major part of a meal (worst case).

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Potato

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1. Conceptual points to consider

a) Continua

For potatoes, and for Solanaceae in general, the food safety issue focuses on the glyco-alkaloid content of the tubers. The issue is well-known from classical breeding practice: as a result of the attempts to get disease-resistant varieties through crossing with a wild relative of *Solanum tuberosum*, glyco-alkaloids of the wild relatives have appeared in the potato. This is mentioned briefly in Section 3 below. New biotechnology may add to this concern, as the glyco-alkaloid content of a new variety may vary substantially from the parent variety through somaclonal variation. As all present-day genetically modified potato variants have gone through a stage of somatic cloning, variations of glyco-alkaloid content will most probably be due to this effect, the chance of pleiotropic effects of the actual genetic modification being much lower.

b) Temporal considerations

PVX coat protein was present in commercial lots of potatoes even before this was realised by breeders or growers. The general public will not be aware of its presence, as it does not influence the food quality of the potatoes. (See Section 5.)

c) Reasonable certainty of no harm

As potatoes are under no circumstances eaten raw (at least not in large quantities), it can be taken for granted that any introduced foreign gene product will be denatured in the consumed food. (See Section 6.)

d) Concept of substantial equivalence

Substantial equivalence of PVX-resistant potatoes is the main issue of Section 5.

e) Concept of variability

The variability of glyco-alkaloid levels is covered in Section 3 and in "Continua" above.

f) Sequential review

In the case of PVX-resistant potatoes, review would start with the classical criterion of glyco-alkaloid content (Section 3), followed by a consideration of the level of expression of PVX coat protein, compared with the levels in "classical" expression through viral infection (Section 5), and with expression of the selective marker gene neomycin phosphotransferase II (NPT-II) (Section 6).

g) Evaluation of marker genes

This is the last step in the sequential review (Section 6).

2. Organism/product

Potatoes and potato products are used as food in many and varied ways. In all cases the potatoes are heated (for example, cooked, deep fried) before use. Whole tubers are consumed ("in the peel") in some cases. Specific cultivars are used for industrial starch production.

3. Traditional product evaluation

In the Netherlands, new potato varieties can only be marketed after they have been included in the List of Varieties of Agricultural Crops. This is also required for marketing of a variety within the European Community, and, reciprocally, any variety put on this list is allowed on the European market. The first principle for admission to the List is that a new variety should be truly novel, *i.e.* it should have traits that distinguish it sufficiently from other varieties for some use, be it ordinary consumption, processing for special consumption (for example, chips, crisps), or as industrial potatoes.

Thousands of trials for the development of a new variety are conducted each year. Testing is done over three consecutive years in small-scale test plots at about 30 sites in the Netherlands.

Of the traits considered in testing, only the determination of the total glyco-alkaloid content of the tubers relates to food safety. As the content depends very much on a variety of factors [location of growth, time of harvesting, condition of storage, part of the tuber (skin, core) tested], the maximum allowed levels in potatoes for consumption are given as (the mean of) the levels in two "standard varieties" (Irene and Eersteling). Higher levels of glyco-alkaloids are allowed in industrial potatoes than in potatoes for consumption. There is an "absolute limit", although this is not set by law, of 100 mg/kg. Too high content of glyco-alkaloids is in fact the only trait that may definitely bar a potato from the market. The safety concern has already been identified in classical breeding, as glyco-alkaloid levels have been known to be affected in the outcome of crosses between *Solanum tuberosum* and wild *Solanum* relatives.

All other traits tested are more or less desirable. They may render a potato variety more appropriate for some type of use, or for growth in certain soils. The traits tested include: early maturity, development of foliage; colour of skin, yellowness of flesh; number, size, shape and uniformity of tubers, frequency of outgrades, marketable yield;

dry matter content; sprouting during conservation; consumption quality (cooked to Dutch taste); resistance against viral, bacterial and fungal infections; resistance against nematodes; sensitivity to harvest damage; resistance to second growth and drought.

The data in the List of Varieties provide a clear framework for the determination of substantial equivalence.

4. Database available for traditional evaluation

The Descriptive List of Varieties of Agricultural Crops includes compilations of the test data for different varieties. Absolute values for the total glyco-alkaloid content of different varieties are not directly available, as these values are too much dependent on test conditions and are in fact of no use to the non-expert.

COBA (Database of Contaminants in Food Products), a Dutch database belonging to the State Institute for Quality Control of Agricultural Products, contains public information on contaminants in food on the market. The main data available on contaminants in potato are on levels of heavy metals and nitrates. Data are derived from monitoring of agricultural products on the market.

5. Novel product

A number of genetically modified potatoes are being tested, or will be tested in the very near future, in small-scale field experiments in the Netherlands. Novel traits introduced by genetic modification include herbicide resistance, virus resistance (viral coat proteins), pesticide production (Bt toxin), bacteriocide production (apidaccine, cropine), and modification of pathways of starch biosynthesis. Of these genetic modifications, only the modification of starch biosynthesis would not influence the food properties of the potato involved. In the other cases, a novel gene that has not been present in the gene pool of *Solanum tuberosum*, or the Solanaceae in general, is introduced.

The case of resistance in potatoes against potato virus X (PVX), through expression of a cloned PVX coat protein, offers a clear-cut example of the application of substantial equivalence in the evaluation of novel food obtained through biotechnology, and one that will reach the market in the very near future. In the transgenic lines tested, the viral coat protein is expressed in all tissues of the plant by means of a constitutive promoter (the cauliflower mosaic virus 35S promoter). Expression of the same gene occurs in natural virus infection, which has always been common as the virus is endemic in the Netherlands. Expression of coat protein during natural infection is higher by at least an order of magnitude, compared with the expression in the transgenic potato variant under consideration for commercialisation. The transgenic potato should be considered novel in that the viral coat protein has never been consciously added to food although it has been present even if in a different form, *i.e.* as a structural component of the viral coat, without any adverse effects from the point of food safety.

6. Additional evaluation procedures

Although new regulation for the commercialisation of novel food is still under discussion in two committees (Food Council and Public Health Council) in the Netherlands, it is to be expected that potatoes expressing the PVX viral coat protein will be considered substantially equivalent, and will therefore not require extensive pre-market testing.

Evaluation of the selective marker gene NPT-II might also follow the paradigm of substantial equivalence (see Section 7); if this were not acceptable, toxicological testing (90-day feeding study) might be required for this aspect.

7. Rationale for evaluation procedures

PVX coat protein has been present in classical potatoes due to viral infection in the viral coat. Although this may be structurally different from the soluble protein as expressed in the transgenic plant, both proteins will be denatured in the food as eaten (*i.e.* cooked), and therefore in all probability it will be substantially equivalent. Levels of expression are substantially higher in tubers of infected classical plants than in the transgenic plant.

NPT-II gene product has been present in the human intestine from lysed bacteria carrying Tn5. Levels of expression may have to be taken into consideration here. Post-translational modification of the NPT-II gene product may occur in the eukaryotic background, which might cause allergic effects. Allergenic properties of food are not, however, an issue for pre-market approval of classical food. As the gene product will be denatured under the expected conditions of use, its enzymatic activity is not an issue in regard to the food safety of transgenic potatoes.

Rice

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1. Conceptual points to consider

The coat protein gene of the rice stripe virus (RSV) has been introduced into rice plants by electroporation. The resultant transgenic rice plants have expressed the coat protein and exhibited a significant level of resistance to virus infection.

Rice (*Oryza sativa L.*) is one of the world's most important food crops and is produced in more than 70 countries.[1,2] It was an important food even before the beginning of written history. Rice cultivation in Japan began more than 2 000 years ago, and rice has been consumed there as the most important staple for centuries. Rice grains are eaten by people of all ages. The most common way of cooking rice is boiling in water.

There are approximately 120 000 rice varieties in the world, comprising three major varietal groups – Indica, Javanica and Japonica. Japan conserves about 20 000 varieties, mainly of the Japonica type. Modern rice breeding, utilising artificial hybridisation, began in Japan in 1904. More than 300 cultivars have so far been developed as a result of the government breeding programme. Recently, prefectural governments and the private sector have started to be involved in rice breeding. The three most important targets have been high yield, resistance to pests and disease, and good eating quality. The latter two targets have been receiving increasing attention.

Rice grain characteristics vary substantially among varieties. They include size, shape, colour, scent and various physical characteristics, as well as chemical composition. Abrasive milling removes the outer layers, producing milled or polished rice and the by-product bran and polish.

The purpose of milling is to improve the rice's palatability and digestibility. Bran may vary from 8.8 to 11.5 per cent of the weight of brown rice, polish from 1.2 to 2.2 per cent, milled rice from 86.0 to 90.0 per cent. The average content of major components of

brown rice is about 74 per cent carbohydrate, 7 per cent protein and 2 per cent fat.[3,4] Protein distribution is 14 per cent in bran, 3 per cent in polish, 83 per cent in milled rice. The protein content of the outer layer is about twice that of the milled kernel: an example indicates 14.8 per cent for the outer layer and 7.4 per cent for the inner part.[5] The protein-rich rice kernel is removed by the milling process before the rice is cooked for human consumption.

RSV is one of the most serious threats to rice plants, causing severe damage not only in Japan but also in Korea, China and elsewhere. Yield and quality may be lowered substantially when the plant is infected by it. RSV is transmitted by the small brown planthopper, *Laodelphax striatellus*. Application of pesticides to kill the insect vector is time consuming and expensive, and still not wholly successful.

In the transgenic RSV-resistant rice plant, the content of coat protein produced by the gene transferred from RSV is 0.5 per cent in total soluble protein in the leaves, but has not yet been reported in the rice grain. It is very unlikely that genetic modification has brought about a significant change in the composition of the rice grains in comparison with the traditional counterpart *i.e.* naturally infected rice plants. In fact, rice grains from naturally infected rice plants have been eaten by humans for centuries without any harm. Consequently, in the present case study coat protein in the transgenic rice grains can be considered substantially equivalent to coat protein in the infected rice grains that have a long history of safe use and consumption, taking into account the characteristics of the new trait and the extent of dietary exposure.

Safety considerations may also include the need to evaluate the potential for and human health effects of transferring the new genetic marker (hygromycin-resistant marker in this case study).[6,7]

2. Organism/product

In this case study, the product that will be eaten by the consumer is the rice grains derived from a genetically engineered rice plant that is resistant to RSV.

3. Traditional product evaluation

Since milled rice grain is the ultimate product eaten by humans, particular attention is paid to the examination of grain characteristics when a new rice variety is being developed in Japan. The characteristics examined include shape, size, colour, weight (1 000 grains), appearance, and content of protein and amylose. These together with other characteristics comprise a set of information necessary for the registration of new cultivars. When growers sell rice grains to the government, there is a grade standardisation system. The inspected characteristics include volume weight, ratio of perfect grains, water content, ratio of foreign materials mixed in, etc.[8]

Rice grains have been eaten by humans world-wide for centuries, yet no harmful effects or toxicity have been reported. On the basis of traditional and long-experienced consumption, it is strongly believed by the public that rice grains are a safe product. Therefore, under the Seed and Seedling Law (Ministry of Agriculture, Forestry and Fisheries, or MAFF), no safety evaluation is required for rice grains produced on natu-

rally growing rice plants. Under the Food Sanitation Law (Ministry of Health and Welfare), there are regulations in regard to pesticide residues and heavy metals.

4. Database available for traditional evaluation

At MAFF's Hokuriku National Agricultural Experiment Station, 6 000 cultivars including Japonica were collected and evaluated. In addition, at MAFF's National Institute of Agrobiological Resources, 5 000 foreign rice cultivars were collected and evaluated. A computerised database is available at both locations. Preparatory work is in progress at MAFF's National Germ Plasm Centre for establishing a nation-wide database that can be accessed by breeding stations across the country.

5. Novel component(s)/product

A genetically engineered rice plant has been developed from protoplast on which electroporation was carried out in order to insert a gene encoding protein of rice stripe virus (RSV) (1.8 Kb) and a gene encoding hygromycin tolerance (1.0 Kb) as a marker.[9, 10]

Incorporation of the RSV coat protein gene into a plant genome was confirmed by Southern hybridisation analysis, and a genetically stable transmission and expression of the gene was detected by Western blot analysis. In addition, in the artificial inoculation test the transformed rice plants exhibited a significant level of resistance to RSV infection. The transformed rice plants are phenotypically normal, and fertile in seed setting.

Thus, the novel feature in the present case study is that the rice grains contain foreign DNA of a coat protein gene of RSV and of a hygromycin tolerance gene.

6. Additional evaluation procedures

As indicated above, rice grains produced on naturally growing rice plants, whether infected by RSV or not, have been believed safe and have been eaten by humans of all ages for centuries. Moreover, as indicated, the transformed rice plants are normal in growth as well as fertility and are indistinguishable from the parental rice plants.

If the rice grains containing new traits are considered to be substantially equivalent, there will be no additional evaluations. If not, additional evaluation will be needed on the basis of domestic regulatory procedures.

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Animals

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Case No. 1 Animals from transgenesis experiments

1. Conceptual points to consider

This case study deals with meat derived from apparently untransformed individuals in transgenesis experiments involving traditionally bred food animals. It assumes that the transforming material is well-characterised and is not infectious.

At the time of writing, most transgenic animals were produced by the injection of DNA into the pronucleus of a fertilised ovum. The success rate of this technique in swine and cattle is low. Usually not more than 5 per cent of the animals produced clearly exhibit the trait associated with the injected DNA and can therefore be readily identified as transgenic. If it were possible to demonstrate that the remaining 95 per cent were not transgenic in any way, it could be argued that they are substantially equivalent to traditionally bred animals and that consequently it should be possible to market them for food. In principle, it is not possible to demonstrate unequivocally that every cell in an animal's body is free from a given transgene. But if the criteria described below are met, this would not be necessary and the animal could be considered substantially equivalent to untreated animals.

2. Organism/product

The product considered in this case study is edible portions of swine and cattle, though the case study may be applicable to other meat and poultry products derived from common food animals.

3. Traditional product evaluation

Records of the breeding of traditional food animals, including swine and cattle, go back to antiquity. During this protracted history, food safety problems have never been traced to a specific animal breed or to a specific line of descent of a domestic food animal.

Traditionally bred animals are inspected for disease or "unwholesomeness". Furthermore, if animals have been treated with drugs, the tissue levels of these substances must be safe, or below established safe tolerance levels, before the animals are marketed as food.

Food animals selected through traditional breeding programmes have not been subjected to special food safety reviews.

4. Databases available for traditional evaluation

The OECD countries have similar standards for the *ante-mortem* and *post-mortem* inspection of animals at slaughter, as well as for the processing of the corresponding animal products. The organoleptic standards are well-established, and are augmented by laboratory support for the detection of infectious agents and drugs or chemical residues. There is a large amount of baseline data available on the composition and concentrations of normal constituents in meat products.

5. Novel component(s)/product

Animals may be considered non-transgenic and therefore substantially equivalent to traditionally bred animals if the presence of the transgene is not directly detected, or if its absence is inferred from a number of additional criteria.

The polymerase chain reaction (PCR) enables the detection of inserted genes at very low levels, easily in as few as 0.1 per cent of the cells examined.[1] In other words, a transgene is almost certain to be detected if it is present in more than a few cells. Animals that have incorporated the transgene into some but not all of their cells are said to be "mosaic". Mosaicism cannot be ruled out definitively using PCR, as it is always possible that a small fraction of cells, or even one cell in an animal, carries the transgene.

The presence of a small percentage of transgenic cells in a mosaic animal is likely to be of little consequence as regards food safety. The use of the following three criteria, in addition to PCR, should strengthen arguments for substantial equivalence to the untransformed parental animals:

a) the product of the inserted gene is not detected;
b) there is no obvious phenotypic expression of the transgene;
c) the animal is healthy.

Because genes exert their effects through their products, failure to detect the gene product is another indication that a gene is not present or that it is not being expressed. The same conclusion can be made if none of the transgene-associated phenotypic charac-

teristics are present. Finally, the requirement for a healthy animal makes it unlikely that an undetected transgene has caused some unexpected secondary or pleiotropic effect.

Thus, animals from transgenesis experiments may be considered substantially equivalent to traditionally bred animals if the transgene is not detected by direct measurement, and if the other three criteria above are met.

Case No. 2 Swine transgenic for porcine somatotropin

1. Conceptual points to consider

This case study describes establishing the substantial equivalence of swine carrying a porcine somatotropin transgene. These transgenic animals can be said to be substantially equivalent to traditionally bred animals, based on an evaluation of four features: the gene product; the DNA; the organism; and possible pleiotropic effects. The case study illustrates the use of "reasonable certainty" and sequential review.

2. Organism/product

The product considered in this case study is edible portions of swine carrying a porcine somatotropin transgene.

3. Traditional product evaluation

As in Case No. 1 above.

4. Database available for traditional evaluation

This is also the same as in Case No. 1 above. However, animals traditionally selected for leanness or rapid growth should be included in the database. Some of these animals (for example, dairy cows) have elevated somatotropin levels.

5. Novel component(s)/product

Healthy transgenic swine carrying a swine somatotropin gene have been produced in Australia. This gene produces a somatotropin molecule identical to the native swine somatotropin. Furthermore, the gene is linked to a promoter made by modifying the metallothionein prometer, and this makes it possible to stop expression of the gene by removing zinc and copper from the diet. Substantial equivalence may be established by sequentially reviewing the gene products, the DNA, the organism, and possible pleiotropic effects.

The transgene product is swine somatotropin. However, because the metallothionein promoter allows the transgene to be switched off by removing zinc from the diet, the somatotropin level is no different from that of the control animals at the time of marketing. The metallothionein promoter produces no products.

The DNA inserted in these swine is not infectious. Non-infectious DNA is substantially equivalent to other DNA in the human diet, which includes all the genetic material from all organisms which are consumed.

The transgenic animals have all the characteristics, and the same appearance, as the traditional organisms except for the modified traits associated with the somatotropin gene. Consequently, the animals can be considered substantially equivalent to traditional animals.

Pleiotropic effects are unlikely to be food safety concerns in healthy animals. These unexpected effects are caused by the random insertion of the transgene in chromosomal locations, which increases or decreases the expression of one of the host animal's genes, may inactivate a gene, or may cause the normal metabolism of the cell or cell replication to be altered. If the animal is healthy, there is reasonable certainty that it is likely to be safe as food since unsafe levels of a pharmacologically active gene product would affect the health of the animal itself.

Substantial equivalence is established by determining that the gene product is structurally identical to normal porcine somatotropin, and that the concentration does not exceed normal levels. The transgene DNA is not infectious, the animal is normal in appearance, and there are no indications of pleiotropic effects.

Notes and References

1. Stetler-Stevenson, M., *et al.* (1988). *Blood*, 72: 1822-1825. The authors claim a potential sensitivity of detection of one gene in 2×10^5 cells.

Annex I

Terms of reference for a working group on food safety

1. Scope and objectives

The Working Group on Food Safety of the Group of National Experts on Safety in Biotechnology (GNE) will address the scientific issues and principles involved in assessing the safe use of new foods or food components. Particular attention will be given to new foods and food components produced by means of biotechnology. The Working Group will not address the scientific principles for assessing the safety of food additives, contaminants, processing aids and packaging materials. Such principles are well established both nationally and internationally. Also, this Working Group will not address principles of the environmental safety of these products as they are already addressed in documents of the OECD and by other working parties of the GNE.

2. Concepts underlying the work

Recognising the numerous benefits to health, nutrition, food preservation and food production to be obtained by introducing new foods and food components produced by biotechnology into the food supply, the aim of this work is to:

a) elaborate the scientific principles necessary to assure that the safety of new foods and food components will be at least substantially equivalent to that of the widely accepted conventional counterparts;

b) develop scientific principles to focus on the safe use of new food or food components of microbial, plant or animal origin;

c) explore procedures for maintaining the flexibility and timeliness of the principles, once developed;

d) take into account the principles, criteria, procedures, decision trees, methods, guidelines and the results of recent scientific endeavours related to food safety evaluation already available or in preparation; and

e) share ideas, data and information among experts, Member countries and other international organisations, in particular the World Health Organisation (WHO) and the Food and Agriculture Organisation (FAO) and other relevant consultative bodies on food safety, to enhance co-operation and harmonise the results of this work.

3. Issues to be addressed

Among the issues to be addressed by the Working Group are:

a) the scientific principles which underlie the definition of a new food or food component;

b) identification of methods to distinguish between new foods or food components and their conventional counterparts;

c) considering the safety of conventional foods and food components, establish whether such foods and associated safety judgements are a good benchmark for assessing the safety of new foods or food components;

d) determination of the methods for establishing the substantial equivalence of safety of new foods or food components as compared to their conventional counterparts; and

e) identification of methods to be used[1] to establish the safety of new foods or food components for which there is no conventional counterpart

4. Approaches and processes to be employed

a) Models or examples of new foods or food components will be identified and existing information related to their safety assessment will be collected and used to assist in the development and/or demonstration of the applicability of the proposed scientific principles and associated methods for assessing the use of new foods or food components.

b) The Working Group will assist the Secretariat to rapidly collect and disseminate existing documentation. Following a review of the information available, the Secretariat in consultation with the chairman will prepare a document to facilitate the first meeting of the Working Group.

c) The Working Group would meet in the Spring 1991 to develop a draft of the scientific principles and methods. Prior to the meeting the group members will co-ordinate with relevant national authorities to obtain comments regarding the information and preparatory documents available. Subsequent to the meeting, the Working Group experts should solicit comments from appropriate agencies or departments and send them to the chairman and Secretariat so that they can be used to revise the draft principles.

d) The draft principles document will be transmitted to the members of the Group of National Experts of Safety in Biotechnology in August 1991. The members of the GNE can then review and comment of the draft in advance of the following plenary meeting.

Notes and References

1 Safe use is based on the concept that there should be a reasonable probability that no harm will result from intended uses. With respect to food and food components, safe use is that which presents a socially acceptable risk under the expected conditions of consumption

Annex II

Selected list of documents or publications relating to the assessment of food safety

As background for its discussions, the Working Group on Food Safety and Biotechnology examined a number of documents and publications available in OECD Member countries relating to the assessment of food safety. These included:

Advisory Committee on Novel Foods and Processes (1991). *Department of Health Report on Health and Social Subjects No. 38. Guidelines on the Assessment of Novel Foods and Processes*. London (HMSO). Address: Administrative Secretary, Advisory Committee on Novel Foods and Processes, Room 609, Eileen House, 80/94 Newington Causeway, London SE1 6EF, UK.

ILSI (International Life Sciences Institute) (1989). *Assessment of Novel Foods. A Discussion PaperPrepared by an ILSI Europe Technical Committee on Novel Foods*. Address: ILSI European Branch, 83 Avenue E. Mounier, Box 6, B-1200, Brussels, Belgium. Telefax: (32)(2) 762-00-44.

International Food Biotechnology Council (1990). *Biotechnologies and Food: Assuring the Safety of Foods Produced by Genetic Modification*. Part 2 of *Regulatory Toxicology and Pharmacology*, Vol. 12, No. 3 (December). Address: International Food Biotechnology Council, 1126 Sixteenth Street, NW, Suite 300, Washington, D.C. 20036, USA.

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Nordic Working Group on Food Toxicology and Risk Evaluation (1991). *Food and New Biotechnology – Novelty, safety and control aspects of foods made by new biotechnology*. Nord 1991: 18. Address: Nordic Council of Ministers, Store Strandstraede 18, DK-1255 Copenhagen K, Denmark.

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World Health Organization (1987), *Principles for the Safety Assessment of Food Additives and Contaminants in Food*, IPCS Environmental Health Criteria 70. Address: Distribution and Sales, World Health Organization, 1211 Geneva 27, Switzerland.

World Health Organization (1991), *Strategies for Assessing the Safety of Foods Produced by Biotechnology. Report of a Joint FAO/WHO Consultation*. Address: Distribution and Sales, World Health Organization, 1211 Geneva 27, Switzerland.

Annex III

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02 1993

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PRINTED IN FRANCE
(93 93 04 1) ISBN 92-64-13859-5 - No. 46565 1993

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