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CONSULTATIONS AND WORKSHOPS

Safety assessment of foods derived from genetically modified microorganisms

Report of a Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology

WHO Headquarters
Geneva, Switzerland
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1. Introduction

A Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology – Safety Assessment of Foods Derived from Genetically Modified Microorganisms (GMMs) was held at the Headquarters of the World Health Organization (WHO) in Geneva from 24 – 28 September 2001. A total of 27 experts, including authors of discussion papers, participated in the Consultation. The complete list of participants is given in Annex 1.

Ms Ann Kern, Executive Director, Cluster of Sustainable Development and Healthy Environments, opened the Consultation on behalf of Directors-General of WHO and the Food and Agriculture Organization of the United Nations (FAO). Ms Kern stated that WHO and FAO have been organizing Consultations of this kind since 1990 to provide scientific and technical guidance to Member States and to the Codex Alimentarius Commission. Ms Kern also expressed the appreciation of the two Organizations to the Government of Japan for its generosity in supplying additional funding for this Consultation. She acknowledged the interest of Member States in these sometimes hotly debated issues, and the need for sound scientific advice developed and formulated by the Expert Consultations upon which Governments can base their discussions. Clear assessment and communication of scientific data is becoming increasingly important so that the scientific risk assessment process is accurately reflected in the risk management process. Ms Kern suggested that the issues of safety and nutritional assessment of foods derived from biotechnology would be even more important in the near future with the rapid development of new foods with potential benefits related to health.

The Consultation elected Dr Ian Munro as Chairperson and Dr Bodil Lund Jacobsen, Professor Ingolf Nes, Dr Ruud Valyasevi and Dr Christopher Viljoen as Rapporteurs. The Consultation also decided to ask all participants to assist Rapporteurs by drafting each of the sections of the report of the Consultation. Dr Thomas Cebula and Dr James Maryanski (USA) and Dr William Yan (Canada) took part in the discussions through teleconference from their countries.

All participants completed a Declaration of Interest as defined by FAO and WHO.

2. Background

FAO and WHO have embarked on an initiative to organize a series of scientific expert Consultations to provide scientific and technical advice to their Member States. The scientific advice derived from the Joint FAO/WHO Expert Consultations can be used by the Member States of FAO and WHO directly. It will also serve as the scientific foundation for the work of the Codex Alimentarius Commission in their deliberation on safety assessment guidelines for foods derived from biotechnology presently being developed by the Codex *ad hoc* Intergovernmental Task Force on Foods Derived from Biotechnology.

FAO and WHO have to date organized two Expert Consultations. The first Consultation held in Geneva in June/July 2000 addressed the overall aspects of safety assessment of genetically modified foods of plant origin and responded to five specific questions raised by the First Session of the Task Force (FAO/WHO, 2000). The second Consultation held in Rome in January 2001 specifically addressed the allergenicity of foods derived from biotechnology (FAO/WHO, 2001).

At the 24th Session of the Codex Alimentarius Commission held in July 2001, it was decided that the Task Force expand its work in starting the work on drafting a Proposed Draft Guideline for the Conduct of Food Safety Assessment of Modified Microorganisms in Food and established a new Working Group to prepare a draft document on this. FAO and WHO at that time announced they would jointly convene a third joint Consultation to consider the scientific aspects of the safety assessment of genetically modified microorganisms in foods in support of this new work.

FAO and WHO convened this Consultation to evaluate and build on experience gained since the previous two FAO/WHO Consultations and to assess whether currently available approaches for assessing the safety of foods and food ingredients derived from genetically modified plants could be applied to genetically modified microorganisms (GMMs). The Consultation examines unique aspects to be considered in the safety assessment of foods produced with the aid of GMMs.

The safety and risk assessment of foods, including genetically modified foods, are generally considered within a framework of risk analysis. Within this framework, reference can be made to the use of precaution in risk management and risk assessment. Ongoing discussions within the Codex system will help guide these considerations further.

3. Scope

The Consultation was convened to consider criteria for the safety assessment of food derived from microorganisms that have been genetically modified using recombinant DNA techniques. Specifically, the Consultation was requested to provide FAO, WHO and their Member Countries with scientific advice in relation to the safety assessment of GMMs in food.

The Consultation agreed to the following definitions for the purposes of this Consultation:

"Genetically modified microorganisms" (GMMs) means:

Bacteria, yeasts or filamentous fungi in which the genetic material has been changed through modern biotechnology in a way that does not occur naturally by multiplication and/or natural recombination.

"Modern biotechnology"[1] means the application of:

- In vitro nucleic acid techniques[2], including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles, or
- Fusion of cells[3] beyond the taxonomic family that overcome natural physiological, reproductive or recombination barriers and that are not techniques used in traditional breeding and selection.

[1] This definition is based on the Cartagena Protocol on Biosafety under the Convention on Biological Diversity.
[2] These include but are not limited to: recombinant DNA techniques that use vector systems and techniques involving the direct introduction into the organism of hereditary materials prepared outside the organism such as microinjection, macroinjection, chemoporation, electroporation, microencapsulation and liposome fusion.
[3] Including protoplast fusion and hybridization.

The Consultation agreed to confine its discussion to food produced with the aid of GMMs, namely:

- Foods and food ingredients consisting of or containing viable GMMs.
- Foods and food ingredients consisting of or containing non-viable GMMs.
- Foods and food ingredients produced by fermentation using GMMs and from which the GMM has been removed.

The Consultation did not consider highly purified products such as food additives, enzymes, polysaccharides, flavours etc. derived from GMMs. The Consultation noted that such purified food additives have been produced and permitted in a number of countries for over a decade (e.g. chymosin) and the safety of many has been assessed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1999). However, the Consultation agreed that if viable GMMs and/or microbial components are present in such products, the general principles for the safety assessment of foods derived from GMMs outlined in this report could be applied.

The Consultation agreed that, although beyond its scope, the concepts and principles described in this report are equally applicable to all foods produced with the aid of microorganisms.

The report does not specifically address the presence of GMMs used for agricultural purposes such as microbial plant protection agents, feed additives, bio-fertilizers etc. that may find their way into food. However, the Consultation agreed that the general principles for the safety assessment of foods produced with the aid of GMMs could be applied in such cases.

The Consultation also recognized safety issues exist in relation to the exposure of workers (occupational health) involved in the production of the food using GMMs but found that this issue was not within the scope of this Consultation.

The Consultation did not consider environmental safety issues related to the release of GMMs into the environment as these were outside its defined scope. Similarly, it did not consider socioeconomics, risk management and public perception issues.

As background to its discussions, the Consultation received papers and/or presentations on:

1. General consideration of the safety assessment of GMMs in foods, including:

- Use of living microorganisms in food and perspectives on the application of genetic modification in such microorganisms;
- Methodologies available for the safety assessment of the GMMs;
- Conventional safety assessment methodologies used for foods derived from biotechnology including foods of plant origin;
- Issues specific to foods produced with the aid of GMMs, and

2. Other specific topics arising in relation to the safety assessment of GMMs in food including:

- Gene transfer;
- Genetic familiarity and stability of microorganisms;
- Colonization and persistence of microorganisms in the gut and their impact on the gut ecosystem with special reference to effects on human health;
- Genetic basis of pathogenicity of microorganisms and its possible change by genetic modification;
- Nutritional and toxicological aspects of bacterial or fungal metabolites and their possible alteration by genetic modification;
- The allergenicity of microorganisms and its potential for induction or change by genetic modification; and
- Survival/propagation of microorganisms, including GMMs, in the environment and the implications to public health.

A list of agenda items and documents is given in Annex 2.

4. Safety Assessment

4.1 General approach to safety evaluation

Roughly one-quarter of food production involves microbial fermentation processes. This includes foods such as bread, sour dough, sour milk and cream, yoghurt, cheese, sour vegetables, fermented meat, vinegar, wine and beer. Fermentation provides a simple technique to produce food of high nutritional and hygienic quality. This technology has a long history of food use and is especially important in developing countries. The influence of microorganisms is thus of great importance to the safety and nutritional status of food. The introduction of foods produced with the aid of GMMs into the food supply brings potentially new issues of food safety. This section outlines general principles for the safety assessment of GMMs in food with reference to the established principles applied to the safety assessment of genetically modified plants and food safety issues specific to the nature and use of microorganisms in food.

Several international organizations have already addressed the issues associated with the safety assessment of novel foods including genetically modified plants and microorganisms (FAO/WHO, 1991; OECD, 1993; WHO, 1995; FAO/WHO, 1996; ILSI, 1995; Commission of the European Communities, 1997). It is generally agreed that such an assessment requires an integrated and stepwise, case-by-case approach using the concept of *substantial equivalence* (see section 4.3) that is directed by the results of a comparison between the genetically modified plant or microorganism and its conventional counterpart. The FAO/WHO Expert Consultation on Safety Aspects of Genetically Modified Foods of Plant Origin (FAO/WHO, 2000) provides important recommendations on the use of *substantial equivalence* as a concept to guide the further safety assessment process. Some authorities have developed decision trees to assist in determining the extent of testing required in specific cases (UK ACNFP, 1995; Commission of the European Communities, 1997; ILSI, 1999).

The following general principles are important to consider in the safety evaluation of food produced from GMMs:

- The safety assessment should address the health aspects for the whole population, including immuno-compromised individuals, infants and the elderly.
- The safety assessment should be based on sound scientific data and should use the most appropriate safety assessment methods. If new information becomes available, the safety assessment may need to be revised.
- The safety assessment should involve the characterization of the genetic modification, including deletion or insertion of DNA sequences, characterization of recipient microorganisms, the ultimate donor organism, the vector(s) used in the construction of the GMM, the construct and the GMM itself.

Other important considerations in the safety assessment of food produced with the aid of GMMs include:

- Information on the direct and indirect exposure[4] of humans to the food or to the GMM itself. This should also consider the potential level of intake and dietary impact of the GMM.
- Possible secondary effects from expression of the inserted gene or the disruption of host DNA or metabolic pathways, including composition of critical macro- and micronutrients, antinutrients, endogenous toxicants, allergens, and physiologically active substances.
- The inherent differences between microbes and plants, and the influence of the food matrix on the GMM, should be taken into consideration when applying the concept of *substantial equivalence* (discussed in section 4.3).

In addition, the following elements should be taken into account in the safety assessment. This list is not exhaustive and, in some cases, not all elements mentioned will be relevant.

- Techniques used for genetic modification
- Strain identification and characterization (recipient, donor [if appropriate] and the GMM itself)
- Natural habitat
- History of use
- Gene transfer
- Genetic stability
- Pathogenic potential
- Characterisation and verification of the expected protein expression product of the novel DNA
- Composition of the food containing the GMM
- Safety and nutritional assessment (including potential toxicity and nutritional aspects)

[4] Direct exposure refers to GMMs used in food production, processing or consumed as such while indirect exposure refers to exposure encountered through the application of GMMs elsewhere in the food chain (e.g. animal feeds).

- Interactions between the GMM, the gastrointestinal flora and the mammalian host
- Impact on the immune system
- Effects of processing, cooking and storage.

4.2 Aspects specific to microorganisms

The genetic modification of microorganisms involves similar recombinant DNA techniques to those used in the production of GM plants. However, there are distinct genetic characteristics of microorganisms that require specific aspects of their safety to be addressed. Microorganisms that are used in food production include Gram-positive and Gram-negative bacteria, yeasts and filamentous fungi. The genome structures and the available genetic technologies differ for bacteria, yeasts and filamentous fungi, although some common techniques are used.

The ready exploitation of homologous recombination in bacteria is a major advantage that facilitates good control over genetic modification procedures. Integration sites can be used by design and unwanted DNA can be removed with relative ease. Systems for the selection and maintenance of introduced DNA can be designed using homologous genes and selection methods developed that are compatible with safe food use.

Microbial genomes are relatively small and several bacterial genomes and some yeast genomes have been sequenced, including that of *Saccharomyces cerevisiae*. The acquisition of the complete genome sequence for a particular bacterial species is now a realisable scientific objective. The availability of such genome sequence data greatly enhances the knowledge base that is available to support safety evaluation. Post-genomic analytical techniques provide a valuable opportunity to analyse gene expression at the level of the entire genome. DNA microarray technology involves the use of nucleic acid probes for all of the genes in the genome. This can be used to compare the presence of individual genes in different strains and gene expression in different strains and different environments. Proteomics separates proteins isolated from the whole cell using two-dimensional gel electrophoresis and allows comparisons to be made between strains and in different environments. Individual proteins can be identified using mass spectrometry to relate separated protein spots to specific genes.

Microorganisms used in food may remain viable during food production and following their consumption. For this reason, they have a potential to interact with the consumer both directly and indirectly. It is important to ensure that the recipient microorganisms are not pathogenic, toxigenic or allergenic and that the genetic modification does nothing to compromise this status. Also, the fate of consumed GMM and its impact on both the gastro-intestinal (GI) tract and its microflora need to be considered.

4.3 Application of the concept of *Substantial Equivalence* to GMMs

The concept of *substantial equivalence* was developed by WHO, OECD and FAO following recognition of the limitations of conventional toxicology for the safety evaluation of novel foods. Quoting the OECD publication (1993), the concept ‘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.'

The FAO/WHO report on 'Safety aspects of genetically modified food of plant origin' (2000) addressed criticism of the application of the concept of *substantial equivalence* and reaffirmed its usefulness. It emphasized that the determination of *substantial equivalence* is not in itself an end-point but rather the starting point for safety evaluation.

The concept of *substantial equivalence* is also of value for the safety assessment of GMMs. Microbial genomes have natural genetic plasticity and this may become apparent in foods or during food processing and where complex communities are involved. In addition, the gene expression of a microorganism is expected to vary according to its environment. This is especially pertinent for safety assessment where a variety of data might be gathered under *in vitro* laboratory conditions, in a food matrix, or in the GI tract following consumption. These constraints suggest that the concept of *substantial equivalence* should be applied both to the GMM itself and food produced with its aid. In applying this concept it is important to remember that minor differences can separate pathogenic strains from non-pathogenic strains of microorganisms.

The FAO/WHO report (2000) recognized the need to keep abreast of developments in genetic modification technology and noted that new methodologies, such as molecular profiling techniques, may provide a more detailed analytical comparison. The concept of *substantial equivalence* involves a targeted analysis of the composition and phenotype of the GMM compared to its conventional counterpart. Molecular profiling provides a non-targeted and more holistic approach to this analysis. Microorganisms are especially amenable to the use of DNA microarrays and proteomics. Metabolic profiling has advanced, using a range of analytical techniques, and it may be of special value in the assessment of GMMs where metabolic rerouting is the intended outcome. The major limitation of profiling is the need to accommodate the background of normal variation and the need to interpret the significance of any differences that are detected. Several steps must be taken before the full potential of these techniques can be realized in routine safety assessments. First, the methodologies must be validated to ensure their reproducibility and robustness. Then, agreement must be reached in assessing their performance. That is, what is the range of differences in a given array or profile that will be considered as "normal variation". Any profile differences considered not to be within this normal variation must be evaluated from a safety perspective.

5. Specific Food Safety Issues

5.1 Introduction

This section deals with specific issues that are relevant to the safety of foods produced with the aid of GMMs. These issues include the potential of gene transfer between the GMM and other organisms found in food and the GI tract. Also, the safety of genetic markers used for selection (such as antimicrobial resistance genes), and the potential of GMM interaction with the intestinal microflora and the immune system are evaluated. The discussion that follows provides an appraisal of existing knowledge on these topics and suggests scientific approaches that may be used to assess possible health risks.

5.2 Techniques for genetic modification

In general, the techniques for genetic modification of microorganisms are better understood than in plants. For example, in bacteria, the inserted gene recombinant constructs can be integrated at specific sites on the chromosome or on plasmids. However, the techniques for genetic modification applied to GMMs remain an important safety consideration. The factors to be considered are discussed below.

5.2.1 Bacteria

5.2.1.1 Host

The host microorganism should have a history of safe consumption either as a food or as a food component or its safety must otherwise be established.

5.2.1.2 Inserted gene

The inserted gene(s) may be sourced from the same microbial species or from evolutionarily more distant organisms. The gene product of any inserted gene(s) should have a history of safe use in food or its safety should otherwise be established. The food safety evaluation will be facilitated by reducing to a minimum any extraneous DNA sequences.

5.2.1.3 Vector and construct

The DNA sequence of the whole vector should be characterized, including replicons, promoters, selective markers, linkers, and any foreign DNA, in case the vector is part of the GMM. It is recommended that vectors consisting only of nucleotide sequences from microorganisms with a history of safe use in food should be used. Any selectable markers should be carefully chosen and based on safe use. Information on the sequence similarities and the protein function of the selective marker should be available to assess any safety hazard. In particular, antimicrobial resistance markers should be avoided and not be present in the final GMM. Several techniques are available to remove selectable markers in GMMs, such as sequence specific recombination.

5.2.1.4 Methods of DNA transfer to the host

Methods of transfer of DNA into microorganisms can involve physical, chemical or biological approaches. DNA transformation methods that minimize major genetic rearrangement in the host genome should be used. When the inserted gene is integrated into the genome, the nucleotide sequence of the flanking regions at the integration site of the chromosome should be characterized.

5.2.2 Yeast and filamentous fungi

Most methods of genetic manipulation for bacterial systems can also be applied to yeast and filamentous fungi used in food production, thus similar safety considerations as in bacteria should be applied. Specific cloning vectors (e.g. centromeric plasmids, yeast artificial chromosomes, plasmids based on killer factor determinants etc.) have also been constructed. Reliable methods are available in certain species for directed integration of *in vitro* modified or

composed gene constructs into specific chromosomal sites and for deletion of genes. Transgenic constructs made by these methods are highly stable during vegetative propagation of cells. They can, however, recombine at mating with related strains of the native mycoflora. In species with less developed genetics, there is insufficient information available on the recombinant processes to precisely predict the mechanism and the site of integration of novel genes. For this reason, methods currently used for genetic manipulation of yeast and filamentous fungi may allow integration at variable sites, resulting in transgenic strains with variable biotechnological performance and genetic stability.

5.3 Strain identification and characterization

First and foremost, the host microorganism should be safe, well characterized and stable. The origin of the strain should be known. Proper state-of-art taxonomy should be applied to describe the strain. Although many genotypic and phenotypic methods exist, each with its own merits and limitations, the Consultation recognized the need for the host microbial strain to be adequately characterized from a scientific, manufacturing, and safety perspective. This currently includes DNA/DNA hybridization and 16S rRNA sequence determination which provide crucial information on taxonomic classification of the organism. Standard physiological/biochemical methods for phenotypic characterization are commercially available and are commonly used. Information on pathogenic traits within the host genus can also provide important guidance for the characterization.

Secondly, the GMM strain produced should be as safe as the host organism. The novel status of the GMM strain, including its phenotypic and genotypic characteristics, should be characterized in order to assess its safety. Existing molecular techniques provide precise tools for such characterization and for the comparison of microorganisms at species and strain level. Comparative assessment of the GMM strain with its host strain can be undertaken using approaches such as restriction analysis, random amplified polymorphic DNA analysis (RAPD-PCR), amplified fragment length polymorphism analysis (AFLP), protein profiling etc. Further analysis can extend to genome sequencing.

Additionally, the effects of the genetic modification on the properties of the host organism, the desired stability of the genetic system, and the desired functional properties of the gene construct are important factors that should be considered in the GMM.

5.4 Gene transfer

5.4.1 Bacteria

Prokaryotic microorganisms have developed a variety of mechanisms by which they can transfer DNA to other cells. This can result in the transfer of heritable traits. These transfer mechanisms allow bacteria to respond to environmental changes by acquiring new genetic information that might provide a selective advantage under changed environmental conditions. An example would be the worldwide spread of antimicrobial resistance genes among microorganisms since the introduction of antimicrobial agents in human health care, veterinary medicine and agriculture. One mechanism of gene transfer, termed *conjugation*, relies on a plasmid (autonomously replicating DNA molecule) in the donor cell or the presence of conjugative transposons in the chromosome. These genetic elements direct cell-to-cell contact

during which a copy of the plasmid or the transposon is transferred to the recipient cell. Various types of plasmids have been identified in bacteria and some of them can elicit the transfer of other plasmids that do not have capability for their own transfer (Clewell, 1993).

In nature, bacterial populations and communities often contain considerable fractions of cells with plasmids, and several different plasmids may be present in the same cell. Plasmids and transposons may confer new properties to the cells. Conjugative gene transfer from bacteria to eukaryotic cells, including plant cells, yeast, filamentous fungi and animal cells, has been observed (in nature or in experimental systems).

Natural transformation is another gene transfer process that involves the active uptake of extracellular DNA by bacteria into their cytoplasm. This process has so far been identified in a limited number of bacteria belonging to major trophic and taxonomic groups (Lorenz and Wackernagel, 1994). DNA uptake occurs mostly during a specific growth phase of cells (*competence*). Both chromosomal DNA fragments and plasmids can be taken up. Under particular physical or chemical conditions, DNA may enter bacteria that do not actively take up DNA (a type of transformation often used in gene technology).

Finally, transfer of bacterial genes can also occur by *transduction* wherein transfer is mediated by bacterial viruses that have incidentally packaged DNA of the last host cell instead of viral DNA (Masters, 1996).

Conjugative processes, transformations and transduction may occur within members of a species but also between members of different species and genera. Extensive studies, including whole genome sequence analyses, have indicated that horizontal gene transfers have strongly contributed to the genomic structure of bacterial species. Other studies provide evidence that various gene transfer mechanisms are active in natural habitats of bacteria including soil, sediment, river epiliton, rhizosphere, phylloplane, foodstuffs, intestine, mammalian oral cavity (Lorenz and Wackernagel, 1993; Bräutigam et al., 1997; Davison, 1999; Mercer et al., 2001).

DNA transferred to a new host cell may establish by genomic integration (e.g. homologous recombination; de Vries et al., 2001) or by plasmid formation (e.g. when an origin of replication is present). Establishment may be inhibited, for example, by lack of nucleotide sequence homology or the presence of restriction endonucleases (Davison, 1999; Majewski, 2001). When the new genetic information provides a selective advantage to the cell, the trait may become fixed in the population when selection pressure prevails long enough. Gene transfer processes must be considered as part of the nature of prokaryotic organisms (Syvanen and Kado, 1998). Clearly, the spread of a gene or a gene assembly, as well as the formation of new gene assemblies, in the microbial community is mainly driven by the selection pressure in the habitat (Lawrence and Roth, 1998).

In order to reduce the potential for the spread of a recombinant construct, its chromosomal integration may be preferable to localization on a plasmid. To limit the selection of bacterial strains coexisting with the GMM into which the recombinant construct has been unintentionally transferred, genes in the construct that could provide a selective advantage under certain conditions should be avoided (e.g. antimicrobial resistance determinants). Finally, to limit the chance of unintended integration into other genomes, any sequences that would stimulate such integration should be avoided in the construct.

5.4.2 Yeast and filamentous fungi

Due to the more complex structure of the eukaryotic microorganisms, the processes for gene transfer in yeast and filamentous fungi are different to bacteria. Natural cell-hybridization and genetic recombination occur frequently in species that have sexual (mating, meiosis and sporulation) or parasexual (anastomosis, nuclear fusion and haploidisation by gradual loss of chromosomes) life cycles. In certain genera, interspecific hybridization can also occur between closely related species.

Synthetic gene transfer from yeast to mammalian cells has also been successfully demonstrated using yeast artificial chromosomes (YACs) which have significant potential as gene therapy vectors (Giraldo and Montoliu, 2001; Fabb and Ragoussis, 1995).

5.5 Genetic stability

The chromosomes of microorganisms are generally much more fluid than the chromosomes of higher eukaryotes. They grow faster and are required to adapt rapidly to changing environments. They are consequently more prone to genetic change than higher organisms. In bacteria, various mechanisms of horizontal gene transfer have been identified and are frequently seen. Further, mobile genetic elements are actively involved in reorganization of the genetic material of bacteria, and this may result in new phenotypic properties, gene inactivation, destabilization of the integrity of the genetic material, and gene loss. Mobile DNA elements include insertion sequences (IS), transposons, plasmids, and prophages. Many bacteria have large numbers and different types of IS elements and some of these are very active in transposition. Such genetic changes often happen in a non-random way and may include participation of specific DNA sequences.

Genomes of certain yeasts and filamentous fungi are also prone to undergoing rearrangements. These changes take place during cultivation and are probably due to spontaneous transpositions of mobile elements (e.g. Ty retrotransposons) and chromosomal segments (manifested in chromosomal length polymorphism).

The genetic plasticity of microorganisms (Stibitz and Yang, 1999; Brunder and Karch, 2000; Le Bourgeois et al., 2000) may influence the fate of the recombinant DNA in a GMM, and therefore this has to be taken into consideration when the stability of a GMM is evaluated.

The genetic stability of the recombinant DNA molecule is also dependent on the localization of the cloned gene(s), whether it is on the chromosome or on a plasmid. Plasmids can be lost by segregation or integrated into the chromosome or other plasmids. If the vector system is chosen carefully, one will expect that in either of the two cases – high copy number vector and chromosomally integrated insert DNA – the stability of the new genetic information will follow basic biological mechanisms and the stability of the transferred genes should be comparable with that of host genes. If high stability is required, one should not include DNA sequences that may represent a risk for stability, sites for known transposons and IS element insertions, or attachment sites for temperate phages.

5.6 Pathogenic potential

Microorganisms used for the production of fermented food (e.g. acetic, propionic, and lactic acid bacteria, yeasts and certain filamentous fungi) have a long history of safe use. Although some enteric lactic acid bacteria have, in rare instances, been identified as the cause of bacteremia or endocarditis in patients with severe underlying disease, they can by no means be regarded as food-borne pathogens (Gasser, 1994).

Foodborne pathogens are either invasive and/or toxinogenic in the food or in the human intestine. Opportunistic pathogens in food may not be hazardous for the healthy consumer, but may pose a threat to some health-compromised persons. The genomes of many of the important food-borne pathogens and of some opportunistic pathogens have been fully sequenced, and the genes responsible for pathogenicity traits have been identified (Finlay and Cossart, 1997; Morschhauser et al., 2000). This opens the way to identify similar genetic information in the genomes of microorganisms used in food fermentation. Of the several genomes of microorganisms used in food fermentation that have been completely sequenced, two examples (*Saccharomyces cerevisiae* and *Lactococcus lactis*) have been published and reported to be free of known pathogenicity traits. If strains of species known to carry potential toxin genes are subjected to genetic modification, they must not carry such genetic information.

The long history of safe use and the available genetic evidence suggest that the genetic background of the majority of microorganisms used for food fermentation is free from pathogenicity islands and other pathogenicity determinants.

In addition to this, the following need to be considered:

- The genetic modification could produce a metabolic imbalance that may enhance the level of common metabolites that are normally not toxicologically significant in food to levels that are unacceptable (e.g. formic acid, acetaldehyde, biogenic amines in lactic acid bacteria or yeast; cyclopiazonic acid or roquefortin in *Penicillium camemberti/roqueforti*).
- The genetic modification could switch on genes coding for normally unexpressed toxins in the microorganism.
- The genetic modification [i.e. the expression of new protein(s)] could change the "cross-talk" between the microbe and the intestinal immune system of the consumer leading, for example, to an undesirable immune reaction or undesirable reactions with other cells (e.g. enterocytes) of the GI tract.

The first and second of these points may be addressed with *in vitro* studies. The third point may need to be addressed by *in vivo* studies in suitable animal models or human volunteers. If required, such studies will need to be carried out in accordance with good practice guidelines and ethical standards.

5.7 Safety and nutritional assessment

As highlighted in section 4.3, the assessment of the safety and nutritional aspects of GMMs should take into consideration the outcome of comparisons according to the concept of

substantial equivalence. When deemed appropriate, animal studies may be used to evaluate the safety of GMMs where the recipient organism, donor organism or gene or gene product do not have a history of safe use in food. However, as previously noted, animal studies have both strengths and weaknesses (FAO/WHO, 2000). While animal studies may be useful for identifying potential hazards in food produced with the aid of GMMs, and in certain cases to establish dose–response relationships, the major limitation is that the response in the animal has to be correlated with that in humans. Often, differences in the anatomy and physiology of animals and humans lead to substantial differences in dose relationships between the two and this needs to be taken into consideration in the interpretation and assessment of the relevance of animal studies on food produced with the aid of GMMs.

The safety of GMMs intended for use in food should be evaluated in terms of the food matrix in which they are consumed and this raises issues regarding the safety assessment of whole foods using animal safety studies. Guidance regarding the role of animal studies in assessing the safety of whole food products has been provided by previous consultations (FAO/WHO, 1991; 2000). Further advice concerning the role of animal studies in the safety assessment of whole foods has been given by the Scientific Committee for Food (Commission of the European Communities, 1997) and the UK ACNFP (1995, 1999).

In the use of animal studies, it is important that the experimental design addresses the specific safety issue under investigation. The following considerations (*inter alia*) should be taken into account when designing animal feeding studies:

- The use of appropriate and relevant control groups.
- Dose to be administered.
- Endpoints to be measured (for example the impact on the microflora and the GI tract).
- Statistical power.
- Duration of the test.
- Rigorous control of confounding factors.

Previous recommendations with regard to animal testing are not specific for GMMs and it is therefore recommended that guidelines be established to assess when and what kinds of animal studies are appropriate for testing GMMs.

5.8 Interactions between the GMM, the intestinal flora and the mammalian host

Throughout adult life, the human gastrointestinal (GI) tract is populated by a huge number (up to 10^{14}) of live microorganisms, outnumbering the number of somatic cells by 1–2 orders of magnitude. The exact composition of the flora, estimated to consist of at least 400 species, is poorly known, as the majority of species cannot be routinely analyzed by commonly used cultivation methods or molecular biology techniques. The flora fluctuates qualitatively and quantitatively from the oral cavity (dominated by lactic acid bacteria, streptococci and certain anaerobic species, to the stomach (transient acid-tolerant organisms), to the small intestine (with a transition to colon-like flora), to the colon where bacterial densities up to 10^{12}/g dry weight are reached. The colonic microflora is dominated by anaerobes like *Bifidobacterium*, *Eubacterium*,

Bacteroides and *Clostridium*, while the counts of microaerophilic microorganisms and facultative anaerobes such as Lactobacilli, Enterococci and coliforms usually are 3–4 orders of magnitude lower (Mikkelsaar et al., 1997; Willis and Gibson, 2000). Throughout the GI tract, the endogenous microflora represents the main barrier against exogenous microbes (colonization resistance; Araneo et al., 1996).

At any time after birth, the qualitative and quantitative composition of GI microflora depends on previous and ongoing exposure to and interactions with environmental factors (diet, antimicrobial therapy, disinfectants, food additives, occupation, climate etc.), factors associated with the mammalian host (age, gender, intestinal motility, transit time, pH, bile acids defensins etc.), and factors associated with flora itself (competition for nutrients, oxygen, H^+ acceptors/receptors, production of antimicrobials, organic acids, NH_3, H_2S etc.). The relative importance of these factors in the formation of GI ecosystem(s) has not yet been elucidated.

The GI flora may interfere with various mammalian host-associated structures and functions in a compartmentalized way and at an organ, cell, and molecular level (Falk et al., 1998; Midtvedt 1999; Moreau and Gaboriau-Ruthiau 2001). Some important interactions include:

- Role of enterocytic mitosis.
- Intestinal motility.
- Development of gut-associated immune system (GALT).
- Interference with enterohepatic circulation.
- Production of organic acids, nucleotides etc.
- Prokaryotic–eukaryotic cross-talk at the cellular level.

The relative importance of these interactions may vary according to the age and health status of the individual. The ability of an exogenous microbe (including a GMM) to survive in the GI tract depends on its ability to tolerate and adapt to the various microflora and host-associated factors mentioned above (survival being defined as the detection of a microorganism for a limited period of time where the growth rate is lower than the rate of elimination; Benbadis et al., 1995). In relation to colonization resistance, other strain-specific properties necessary for survival are not very well understood (Cesena et al., 2001). The strain may even leave the gut lumen and end up elsewhere by mechanisms usually termed translocation. Consequently, simple *in vitro* trials are not sufficient to predict the intestinal survival and suitable animal models, simulated human GI systems, and human trials may be required. Reliable strain identification is also essential for these studies.

A GMM that survives ingestion may only be a transient passenger or may establish itself for a variable time in the GI system. Colonization has been defined as the detection of a microorganism for a relevant period of time at a constant level (Benbadis et al., 1995).

While a permanent, life-long colonization of an adult by an exogenous microorganism is apparently a rare event, experiences with certain probiotic strains have shown that strains can be recovered in the faeces and colonic mucosa for weeks after discontinuation of oral administration (von Wright and Salminen, 1999). The term “persistence” has been introduced

to describe the survival of a microorganism in the GI tract for longer than two intestinal transit times (ILSI, 1999).

Whether the GMM is established in the GI tract or not, the possibility remains that it might influence the microflora or the mammalian host. The effects on the flora might partly depend on the functions expressed by the GMM (phenotypic expression), and potentially on horizontal gene transfer. The influences on the host can be direct or indirect. A direct effect can take place on all structures and functions listed previously, and indirect effects can be mediated through interference with the active part of endogenous flora. Even non-viable microorganisms are known to retain functional properties (i.e. cell adhesion, binding of chemicals, immunomodulating activities), which can have direct or indirect on both microflora- and host-associated functions (reviewed by Ouwehand and Salminen, 1998). Additional liberation of biologically active compounds (toxins, enzymes etc.) may take place.

Conjugative transfer between microorganisms in the gut is known to occur. It is reasonable to assume that its probability depends both on the relatedness of the GMM to the intestinal microflora and on its residence time in the GI tract, this being more likely with a persistent or colonizing strain than with a transient strain. The possibility of transfer of DNA from lysed GMMs should not be overlooked, since bacterial transformation in the human oral cavity has been demonstrated (Mercer et al., 2001). Furthermore, the work of Schubbert et al. (1997) indicated measurable persistence of DNA in the intestinal tract, and studies on the fate of DNA from food in the mammalian GI tract showed that plant and recombinant DNA could enter the blood stream, tissue cells and even nuclei (Schubbert et al., 1997; Einspanier et al., 2001; Hohlweg and Doerfler, 2001).

5.9 Exposure

The degree of intake of food GMMs needs to be considered in the pre-market safety evaluation and for monitoring any impact in the food chain.

The following factors should be considered when assessing exposure to such food GMMs:

- The food matrices in which the GMM or components thereof are consumed.
- Whether the exposure is to viable or non-viable GMMs, genes or gene products.
- The dose and duration of exposure (in assessing the potential hazard).
- Exposure should be assessed within the entire population, taking into consideration special population groups (e.g. immune-deficient individuals, children and the elderly) and different geographical regions.
- Exposure to GMMs and their components during their production and preparation in the food chain.

As GMMs gain use in the food supply, consideration needs to be given to methods that measure the potential effects of exposure on the general population. The issues relating to conducting health surveillance for genetically modified foods have been dealt with by a previous Consultation who found that: *"The change in nutrient levels in a particular crop plant may impact on overall dietary intake. In such cases, it is important to determine alterations in nutrient content and bio-availability, and their stability with time, processing and storage, as well*

as to monitor changes in dietary patterns as a result of the introduction of the genetically modified food and evaluate its potential effect on nutritional and health status of consumers. However, an assessment of the impact on nutritional status of consumers is important for all significant dietary changes and not specific to the introduction of genetically modified foods" (FAO/WHO, 2000).

As noted by a previous Consultation (FAO/WHO, 2001), very little is known about the potential long-term health effect of any food and this situation is compounded by the wide genetic variability in human populations. Given the complexity of monitoring the effects of human exposure to food produced with the aid of GMMs, it is recognized that it would be difficult to identify any effects against the background of conventional foods, unless studies were designed to answer very specific questions. Nevertheless, it is also recognized that it is important to develop methods to monitor (trace) exposure to GMMs.

5.10 Impact on the immune system

To assess the immune-modulating potential of a transgene in a GMM, a case-by-case consideration is recommended. A separate Consultation dealing with allergenicity in terms of genetically modified organisms has already made several recommendations in this regard (FAO/WHO, 2001).

It must be noted that interactions of gut microflora with the immune system occur. Gut-associated lymphoid tissue (GALT) has important interactions with the immune system and it is well established that microbial stimuli are the main antigenic forces in the development and maintenance of GALT and acquired immunity. It should be emphasized that GMMs, in contrast to food derived from GM plants, may establish themselves within the GI tract, thereby prolonging potential immune-modulating effects.

6. Conclusions

i. The Consultation agreed that the safety assessment of GMMs should proceed on a case-by-case basis aided by a series of well-defined questions. The Consultation confirmed that a comparative approach, using the concept of *substantial equivalence*, provided a practical means of identifying similarities and differences between food produced with the aid of GMMs and their appropriate comparators. These differences would then be the focus of the safety evaluation.

ii. The Consultation noted that there are intrinsic properties of microorganisms that require special consideration in the application of the concept of *substantial equivalence*. In particular, it noted that the food matrix in which GMMs may be consumed could influence its safety, thus the impact of the food matrix needs to be considered. It may therefore be necessary to apply the concept of *substantial equivalence* both to the GMM and to the food produced with the aid of GMMs. In doing this, it may be necessary to examine additional parameters such as pathogenicity and persistence in the mammalian host GI tract.

iii. The Consultation noted that the potential range of GMMs in food included viable and non-viable microorganisms that may be consumed as such or may be integral components of foods. The Consultation noted that because of the wide range of products involved, the safety assessment needed to take into consideration the specific uses and exposures to the GMM being considered.

iv. The Consultation noted that the use of microorganisms in food production is of great importance to the nutritional quality and safety of the food supply. Therefore, the evaluation of GMMs should encompass both safety and nutritional aspects.

v. The Consultation noted that microorganisms in the GI tract exert important effects on the immune system. While previous recommendations (FAO/WHO, 2001) relating to the allergenicity of new proteins expressed in GM plants can be used in the safety assessment of foods produced with the aid of GMMs, it must be noted that the possible effect of GMMs or their components on the immune system in the mammalian host requires additional consideration.

vi. The Consultation noted that genetic material from food has the potential to transfer to gut microflora and to cells of the mammalian host *in vivo*. The safety concerns of such gene transfer need careful evaluation based on the properties of the GMM and its components.

vii. The Consultation concluded that in developing a GMM for use in food production, vectors should be used which consist only of nucleotide sequences from microorganisms with a history of safe use in food. Any selectable markers should be carefully chosen and based on safe use. In particular, antimicrobial resistance marker genes should be avoided and not be present in the final GMM.

7. Recommendations

i. It is recognized that the complex ecosystem of the human gastrointestinal tract is subject to increasing and successful analysis. It is recommended that this analysis should be enhanced, including examination of the ecological components of the GI tract, the prevailing selective conditions, and the effect of nutritional conditions and host factors on the interactions. These studies would provide the basis for improved risk assessment.

ii. While a permanent life-long colonization of an adult by an exogenous microbe is apparently a rare event, strains can be recovered from the intestinal tract for weeks after the discontinuation of exposure through food. Whether a GMM is established in the GI tract or not, the possibility remains that it might influence the microflora and/or the mammalian host. The effects on the flora might relate to functions expressed or to horizontal gene transfer. There is a need to improve the methods for evaluating the function of microorganisms in the GI tract.

iii. Fermentation provides a simple technique to produce food of high nutritional and hygienic quality. This technology is used globally and is especially important in developing countries. The continued improvement of such technologies could involve the use of GMMs. The Consultation recommended that FAO/WHO promote capacity-

building efforts to support the needs of developing countries in improving and evaluating this technology.

iv. The Consultation recognized the need for efficient communication of issues related to the development and safety evaluation of GMM food. Specific communication of the principles guiding the safety assessment to the public etc. would enable efficient interaction and transparency in the evaluation process. The Consultation recommended that FAO/WHO coordinate the efforts to achieve this.

v. The Consultation noted that specific methodologies are available which enhance the safety of GMMs through an improved understanding of the biology of microorganisms. New technologies are developing rapidly with a potential to enhance the safety evaluation of GMMs, especially bacteria. These are described within the report and include molecular profiling. The Consultation encourages further development and validation of such methods.

vi. The Consultation identified a number of aspects that it recommends should be taken into account in assessing the safety of food produced with the aid of GMMs. Details of these are given in the body of the report and include:

- application of the concept of *substantial equivalence* to the GMM and foods produced from the GMM;
- consideration of the techniques used for the development of the GMM – especially the history of safe use of host microorganisms and also of the microorganisms from which inserted gene(s) and vector(s) are derived – and avoidance of the use of antimicrobial resistance marker genes;
- strain identification and characterization;
- transfer of genetic material from the food to the gut microflora and mammalian host cells;
- genetic stability of the GMM;
- pathogenic potential of the GMM;
- impact of the GMM on the human immune system; and
- human exposure to the GMM and the effects of food processing, production and storage.

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Annex II: Agenda Items and List of Consultation Room Documents

* denotes agenda item consisted of submitted paper only.

† denotes no paper was submitted against this agenda item.

Documents No	Agenda Items	Titles
Biotech 01A/01	Agenda Item 3	Provisional Agenda and Timetable
Biotech 01A/02	Agenda Item 3	Provisional Annotated Agenda
Biotech 01A/03	Agenda Item 4 Topic 1	Use of living microorganisms in food and perspectives on the application of genetic modification in such microorganisms
Biotech 01A/04	Agenda Item 4 Topic 2-a	Conventional safety assessment methodologies used for foods derived from biotechnology including foods of plant origin
†	Agenda Item 4 Topic 2-b	Issues specific to foods using genetically modified microorganisms
†	Agenda Item 4 Topic 3	Genetic stability of microorganisms and their familiarity
Biotech 01A/07	Agenda Item 4 Topic 4	Gene Transfer
Biotech 01A/08	Agenda Item 4 Topic 5-a	Intestinal flora and gut ecosystems
Biotech 01A/09	Agenda Item 4 Topic 5-b	Gut colonization and effects on health (beneficial and adverse)
Biotech 01A/10	Agenda Item 4 * Topic 6	Genetic determinants of pathogenecity of microorganisms and its possible change by genetic modification Published papers: Brett Finlay, B. and P. Cossart (1997) Exploitation of Mammalian Host Cell Functions by Bacterial Pathogens. Science; 276: 718-725.
Biotech 01A/11	Agenda Item 4 * Topic 7	Nutritional and toxicological aspects of bacterial or fungal metabolites, their possible alternations by genetic modification
†	Agenda Item 4 Topic 8	Allergenicity of microorganisms and its possible induction or change by genetic modification.
Biotech 01A/12	Agenda Item 4 * Topic 9	Survival/propagation of microorganisms including GMM in the environment and its implication on public health