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The Safety Assessment of Novel Foods

GUIDELINES PREPARED BY ILSI EUROPE NOVEL FOOD TASK FORCE

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2. Introduction

The contents of this document have been achieved through an extensive exchange of views and experiences by an expert group composed of scientists from academia, government agencies and industry, under the auspices of ILSI Europe. The philosophy behind the Guidelines resulting from this collective effort has also drawn on several scientific documents from international and national institutions [Organisation for Economic Cooperation and Development (OECD), World Health Organization (WHO), Food and Agriculture Organization (FAO), Codex Alimentarius Commission, Food and Drug Administration (FDA), etc.].

These Guidelines should not be considered as being a set of stringent rules to be applied in approval procedures for products falling within the scope of the proposed EU Regulation on Novel Foods and Novel Food Ingredients. Rather, they are to be considered as a common background against which a case-by-case approach may be applied. As such, they are intended to assist industry in the preparation of adequate documentation and data when seeking approval for a novel product. Similarly, they should provide guidance to government authorities on what may reasonably be required in terms of safety assessment for novel food products.

Innovation in food production is not a recent event. There are more than a hundred years of experience of introducing new products and processes. During the last decade, science and technology have contributed at an accelerated pace to the introduction of new products so as to satisfy nutritional, technological, socio-economic and quality requirements of consumers. The development of the modern food product market has led to the availability of a large diversity of food products.

When new products enter the market, the consumer needs to be assured of their quality and safety. Therefore, the food industry needs toxicological and nutritional guidance in the evaluation of novel foods and food ingredients to identify any potential risks, so that these may be appropriately managed. As in the case of traditional foods and food ingredients, the use of novel foods and food ingredients may not necessarily affect the composition of the diet—it depends on the nature of the novel food or ingredient, its place in the diet and the way in which it is used by the consumer.

It is hoped that these Guidelines will make an effective contribution to a scientifically-based commonsense approach to food safety assessment.

3. Categories of novel food covered by the EU Novel Foods Regulation

The EU Regulation on novel foods and novel food ingredients applies to the placing on the market of foods or food ingredients which have not hitherto been used for human consumption to a significant degree within the EU and which fall under the following categories:

(a) foods and food ingredients containing or consisting of genetically modified organisms within the meaning of Directive 90/220/EEC;
(b) foods and food ingredients produced from, but not containing, genetically modified organisms;
(c) foods and food ingredients with a new or intentionally modified primary molecular structure;
(d) foods and food ingredients consisting of or isolated from micro-organisms, fungi or algae;
(e) foods and food ingredients consisting of or isolated from plants and food ingredients isolated from animals, except for foods and food ingredients obtained by traditional propagating or breeding practices and which have a history of safe use;
(f) foods and food ingredients to which has been applied a production process not currently used, where that process gives rise to significant changes in the composition or structure of the foods or food ingredients which affect their nutritional value, metabolism or level of undesirable substances.

The Regulation does not apply to:

(a) food additives falling within the scope of Council Directive 89/107/EEC;
(b) flavourings for use in foodstuffs, falling within the scope of Council Directive 88/338/EEC;
(c) extraction solvents used in the production of foodstuffs, falling within the scope of Council Directive 88/344/EEC.

4. The safety assessment of novel foods

4.1 Introduction

A wide range of different novel foods and ingredients is covered by the scope of the EU Regulation and their safety assessment requires a case-by-case approach embracing both a toxicological assessment and a nutritional assessment taking into account the way in which the novel food will be processed and used and the potential intake.

Certain background information is an essential prerequisite for the safety assessment of any novel food or food ingredient and this is outlined in Section 4.2. On the basis of this information the need, if any, for further nutritional or toxicological studies can be determined using the SAFEST principles outlined in Section 4.3. Sections 4.4 and 4.5 respectively discuss some of the nutritional and toxicological studies which, depending on the nature of the novel food or food ingredient, the way in which it will be used and its anticipated intake, may be necessary before the safety assessment can be completed.

4.2 Background information

Certain background information on the presentation and use of a novel food or food ingredient is necessary to establish its safety and help identify the need, if any, for additional studies to facilitate the assessment of toxicological and nutritional safety.

4.2.1 Name or denomination

This should include, as appropriate, details of the scientific, trivial or chemical name of the novel food or ingredient.

4.2.2 Source

Essential details of the source include whether the novel food or ingredient is, or is obtained from, a plant, animal or micro-organism or whether it is a product of chemical synthesis. In the case of novel foods and ingredients from biological sources the full taxonomic classification of the source should be given.

4.2.3 Origin

This should include, for novel foods and ingredients obtained from a plant, animal or micro-organism, details of whether the source is naturally occurring, has been developed by traditional breeding and selection techniques or whether genetic modification has been used. If the source has been developed using traditional breeding and/or selection techniques details of the original organism (taxon, variety, strain, etc.) should be given. In the case of foods which are, or are derived from, genetically modified organisms the data should include characterization of the host organism, the vector/inserted genes and the recombinant organism:

Host organism: genotypic and phenotypic characteristics (including, in the case of micro-organisms, any history of pathogenicity); the presence of secondary metabolites or other potentially toxic and/or antinutritional components; and any history of use in food production.

Vector/inserted gene(s): sequence characterization; size, stability and mobility; the presence of resistance markers; any history of use in food production; and any potential allergenicity of the gene product(s).

Recombinant organism: genetic stability; specificity of expression of the new gene(s); predicted secondary effects; levels of expression of known toxicants, anti-nutrients and potentially significant nutrients; and phenotypic comparison (agronomic traits, growth characteristics, metabolism, nutritional value, etc.) with the host organism and with other commercially important varieties of the species.

4.2.4 Method of production and/or preparation

For all novel foods and ingredients it is necessary to describe the method of production and/or processing. This should be sufficiently detailed to enable consideration of potential effects on the composition of the novel food including major nutrients, toxicants and pathogens present in the food as well as contaminants and by-products that might be introduced by the process. Where particular instructions are required at a processing or domestic level to ensure the safe use of the novel food or ingredient, these should be given.

4.2.5 Previous history

Details of any previous use as a food or as an animal feedingstuff or drug should be given—including, where appropriate, information of uses outside the EU. All data should be capable of verification and should include details of processing and intake and/or exposure levels as well as details of the specification of the previously used material.

4.2.6 Specification

Full details of the specification of the novel food or ingredient are essential to ensure that the product marketed is the same as that on which the safety evaluation is based. In all cases the specification should include the gross composition. Other aspects that might require analysis should be decided on a case-by-case basis, taking into account the source of the novel food or ingredient and its processing history. But they will usually include toxins, nutrients and antinutritional factors known to be associated with the source of the novel food or ingredient or contaminants that might arise from the production process. Examples include: natural toxins and antinutritional factors in the case of plant based materials; nucleic acids, D-amino acids or odd carbon chain length fatty acids in microbially derived materials; residues of catalysts or solvents in processed products or, in the case of novel foods with a modified molecular structure, details of related structures posing potential risks. Where the presence of particular components might give rise to a significant toxicological or nutritional risk, safe limits should be specified.

For those novel foods which are expected to provide a significant dietary source of protein, fat, carbohydrate and/or minerals, further information on these aspects will be required.

Protein: amino acid profile, non protein nitrogen and unconventional amino acids.

Fat: fatty acid profile including trans-fatty acids, energy density, unsaponifiable compounds and possible effects on fat soluble vitamins.

Carbohydrate: chemical structure, molecular weight, *in vitro* digestion and fermentation, dietary fibre content.

Vitamins and minerals: analysis of significant nutrients.

4.2.7 Purpose

Details of the rationale behind the development should be given, for example is it for technological reasons, to improve nutritional status of the diet or

to reduce existing dietary risks. This information will help establish the role of the novel food or ingredient in the diet as well as potential intakes and target groups.

4.2.8 Expected use

This should include details of how the product is expected to be processed, prepared and used. It should include the frequency and levels of use by the population as a whole and by particular target groups as well as identifying existing foods that it might be expected to replace in the diet and their nutritional impact in the diet.

4.3 Safety assessment of food by equivalence and similarity targeting (SAFEST)

Any concept to achieve the safety assessment of novel foods and food ingredients must satisfy the needs of producer, manufacturer, legislator and consumer. The framework of the concept must therefore follow accepted lines of scientific argument, the results of the safety assessment must be reproducible and acceptable to the responsible health authorities and the outcome must satisfy and convince the consumer. Novel foods should be at least as safe as traditional counterparts, where these exist, or should not add to risks of dietary origin.

The ILSI Europe Task Force on Novel Foods has developed the SAFEST concept to facilitate the safety assessment of novel foods and food ingredients covered by the EU Regulation on novel foods and novel food ingredients.

4.3.1 The SAFEST concept for novel foods

The idea of using traditional foods, accepted as safe in use, as a basis for comparison in the safety assessment of novel foods was developed into the concept of substantial equivalence in 1992 by the Food Safety Working Group of the OECD's Group of National Experts in Safety in Biotechnology, having been introduced in a joint FAO/WHO consultation on biotechnology and food safety in 1990. The concept was intended to provide a practical approach to the safety evaluation of foods which are, or are produced from, genetically modified organisms. It was not intended by OECD to be applied to other novel foods although the experience of the UK Advisory Committee on Novel Foods and Processes suggests that the approach is more widely applicable than to genetically modified organisms and their products. With regard to traditional foods, a long history of safe use has been the basis of an informal safety assessment although the outcome may be formally recognized in the GRAS (generally recognized as safe) concept, which may have legal acceptance, for instance in the United States.

Many genetically modified organisms used as food or in the production of food are expected to be derived from existing food source organisms. The concept of substantial equivalence is designed to focus the food safety evaluation on differences between the genetically modified organisms and its parent organism. It is, essentially, an analytical and functional comparison of the parent and the genetically modified organisms. Application of the concept will also provide reassurance that gene insertion has resulted in no non-specific or unpredictable effects in the host organism. If such effects are found to occur then they will be identified and will need to be the target for the safety assessment programme for the novel food.

Extending the concept of substantial equivalence to other novel foods is possible if it can be shown that the novel food is substantially equivalent or sufficiently similar to a traditional, acceptable, reference food so as to allow a reasonable safety assessment without extensive additional toxicological and nutritional testing. The SAFEST concept describes how to target substantial equivalence and sufficient similarity and outlines procedures for safety assessment if sufficient targeting is not possible. Further, the approach of targeting equivalence and similarity may highlight where there are dissimilarities and hence focus the safety assessment on the significance of these.

4.3.2 Application of the SAFEST concept

The SAFEST concept can be applied at the molecular, organism, product, process and/or dietary level. For all novel foods and food ingredients covered by the EU Regulation, the safety assessment requires the appraisal of the background information detailed in Section 4.2 to determine whether the novel food is substantially equivalent to a traditional counterpart (i.e. in SAFEST class 1), sufficiently similar to a traditional counterpart (i.e. in SAFEST class 2) or insufficiently similar to a traditional counterpart (i.e. in SAFEST class 3).

In establishing the SAFEST class of a particular novel food or food ingredient, the traditional counterpart used for comparative purposes should be chosen carefully to reflect not only the chemical composition of the novel food or food ingredient but also its intake, its role in the diet and the effects of processing. For example, for novel foods or food ingredients in EU category (f), the traditionally processed food or food ingredient may serve as the traditional counterpart. In the case of novel foods in EU categories (a) or (b), the traditional counterpart will often be the traditional food or ingredient obtained from a non-modified organism (often the organism used as the host for the genetic modification). Comparison of the genetically modified organism with the host organism will also help to establish that there are no consequential, but unforeseen, effects of the genetic modification that might impact on food safety. This is done by comparing the genetically modified organism with the host organism and ensuring that any differences are as predicted. The comparison should be carried out

at a phenotypic level, including a comparison of appearance and growth characteristics, and also at an analytical level, including an analytical profile of major components, nutrients and toxicants.

Class 1. Foods or food ingredients which are substantially equivalent to a traditional reference food or ingredient

For a single, biochemically defined food or food ingredient, substantial equivalence means biochemical identity within the limits of natural diversity of the traditional counterpart of commerce, for example as caused by naturally occurring mutations or by naturally occurring spontaneous chemical reactions. For a complex food or ingredient, substantial equivalence means identity with a traditional food or ingredient as regards composition, nutritional value, metabolism, intended use and the level of undesirable substances contained therein within the limits of known and measurable natural diversity of the traditional counterpart of commerce.

If, on the basis of the information available, the novel food or food ingredient can be shown to be substantially equivalent to a traditional counterpart, namely it is in SAFEST class 1, no further information is necessary to establish its safety.

Examples of novel foods and food ingredients in SAFEST class 1 are given below:

(A) Well defined isolated metabolites produced from genetically modified organisms and falling within EU category (b) may be shown to substantially equivalent to their conventional counterparts provided that:

  (i) the host organism has a history of safe food use or a history of safe use in the production of foods or food ingredients;
  (ii) the gene(s) encoding the food or food ingredient come from the host organism or from the source from which the ingredient is normally obtained;
  (iii) it can be shown that there are no post-transcriptional changes to the food or food ingredient when expressed in the modified organism or that if they do occur they are of no food safety significance;
  (iv) the chemical composition of the food ingredient and that of its conventional counterpart are, within the limits of biological diversity of edible varieties of the host organism, sources or ingredients, identical; and
  (v) as a result of the production of the food or ingredient using the genetically modified organism, intakes will not be significantly changed with respect to the conventional counterpart.

(B) Although novel foods and food ingredients in EU category (a) are unlikely to be substantially equivalent to their parent organisms at a molecular level, as consumed, substantial equivalence may exist provided that:

  (i) all of the DNA (apart from any non-coding linker sequences) comes from the host organism;
  (ii) no gene products are expressed in the modified organism that are not expressed in the host; and
  (iii) levels of all gene products are, within the limits of natural diversity of existing edible varieties of the host organism, the same in the modified and host organisms.

(C) It may be possible to demonstrate substantial equivalence to existing foods or food ingredients for complex and ill-defined novel foods and food ingredients in EU category (b), for example: flour, meat or autolysed yeast, provided that:

  (i) the host organism used for the modification has a history of safe food use; and
  (ii) levels of all gene products are, within the limits of natural diversity of existing edible varieties of the host organism, the same as those in the foods or food ingredients derived from traditional sources.

(D) Novel foods and food ingredients in EU category (c) cannot, by definition, be substantially equivalent to traditional foods or food ingredients and it is unlikely that novel foods *consisting of* micro-organisms, fungi, algae and in category (d) or *consisting of* plants and in category (e) will be substantially equivalent to existing foods or food ingredients. However, novel foods *isolated from* micro-organisms, fungi, algae, animals or plants may be shown to be substantially equivalent to existing foods or food ingredients, particularly if they are purified and well defined.

Class 2. Foods or food ingredients which are sufficiently similar to a traditional reference food

Sufficient similarity of a novel food or ingredient means that it is substantially equivalent to a traditional counterpart except in certain identifiable aspects. Thus, novelty may be characterized by the presence of new components or new properties as well as by the absence of particular components or properties (including, in the case of micro-organisms, pathogenicity). The differing properties with respect to the traditional food or ingredient serve as the focus of further investigations using appropriate analytical, experimental and other procedures.

For those components of the novel food or food ingredient which are not substantially equivalent to components of existing foods, a focused safety evaluation will be necessary. This will need to take into account available literature on the safety of the

component and further testing may be necessary. If the testing is done on the isolated component it will be necessary to show that the component tested is structurally and functionally identical to that present in the novel food. Account will also need to be taken of any interaction(s) between the component and others in the novel food.

It is anticipated that many novel foods and food ingredients will be in SAFEST class 2 and some examples are given below:

(A) Many novel foods and food ingredients in EU categories (a) and (b) are likely to be in SAFEST class 2 since the only differences between them and the traditional counterpart will be the intended effects of the genetic modification.

(B) Foods or food ingredients with an intentionally modified molecular structure and falling in EU category (c) may be sufficiently similar to existing foods or food ingredients to allow the safety assessment to focus on the impact of the structural modification. This might take the form of an analysis of the impact of the modification on the physical properties of the food or food ingredient including its solubility and bioavailability and an assessment of any consequential safety implications.

(C) Many novel foods and food ingredients in EU category (f) will be in SAFEST class 2 and their safety assessment will focus on differences between the products of the novel process and their traditionally processed counterparts. Thus, in assessing a new heating process, the focus of the assessment will be on any differences consequential on the heat delivery system and not on the effects of the heat generated by the process.

Class 3. Foods or food ingredients which are neither substantially equivalent nor sufficiently similar to a traditional reference food

Single or complex foods and food ingredients which are neither substantially equivalent nor sufficiently similar to a traditional food or ingredient form a separate class for safety assessment purposes. Depending on the nature of the novel food or ingredient the safety assessment may have to be far more extensive than that required for substantially equivalent, sufficiently similar or conventional foods or ingredients.

It is not expected that many novel foods or food ingredients will be in SAFEST class 3, but some examples are given below:

(A) Novel foods or food ingredients which are naturally occurring organisms with no history of safe food use or complex novel foods or food ingredients obtained from such organisms without substantial purification.

(B) Novel foods or food ingredients produced by a process which uses mechanisms not previously used in food production.

Table 1. Examples of how various novel foods would fit into the SAFEST approach

Type of food	Equivalence or similarity	SAFEST class	EU category
Genetically modified baker's yeast, as developed by Gist-Brocades[1]	Substantially equivalent to conventional yeast	1	a
Genetically modified brewer's yeast, as developed by BRF International[2]	Sufficiently similar to conventional yeast	2	a
Genetically modified tomato, as developed by Calgene Inc.[3]	Sufficiently similar to conventional tomato	2	a
Paste from genetically modified tomatoes, as developed by Zeneca[4]			
— novel gene products present in paste	Sufficiently similar to paste from conventional tomatoes	2	b
— novel gene products not present in paste	Substantially equivalent to paste from conventional tomatoes	1	b
Oil from genetically modified oilseed rape, as developed by PGS[4]	Substantially equivalent to oil from conventional oilseed rape	1	b
Carbohydrate polyesters	Not sufficiently similar to a traditional counterpart	3	c
Mycoprotein, as developed by RHM[5]	Not sufficiently similar to a traditional counterpart	3	d
Triticale (a wheat/rye cross)	Sufficiently similar to wheat and rye	2	e
Kiwi fruit	Not sufficiently similar to a traditional counterpart	3	e
Strawberry jam processed by ultra high pressure treatment	Not sufficiently similar to strawberry jam processed by traditional processes	3	f
Chilli-con-carne sterilized using ohmic heating[6]	Sufficiently similar to chilli-con-carne sterilized by other heating processes	2	f

(1) ACNFP Annual report for 1989; (2) ACNFP Annual report for 1993; (3) Calgene submission to the US FDA, 1990; (4) ACNFP Annual report for 1994; (5) OECD report on biotechnology and food safety, 1993; (6) ACNFP Annual report for 1991.

4.3.3 Examples of how various novel foods would fit into the SAFEST approach

Table 1 shows some examples of how a number of specific novel foods might fit into the SAFEST approach if they had been developed after the introduction of these Guidelines.

4.4 Nutritional information

In the case of a novel food or ingredient which, on the basis of the background information, cannot be shown to be substantially equivalent to its traditional counterpart, further nutritional information may be required depending on its anticipated intake and nutritional significance. The nature and extent of any additional studies should be carefully selected and for those novel foods or ingredients in SAFEST class 2 they should focus on the identified differences between the novel food and its traditional counterpart. Studies in human volunteers may be used to confirm the results of nutritional studies but only after the particular novel food or ingredient has been shown to be safe for human consumption.

4.4.1 Nutritional significance of novel foods—a balanced approach

The nutritional evaluation of a novel food or ingredient is most likely to be important for those which are expected to have a significant nutritional impact. The nutritional consequences of the novel food or ingredient for the intended consumer should be assessed at normal and maximum probable levels of consumption. This implies that nutritional reappraisal may be required if there are changes in dietary patterns over time. The background information available on the novel food or ingredient will include details of its nutrient composition taking into account agricultural production, storage, industrial processing and home cooking. The influence of these factors should be carefully considered since there have been occasions in the past when minor process changes have had serious nutritional consequences.

Even in traditional staple foods there is often a balance between components with a nutritional benefit and those with adverse effects and the same is true for novel foods and ingredients. A balanced nutritional risk–benefit evaluation of a novel food or ingredient will need to take into account positive and negative nutritional effects arising to particular groups through novel food-induced nutrient excesses or shortfalls. The nutritional evaluation will have three elements: the composition of the novel food *per se*; the role of the novel food in the diet; and the food(s) in which it will be used. The background information on the novel food will include details of its nature, nutrient composition and purpose and this will help establish its nutritional role in the diet as well as the foods in which it will be used.

Some novel foods or ingredients may have a high percentage content of a particular nutrient(s) yet be of minimal nutritional significance if the intake is likely to be low; if the diet is already more than adequate in respect of the particular nutrient(s); or if other components in the novel food or in the diet are likely to reduce its bioavailability. Conversely, some novel foods or ingredients with a small percentage content of a particular nutrient(s) may be of considerable nutritional significance if diets are close to recommended intake levels for the nutrient(s); if intakes of the novel food might be expected to be large; or if the novel food is expected to replace a significant source of the nutrient(s) in existing diets. Other novel foods or ingredients which are of little nutritional value *per se* may have a significant nutritional impact if they interact with other nutrients or if they replace foods of nutritional significance in the diet.

The nutritional consequences for population subgroups such as children, the elderly, people dependent on institutional catering and those particularly susceptible to the novel food require particular consideration. Many of these groups are at risk in respect of certain nutrients and some examples of this are given in Table 2.

4.4.2 Nutrient bioavailability

For those nutrients, (including protein, lipids, carbohydrate, vitamins and minerals) which are identified as of being of particular significance in relation to the introduction of a particular novel food or ingredient, bioavailability studies may be necessary.

Protein: *in vivo* and/or *in vitro* testing of amino acid bioavailability may be necessary if the nature of the novel food is such as to suggest that this is likely to be low.

Lipids: digestibility and metabolic tests may be necessary if the nature of the novel food is such as to suggest that lipid availability is low.

Vitamins and minerals: if the components of the novel food are likely to affect the availability of minerals and/or vitamins from the novel food; from foods in which it may be an ingredient; or from the diet as a whole, bioavailability studies on the significant nutrients will be required. In this context of particular importance are: the minerals, Fe, Zn, Ca, Mg and Se; the water soluble vitamins B_1, B_2, niacin, B_6, B_{12}, C and folic acid; and the fat soluble vitamins, A, D and E.

Carbohydrate: for those novel foods or ingredients which are sources of unusual carbohydrates and/or

Table 2. Examples of population subgroups and the nutrients at risk

Population subgroup	Nutrients at risk
Pregnant and lactating women	Folate, retinol, Fe
Pre-school children	Vitamin A, Fe, fat
Those with inborn errors of metabolism	Phenylalanine, galactose, etc.
The elderly	Vitamin D, B_{12}, Fe
Vegetarians	Vitamin D, B_{12}, Ca, Fe
Ethnic minorities	Vitamin D, B_{12}, Fe

which interfere with energy metabolism, further studies might include relevant absorption studies, metabolic studies and/or studies of intestinal flora.

4.4.3 Effects on nutrient intake

For all nutrients for which there is an established recommended daily allowance (RDA), the consequences of consumption of the novel food on total dietary intakes should be considered if this is likely to exceed 15% of the RDA. This consideration will need to take account of the quantity and bioavailability of the nutrients in the novel food, the level of use of the novel food and the effects of antinutritional factors in the diet as well as the effects of components of the novel food on other nutrients in the diet.

4.4.4 Micro-organisms

In the case of novel foods or ingredients which are, or which contain, live micro-organisms, details of their dietary impact should be given, including effects on colonic flora, fermentation and short-chain fatty acid production.

4.5 Toxicological information

In the case of novel foods or ingredients which, on the basis of the background information are not shown to be substantially equivalent to their traditional counterpart(s), further toxicological information may be required. The nature and extent of any additional studies should be carefully selected taking into account the source and composition of the novel food, its potential intake and whether it is intended for a specific application or for more general use in the diet.

For those novel foods or ingredients in SAFEST class 2 the toxicological evaluation should focus on the identified differences between the novel food and its traditional counterpart. In some instances it may be appropriate to test isolated components of the novel food rather than the whole food although the results of such tests should be interpreted with care as in some cases a mixture might not produce toxicity while a component of the mixture might produce such an effect. Where specific chemical entities are being tested and these are isolated from sources other than the novel food or ingredient it is essential to ensure that they are structurally and functionally identical to those present in the novel food.

For those novel foods and ingredients in SAFEST class 3, a more extensive programme of tests is likely to be required than for food in class 2 but this will depend on the level of perceived concerns.

The following paragraphs provide a review of the potential types of studies that may be applicable to novel foods or novel ingredients but should not be regarded as a check-list of requirements. The need for specific studies should be established on a case-by-case basis.

4.5.1 Toxicokinetics

Toxicokinetic studies may be required on specific chemical entities found in the novel food or ingredient, for example those chemicals which are the difference between a novel food in class 2 and its conventional counterpart. Suitable studies might cover adsorption, distribution, metabolism and excretion.

4.5.2 Genotoxicity

In vitro and *in vivo* studies of the novel food or of components isolated from it may be required.

4.5.3 Potential allergenicity

It is known that a small number of foods can pose allergenicity problems in a limited number of individuals and tests exist to identify the proteins responsible for causing these adverse reactions. However, there are no validated predictive tests for assessing the allergenicity of proteins from sources that are not commonly recognized as allergens.

If the novel food or ingredient is expected to contain proteins from sources known to be associated with food allergy—for instance it is a genetically modified organism which includes genes from an organism known to be associated with food allergy or if it is the product of a novel process that might affect the potential allergenicity of specific food components—then further information will be required. This might come from specific chemical and immunological tests which exist for identifying the proteins responsible for causing adverse reactions. For example, Western blotting or RAST may be performed using sera from a number of subjects who have been clinically confirmed as allergenic to the particular food source in question. If these *in vitro* tests are negative, confirmation of the absence of allergenic components can be obtained by *in vivo* skin prick tests followed by a double blind placebo controlled food challenge under controlled clinical conditions with patients sensitive to the food in question. The finding of a positive result in any one of the above *in vitro* or *in vivo* tests would require consideration that the novel food should be labelled to indicate the source of the allergenic protein in question.

If the history of the novel food or ingredient does not suggest the presence of proteins from sources known to be associated with food allergy an alternative strategy is required. While it is unlikely that a new protein will elicit an allergic reaction in a large proportion of the population, a comparison of the properties of any new proteins in the novel food with those of known allergens may prove valuable in assessing the likelihood that the new protein will express allergenic potential.

Criteria that could be examined include the following:

(i) molecular weight—most food allergens fall within the molecular weight range 10–70 kDa

although antigenic epitopes can be present in smaller proteins;

(ii) concentration of intact protein in plasma;

(iii) stability to heat—most known food allergens are relatively heat resistant to denaturation by heat;

(iv) stability to processing—many food allergens tend to be stable to food processing conditions;

(v) effect of pH—a feature of allergens is their relative stability to denaturing conditions found in the gastrointestinal tract. The sensitivity of the new protein can be studied using simulated gastric juice *in vitro* (pepsin, HCl);

(vi) digestion with gastrointestinal proteases—can be assessed using *in vitro* methods with gastric juice (pepsin, HCl) and intestinal juice (trypsin, chymotrypsin);

(vii) sequence homology—comparison with the amino acid sequence with that of known food allergens and linear allergenic epitopes. The identification of an immunologically significant sequence identity would require a match of at least eight contiguous identical amino acids; and

(viii) prevalence in food—many food allergens are present as major food components (1–18% total protein).

No single criterion, or even the complete set of criteria, is sufficient to confirm allergenicity or lack thereof; however, the results of such a review may highlight the need to adhere to a strict test-marketing strategy and a post-marketing surveillance procedure.

Workers exposed to a novel food or food ingredient during development or production may become sensitized towards it. Although this will probably be as a result of inhalation or dermal exposure rather than ingestion and the dose received may be much greater than the oral dose anticipated from consumption of the novel food or food ingredient, it is a possible indicator of potential food allergenicity and thus an assessment of reported and confirmed cases of sensitization should be included in the safety dossier.

4.5.4 Potential for colonization

For those novel foods and ingredients which are, or which contain, live micro-organisms (including genetically modified micro-organisms) the potential for colonization of the gastrointestinal tract and the transfer of genetic material in the gastrointestinal tract is important. Acute and subacute studies in conventional rodents, and/or in germ-free animals colonized with specific or total human gut microflora may be used to study these aspects. Subsequent clinical and toxicological examination of these animals may assist in the design of subsequent toxicity tests.

Colonization: measurement of faecal contents compared to those of the traditional counterpart where this exists.

Gene transfer and stability: examination of faecal micro-organisms.

4.5.5 Pathogenicity

In the case of novel foods or ingredients which are, or which contain, live micro-organisms, consideration should be given to their potential pathogenicity based on knowledge of the source organism and on its close relatives.

4.5.6 90-Day subchronic feeding study in rodents

Studies of the whole food may in some cases be combined with a colonization study if the whole food is consumed as a live micro-organism (see section 4.5.4).

Micro components: conventional studies as for food additives are appropriate. Observations may also be made of indications of genotoxicity, neurotoxicity, immunotoxicity and reproductive function. Adverse indications may suggest the need for directed tests in these areas.

Macro components: the maximal dose not producing nutritional imbalance should be used. The use of basal rodent diets with interchangeable macro constituents may be of some advantage in these studies for maximizing exposure. For toxicologically active uncharacterized compounds (e.g. impurities, by-products) suspected of being present at low levels in association with macro components, tests performed on extracts may be useful in some cases.

The safety assessment of macro components is complicated since it is impossible to obtain sufficiently large safety factors from no effect levels (NOELs) in feeding studies to apply conventional risk assessment procedures for determination of the acceptable daily intake (ADI) for the product in humans. Feeding of macro nutrients in large amounts will almost certainly result in toxicity due to factors such as nutritional imbalances rather than inherent toxicity. In order to overcome this, it is necessary to try to improve the sensitivity and diagnostic capabilities of the traditional subchronic feeding study. A potential approach is to identify early sensitive biomarkers of potential inherent toxicity. These could include markers of preneoplastic changes, general cellular toxicity, apoptosis, oxidant stress, etc. Where the results of the 90-day study are indicative of specific effects, some of these biomarkers may then be applicable to human studies of the macro component in question. Pre marketing human studies should therefore form an important part of the safety evaluation of novel macro components. This general strategy is applicable in conventional toxicity studies of macro components as a means of extrapolating from toxicity in animal studies to potential effects to be investigated in human studies.

In studies of a novel process where general application is envisaged, the use of a human-type diet

in the subchronic study may be of value and may overcome criticisms of the relevance of the application of the novel process to rodent diets.

4.5.7 Other toxicity studies

The necessity for other toxicity studies including second species toxicity studies, reproduction studies and carcinogenicity studies will depend on the concern level which is determined by structure category assignment; the intended levels of human exposure (analogous to food additives); and in the case of novel foods obtained by a novel process, the anticipated changes to the chemical structure of known food components.

4.5.8 Confirmation of safety in humans

Relevant studies include: tolerance, examination of the effects on intestinal microflora spectrum and content; and the effects on biomarkers (see section 4.5.6).

5. Summary and Conclusions

The diversity of novel foods and novel ingredients covered by the scope of the EU regulation is such that a check list approach to safety evaluation is inappropriate. Rather, a case-by-case approach is required taking into account the composition of the novel food, its intake, its role in the diet and the intended target group.

The SAFEST approach provides a means of targeting the safety evaluation on those aspects, nutritional or toxicological, of a novel food which are of particular concern. Using this approach, novel foods are assigned to one of three classes on the basis of certain background information. For those novel foods which can be shown to be in SAFEST class 1, namely those which are substantially equivalent to a traditional counterpart, no further information is required to demonstrate their safety. For those novel foods in SAFEST class 2, i.e. those sufficiently similar to a traditional counterpart or differing from it only in particular, well defined, characteristics, the evaluation will focus on those differences. Only in the case of novel foods which are not in class 1 or class 2 is extensive testing of the whole food likely to be required. Even in these cases, the testing should follow a scientifically-based hierarchical approach involving: literature reviews; chemical analysis; appropriate *in vitro* and *in vivo* tests; and, if necessary, confirmation of safety and nutritional value in humans. Examination of the causes of any adverse effects reported by consumers after the novel food or ingredient has been approved and is introduced into the market may provide additional reassurance of safety.

GLOSSARY

This glossary is not intended to provide a precise scientific definition of the terms used but to illustrate the way in which they are used in these Guidelines.

Gene	The smallest sequence of a DNA molecule capable of directing protein synthesis or performing a regulatory function.
Genetic modification	The addition, deletion, substitution, rearrangement or recombination of heritable genetic material using techniques defined in Directive 90/220/EEC.
Genetic stability	A measure of the resistance to change, with time, of the sequence of genes within a DNA molecule or of the nucleic acid sequence within a gene.
Inserted genes	Genes introduced into the DNA of a recombinant organism which are not present at the same position in the DNA of the organism before genetic modification.
Linear allergenic epitopes	Regions on an antigenic protein that interact with the cells of the human immune system.
Non-coding linker sequences	Short pieces of DNA between genes which do not direct protein synthesis or perform a regulatory function.
Post transcriptional changes	Changes to a protein that occur after DNA has been copied to form messenger RNA and this has been translated to produce protein—for example, to facilitate excretion of the protein from the cell.
Recombinant organism	An organism in which the DNA has been made by joining together segments of DNA using the techniques of genetic modification described in Directive 90/220/EEC.
Sequence characterization	Determination of the order in which the nucleic acids making up a DNA molecule, or the amino acids making up a protein molecule, are linked together.
Specificity of gene expression	A measure of the extent to which the ability of an organism to produce a particular gene product is determined by factors affecting the cell, for example, its function, phase of growth or environmental pressures.
Vector	The agent, such as a plasmid or virus, used to carry new DNA into a cell during genetic modification.