Strategies for assessing the safety of foods produced by biotechnology

Report of a
Joint FAO/WHO Consultation

World Health Organization
Geneva

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The World Health Organization is a specialized agency of the United Nations with primary responsibility for international health matters and public health. Through this organization, which was created in 1948, the health professions of some 165 countries exchange their knowledge and experience with the aim of making possible the attainment by all citizens of the world by the year 2000 of a level of health that will permit them to lead a socially and economically productive life.

By means of direct technical cooperation with its Member States, and by stimulating such cooperation among them, WHO promotes the development of comprehensive health services, the prevention and control of diseases, the improvement of environmental conditions, the development of health manpower, the coordination and development of biomedical and health services research, and the planning and implementation of health programmes.

These broad fields of endeavour encompass a wide variety of activities, such as developing systems of primary health care that reach the whole population of Member countries; promoting the health of mothers and children; combating malnutrition; controlling malaria and other communicable diseases including tuberculosis and leprosy; having achieved the eradication of smallpox, promoting mass immunization against a number of other preventable diseases; improving mental health; providing safe water supplies; and training health personnel of all categories.

Progress towards better health throughout the world also demands international cooperation in such matters as establishing international standards for biological substances, pesticides and pharmaceuticals; formulating environmental health criteria; recommending international nonproprietary names for drugs; administering the International Health Regulations; revising the International Classification of Diseases, Injuries, and Causes of Death; and collecting and disseminating health statistical information.

Further information on many aspects of WHO's work is presented in the Organization's publications.

Other WHO publications on food safety

	Price* (Sw. fr.)
Principles for the safety assessment of food additives and contaminants in food. WHO Environmental Health Criteria, No. 70, 1987 (174 pages)	14.–
Guidelines for the study of dietary intakes of chemical contaminants. WHO Offset Publication, No. 87, 1985 (102 pages)	11.–
Food irradiation. A technique for preserving and improving the safety of food. 1988 (84 pages)	16.–
Wholesomeness of irradiated food. Report of a Joint FAO/IAEA/WHO Expert Committee. WHO Technical Report Series, No. 659, 1981 (34 pages).	3.–
Evaluation of programmes to ensure food safety: guiding principles. 1989 (47 pages)	9.–
Health surveillance and management procedures for food-handling personnel. Report of a WHO Consultation. WHO Technical Report Series, No. 785, 1989 (47 pages)	6.–
Jacob, M. Safe food handling: a training guide for managers of food service establishments. 1989 (148 pages)	25.–
Williams, T. et al. Food, environment and health. A guide for primary school teachers. 1990 (148 pages)	26.–
Salmonellosis control: the role of animal and product hygiene. Report of a WHO Expert Committee. WHO Technical Report Series, No. 774, 1988 (83 pages)	11.–
Charles, R. H. G. Mass catering. WHO Regional Office for Europe, European Series, No. 15, 1983 (80 pages)	13.–

Further information on these and other World Health Organization publications can be obtained from Distribution and Sales, World Health Organization, 1211 Geneva 27, Switzerland.

* Prices in developing countries are 70% of those listed here.

Contents

1. Introduction

A Joint FAO/WHO Consultation on the Assessment of Biotechnology in Food Production and Processing as Related to Food Safety was held in Geneva from 5 to 10 November 1990; the participants are listed in Annex 1. The Consultation was opened by Dr J. Rochon, Director, Division of Health Protection and Promotion, WHO, on behalf of the Directors-General of FAO and WHO. In welcoming the participants, Dr Rochon drew attention to the long history of the application of biotechnology to food production and processing. This went back more than 8000 years, so that the food industry was one of the oldest users of biotechnological products and processes.

Since the nineteenth century, the science of biotechnology had developed more rapidly than ever before and particularly so over the past decade. While there were many applications of biotechnology in areas such as drugs, the new technologies were also potentially capable of revolutionizing the world's food supply. Enormous improvements were possible in both the quantity and quality of food available. Contemporary techniques of genetic modification made it possible both to speed up the classical processes of plant and animal breeding and to effect interspecies gene transfers not possible by classical methods.

Dr Rochon predicted that biotechnology, by changing the character of food sources, would have an enormous impact on our ability to provide food for the world's rapidly increasing population. The challenge would be to develop appropriate safety assessment procedures to ensure that these new food sources were safe for human consumption. An international consensus on the safety assessment of foods derived from biotechnology would be a strong foundation for consistent national regulatory activities and it was hoped that the Consultation would be a valuable initiative in that direction.

Biotechnology raised a number of important nonscientific issues related to ethics, consumer perceptions and food labelling, which would need to be taken into account by national regulatory agencies. Such issues were, however, outside the remit of the Consultation, which would confine its activities to the scientific issues of safety assessment.

In his reply, Dr S. A. Miller, Chairman of the Consultation, emphasized the need for a strong science base for any national and international regulatory activities if biotechnology were to move

forward. A careful balance would be necessary to ensure that any possible problems were neither understated nor overstated; it was important that the public should not be unduly alarmed, and equally that any gaps in available information should be acknowledged.

1.1 Scope of the Consultation

The aim of the Consultation was to outline appropriate strategies and procedures to assist those responsible for assessing the safety of specific applications of biotechnology in food production and processing. It adopted a number of definitions established by other international bodies; these, together with definitions of other terms used in this report, are given in the Glossary (p. 57). The Consultation noted that the definition of biotechnology applied equally to classical and modern techniques, underlining the fact that, from the point of view of safety, there was no fundamental difference between traditional products and contemporary ones obtained by means of biotechnology. It agreed, therefore, that the same broad principles of safety assessment should apply to the products of both the old and the new biotechnologies. The Consultation also noted that, as biotechnology was developing rapidly, there would be a need to re-examine the issues at a later date. The Consultation's report should therefore be seen as a first step in a series of activities aimed at reaching an international consensus and providing guidance on the safety assessment of foods obtained using contemporary techniques of biotechnology.

In addition to its direct application to food production and processing, biotechnology also has applications in the production of veterinary drugs, pesticides and other products used in agriculture, and also in the development of improved methods for use in food analysis. Such aspects, which are of significance in food safety, are briefly considered in sections 2.1.4 and 2.4.

While environmental aspects of biotechnology are also important, they are not dealt with directly in this report as they are being dealt with elsewhere; e.g., both the United Nations Environment Programme and the Organisation for Economic Co-operation and Development are active in this field. The notification requirements that apply to the deliberate introduction into the environment of genetically modified food organisms, whether microbes, plants or animals, will usually have to be satisfied before any consideration is given to food safety. Many of the data necessary for the examination of these environmental aspects will also be of value in assessing food

safety, in particular, information on the characteristics of the host and donor organisms, genetic insert and vector. Environmental information on genetically modified plants is particularly relevant to food safety as it also covers the potential transfer of genetic material to other food crops through pollen transfer.

1.2 History of the use of biotechnology in food production

Biotechnology has been used in food production and processing for thousands of years. Almost every ingredient used in the production of food has as its source a living organism, whether animal, plant or microorganism. The food sources available to early humans, both plant and animal, had evolved through natural selection. Genetic diversity arising from spontaneous genetic changes, including recombination, mutation and reproductive isolation, was exploited when the early farmers began to save the seeds from their best crops for later sowings and to use the best animals for breeding.

Even before the laws of segregation and independent assortment were formulated by Mendel in the 1860s and rediscovered in the early part of this century, their significance was recognized and applied empirically in selective breeding programmes. The simple procedure of maximizing the reproductive efficiency of organisms with advantageous phenotypes, while limiting the reproduction of those with undesirable traits, led to great improvements in the productivity of many animal and plant species. With the recognition of the validity of Mendel's laws, these practices were applied in a more scientific manner, to develop new strains of organisms for use in food production and processing. Although considerable advances have been made, there are limits to what can be achieved by conventional breeding and strain selection. The process is slow and limits are imposed by the genetic diversity of the parent organisms; results are often unpredictable and lengthy back-crossing may be necessary to remove undesirable traits introduced together with the desirable ones.

The value of breeding and selection techniques can be improved by increasing the diversity within the gene pool of the parent organisms. Muller et al., working with *Drosophila* in the 1970s, showed that X-rays could have a point effect on a single gene within the organism (*1*). Other forms of radiation and certain chemicals have been found to have a similar effect. Mutants induced by these treatments have been used successfully in plant breeding, with large

numbers of progeny being produced from plants grown from treated seed. Screening identifies useful mutants which can be incorporated into breeding programmes.

The plant breeder's task has been eased considerably in recent years by the inclusion in breeding programmes of plant cell-culture techniques. In food plants, many of the traits that are the targets for change are controlled by more than one gene, and it will be many years before these systems are fully understood and can be genetically modified. The strategy of locating quantitative trait loci using restriction fragment length polymorphisms is based on the fact that DNA sequence polymorphisms, reflected as alterations in the distribution of restriction endonuclease cleavage sites, can often be identified either within or very close to a gene of interest. In the tomato, for example, genes affecting fruit mass can be identified by crossing a tomato with high fruit mass with another variety of tomato that has a number of polymorphic differences in restriction enzyme sites. Progeny plants can then be screened with probes derived from individual plant chromosomes, and the presence of specific chromosomes from the parent with high fruit mass compared with the appearance of the phenotype. In this way, traits can rapidly be mapped to the one or more chromosomes on which the relevant genes reside. This powerful new technique could be used in the rapid selection of superior plants in traditional plant-breeding programmes. Such mapping can also be used in conjunction with molecular cloning procedures to clone the various genes of interest.

In animal breeding also, new techniques have brought about significant advances. Administration of hormones to increase ovum production, followed by artificial insemination, can provide up to a dozen or so embryos which can then be implanted into surrogate mothers. In a more recent development, ten or more cells may be taken from each fertilized egg before cell differentiation, and their nuclei implanted into unfertilized eggs from which the nuclei have been removed. Each embryo is then implanted into a surrogate mother to produce identical offspring. Thus, in theory, several hundred offspring can result from a single mating, considerably reducing the time necessary to introduce a new strain. However, even these developments are limited by the inherent genetic endowment of each species and by the lack of methods for determining the structure and function of individual genes.

New methods based on molecular biology have aroused considerable interest, because they offer the prospect of more rapid and precisely targeted genetic changes than can be introduced through breeding and selection. They are also not bound by the genetic

diversity of the parent organisms or their sexual compatibility. These new methods have been made possible by a number of major discoveries over the past 50 years which have built on the pioneering work of Darwin and Pasteur. They include the demonstration that DNA is the bearer of genetic information, the elucidation of the structure of DNA, and Cohen et al.'s demonstration in 1973 that DNA could be constructed *in vitro*, and inserted and expressed in a living organism (*2*). Organisms can thus be genetically modified by the introduction of novel genetic material in the form of a DNA construct made *in vitro*. A number of techniques can be used for this purpose, including sexual crossing, protoplast fusion and direct gene transfer.

To be of any practical value, the novel DNA introduced into the cells of the host organism must be genetically stable and properly expressed. Expression of the gene products, if achieved, will reflect precisely the nature of the modification made, although the effects of the gene products on the metabolism of the organism may not be easy to predict, particularly if the foreign gene comes from an unrelated species.

2. Applications of biotechnology in food production and processing

The status of biotechnology in food production and processing is reviewed in this section. For convenience, microorganisms, plants and animals are considered separately although many of the principles involved are similar for all three. Particular attention is given to the newer methods of biotechnology.

2.1 Bacteria and fungi

In this section, the history and current status of the use of microorganisms and their products in food production are discussed. Also included, for the sake of completeness, is a discussion of products used in agriculture, including veterinary drugs, biological pesticides and rhizobia.

2.1.1 Fermented foods

Throughout the world, fermented foods form a major part of the human diet; however, it is impossible in this report to discuss any of them in detail. Examples of fermented plant products include alcoholic drinks, tea, coffee, bread, sauerkraut, miso and tempeh. A wide variety of fermented fish, milk and meat products is also available. Fermentation, which may be brought about by yeasts, moulds or bacteria, not only helps to diversify the diet but can also contribute to increased palatability, acceptability, nutritional value and shelf-life of foods.

Many fermented foods have been known for hundreds, if not thousands, of years. Initially they were made in the home or at local level, and the strains of organisms used were passed down from generation to generation and were selected for desirable characteristics, such as flavour production. Many fermented foods are now produced on an industrial scale, and there is interest in applying genetic modification techniques to the microorganisms that produce them, including those involved in bread and beer production. The

yeasts involved are well researched and potentially easy to modify; developments under way include:

(*a*) the incorporation into commercial brewing strains of genes for glucoamylase production, thus avoiding the need to add exogenous enzymes during beer production;
(*b*) the incorporation into commercial bread-making strains of a more efficient system for metabolizing maltose, thus reducing bread-making time;
(*c*) the incorporation into commercial yeast strains of genes to enable pharmaceutical proteins to be produced after the yeast has been recovered from food production.

In the dairy industry, lactic-acid-producing bacteria, genetically modified to produce strains with improved phage resistance or bacteriocin or flavour production, are being developed.

In the early 1970s, single-cell (i.e., bacterial or fungal) protein was thought to have considerable potential as a source of human and animal food. One project that has become a commercial success produces human food from the organism *Fusarium graminearum* grown on hydrolysed starch. The bacterium *Methylophilus methylotrophus* has also been used to convert methanol into animal feed protein, but the project has been terminated because of economic difficulties.

The most abundant renewable biomass on earth is cellulose, an estimated 5–15 tonnes being produced per capita per year. Much of the cellulose is bound physicochemically to lignin. Some higher fungi, including some edible species, can be used to convert lignocellulose directly into fungal protein suitable for human consumption.

Although there is a considerable body of literature on the production of fats and lipids by microorganisms, microbial lipids are not being produced commercially for use in food at present. This situation could change, however, with the growing consumer demand for "speciality" oils and the possible use of genetic modification, in the long term, to manipulate the fatty acid composition of microbial lipids.

2.1.2 Food additives and processing aids

A wide range of food additives, including amino acids, citric acid, vitamins, enzymes and polysaccharides such as xantham gum, has been produced for many years using microorganisms. For some products, the use of microorganisms has replaced chemical synthesis

or extraction from animal or plant sources. Thus, for example, microbes have largely replaced lemons as a source of food-grade citric acid. Microorganisms have advantages as compared with animal or plant sources in terms of continuity of supply of the product and often also in terms of ease of product recovery. Products obtained from microorganisms will differ in their spectrum of impurities as compared with the corresponding chemically produced product.

Traditionally, the production of food additives from microorganisms has been optimized by the correct choice of fermentation parameters and the use of selected high-yielding strains. Many strains of microorganism used in the commercial production of food additives have been improved by processes involving radiation or mutagenic agents. Thus, for example, strategies for maximizing the efficiency of bacterial production of amino acids include inducing the bacteria to excrete glutamate by excluding biotin from the nutrient medium.

There is considerable potential for the exploitation of the new techniques of genetic modification in the production of food additives. They are already being used in a small number of instances both to increase yields and reduce processing costs of existing additives (e.g., L-tryptophan) and to develop new sources of existing additives or processing aids (e.g., chymosin). Future possibilities include the production, in microorganisms, of certain flavour substances currently synthesized chemically or obtained from plant sources.

Although plant cells are not microorganisms, their culture in fermenters is analogous to microorganism fermentation. Plant cell-culture techniques have improved dramatically over the past five years but there are still some technical problems. If these can be overcome, the rapid screening and strain improvement possible with plant cells suggest that such cells grown in culture have considerable potential for use in the production of food additives. In particular, high-value products, such as colours and flavours, might be produced, thus reducing dependence on the agricultural sources from which they are currently extracted.

Protein engineering techniques developed in the past decade make it possible to alter the structure of genes and thus to modify the properties of the corresponding gene products. It should be possible to apply this technology to alter not only the properties of enzymes but also those of other gene products used in food production and processing.

2.1.3 Applications using enzymes

Starch is by far the most abundant polysaccharide used in food. The starch industry is at present almost completely enzyme-based, the starch-degrading enzymes being derived from plants, animals or microbes.

The use of rennet, the common milk-clotting preparation, to produce cheese and other dairy products is by far the largest single use of enzymes by the dairy industry. Extracellular fungal proteinases can serve as rennet substitutes.

Fungi serve as sources of the lactase used to produce low-lactose milk for individuals who are lactose-intolerant. It may be possible in the future to introduce lactase-containing organisms into the milk fermentation process for the purpose of hydrolysing, within the human gut, the residual lactose in yoghurt.

The plastein reaction provides a way of using currently underused protein sources, such as leaf protein. This new technique, which involves controlled proteolysis followed by adjustment of the protein and enzyme concentrations and pH, results in resynthesis of proteins or rearrangement of peptide bonds. It has yet to be applied on a commercial scale.

The important technique of enzyme or microorganism immobilization allows continuous processing of large amounts of material. It entails the immobilization of enzymes or microorganisms on a matrix, such as membrane filters or diatomaceous earth.

2.1.4 Products used in agriculture

Biological pesticides

In recent years, interest in biological pest-control agents has increased because they have relatively narrow host ranges and thus do not affect natural predators and beneficial species. Another advantage is that pests are generally slow to develop resistance to them. Their biodegradability also means that their effect on the environment is relatively small. However, they are less stable than chemical agents, so that shelf-life is reduced and storage and handling are more costly; other disadvantages are the fact that they act more slowly on pests and that the conditions of application are more stringent.

The most commonly used biological pest-control agents include bacteria, viruses, fungi, nematodes and insects; the toxins produced by these organisms are also used. Many viruses are known to be

pathogenic to insects and a dozen or more of the bacculovirus subgroup have been developed commercially. Fungal insecticides have been used successfully since the early 1900s. Other examples of organisms used as insecticides include a nematode that carries bacteria active against the black vine weevil and a wasp that is used to control a sugar-cane pest.

Biotechnology can be used to develop more efficient and potent or virulent strains, to improve the physiological tolerance of biological pest-control agents to stresses encountered in nature, and to expand the host range. The most widely used method is the cloning of the *Bacillus thuringiensis* gene for toxin production, and its transfer to another strain or species with the aim of producing a more efficient insecticide. Research is also being directed towards increasing the efficacy of the bacculoviruses through the introduction of insect-specific toxins. Many bacteria produce extracellular chitinases, i.e., enzymes that destroy chitin, a structural component of many plant pests, including fungi and insects. Chitinases have been cloned and transferred to efficient plant-colonizing bacteria.

Veterinary drugs

Biotechnological processes have been used for many years to produce veterinary vaccines and other products. Recent developments in molecular biology have made it possible to determine the structure of complex molecules of veterinary significance, such as hormones, and of the genes that control their synthesis. Thus, for example, the genes responsible for the production of porcine and bovine growth hormone have been cloned. Expression of these genes in microbial sources makes it commercially feasible to produce protein hormones, such as the somatotropins or their precursors, by the genetic modification of bacterial DNA. The production of interferon by recombinant microorganisms holds promise for alleviating the effects of viral diseases on livestock.

Genetically modified bacteria have been developed to produce products — such as vaccines and monoclonal antibodies — used in the prevention of animal diseases. While these products and their use do not constitute direct genetic modification of animals, they are noted here in order to ensure complete coverage of the broad range of possible biotechnological advances.

Rhizobia

Improvement of the nitrogen-fixing ability of cereals was an early target for those working on the genetic modification of plants. The