# 11
# Safety Testing of Novel Food Products Generated by Biotechnology and Genetic Manipulation

DIANA ANDERSON* AND W.F.J. CUTHBERTSON†

*British Industrial Biological Research Association, Woodmansterne Road, Carshalton, Surrey SM5 4DS, UK and †Harefield, Middlesex UB9 6JA, UK

## Introduction

Within the last decade, genetic manipulation of micro-organisms has been used in a multitude of instances to modify markedly their behaviour, metabolism and productivity. Now there is every indication that it will be possible similarly to change the genetic basis of higher plants and animals, as well as of the micro-organisms used for foods or in food production. There is thus concern to establish appropriate controls relating to application of genetically manipulated organisms for food purposes. The basic problems relating to the safety of biotechnological applications concerning foods are the same as those which apply to all toxicological investigations. The most important of these problems are the impossibility of proving the absence of harmful effects (whether known or not yet experienced) and our ignorance of many biological mechanisms and how they are affected by external factors. There are further complications, for foods, in that many common foods, e.g. beans, wheat, milk, eggs and fish, are not universally tolerated, while numerous carcinogens, mutagens and other toxins are present in many common components of our diet (Ames, 1983).

Any regulations proposed must have sufficient power to ensure the safeguards essential to prevent the release or general use of products or organisms which could harm the consumer or the environment, and yet they must not

Abbreviations: ACINF, Advisory Committee on Irradiated and Novel Foods; ADI, acceptable daily intake; DNA, deoxyribonucleic acid; OECD, Organization for Economic Co-operation and Development; SCP, single-cell protein.

*Biotechnology and Genetic Engineering Reviews*—Vol. 5, September 1987

be so restrictive as to stultify innovation and the benefits which applications could bring. No matter what detailed controls are formulated, therefore, they should be amenable to rapid modification in the light of new knowledge and experience.

This chapter reviews some of the guidelines and regulations which have been published and discusses the problems which may be met and the types of control which may be necessary if the genetic manipulations now forecast for higher plants and animals are to be applied on the commercial scale.

The techniques of biotechnology have been applied to foods for many years. Raw materials may be modified by enzymes (as in cheese manufacture) or by growth of micro-organisms (as in sauerkraut, soy sauce and salami), which may also be used in the production of simple chemicals (such as acetic and citric acids).

Biotechnological developments over the past 5–8 decades now enable micro-organisms to be grown on a manufacturing scale on a wide variety of substrates, for example methanol, carbohydrates and paraffins. The organisms—yeasts, bacteria or fungi—yield products of value in the preparation and processing of many foods and drinks: such substances include specific proteins such as enzymes and many secondary metabolites; alternatively, biomass may be produced which can be processed for animal or human food.

The development of new varieties of organisms, ranging from microbes to vertebrates, has until recently been restricted to selection from populations derived by recombination of a gene pool limited to within a group of organisms sufficiently closely related to permit sexual or parasexual recombination of genetic potential. The DNA of the genes may be modified by mutagenesis, using physical or chemical means, but the effects are not easily predictable and desirable changes usually occur only at very low frequencies. These traits, even when identified, can be transferred to the desired organism only by sexual or parasexual methods.

Novel strains developed in such traditional ways may demonstrate toxic effects (e.g. potatoes; Maga, 1980); however, such phenomena are rarely experienced, and are usually easily detectable because much is known of potentially toxic substances in foods—carcinogens and mutagens (Ames, 1983) and other toxins (Bender, 1987; Heaney and Fenwick, 1987; Watson, 1987). Full toxicological investigations are thus not thought to be necessary for novel strains or cultivars derived by traditional means from species of plants or animals which are in common use for food purposes.

Recent developments in genetic manipulation are now the subject of particular concern. Over the past three decades, means have been devised to introduce genes, as DNA, and to facilitate their replication and expression, in micro-organisms, plants or higher animals. Modern techniques permit the precise insertion of DNA, with well-characterized properties, into the genome. This enables known, undesirable features to be more effectively avoided with some types of genetic manipulation than when the traditionally available uncontrollable mutagenic procedures are used.

The DNA thus introduced need not be derived from a closely related

species but can come from any organism or, indeed, be wholly synthetic (Edge and Camble, 1984; Efimov, Chakhmakhcheva and Ovchinnikov, 1986). In this way the potential gene pool available, and hence the possibility of variation, has been enormously increased. This has caused concern because, as mentioned earlier, the effects of genetic manipulation are not entirely predictable. In addition, it is possible that undesirable attributes may now, wittingly or unwittingly, be transferred more readily than was possible previously, so that increased safeguards are necessary.

## Types of biotechnological products which may be used in foods

The products which may be produced by biotechnology for use in foods, as constituents or in processing, are usually considered under the following headings: (1) metabolites—substances other than protein formed by the metabolic activities of the organism; (2) proteins; (3) biomass, i.e. microorganisms, higher plants or animals which can be harvested for use in foods, usually after processing.

### METABOLITES

The substances of interest are almost all of low molecular weight and are capable of economic production in a reproducible and purified state. Numerous compounds of this type are manufactured for use in foods—as nutrients, flavouring agents, processing aids or preservatives — examples being ascorbic acid, riboflavin, lysine, methionine, citric acid and glutamic acid. There are legal requirements concerning the utility and safety of any substances to be offered for use in foods, no matter how prepared, whether by chemical or biological means (Food Act (1984); Kahn and Gibbs (1985) note tests to approve substances for food use). Thus there is no reason to suggest any special tests for substances of this type when derived by use of genetically manipulated organisms, provided that they meet the agreed requirements for identity, safety and purity. However, poorly characterized mixtures of secondary metabolites prepared by novel means might well demand special toxicological evaluation, whether produced by organisms derived by genetic manipulation, by traditional strain development methods or, indeed, by novel chemical or physical means.

The ability of the production organism to produce harmful substances, and previous experience of use and variability, could all be relevant in assessing the safety of mixtures of metabolites derived from new strains, whatever their origin. The particular circumstances should dictate whether the procedures used to evaluate food additives or novel foods or enzymes would be appropriate (Food Safety Council Scientific Committee, 1978, 1980; Committee on the Medical Aspects of Food Policy, 1980; Association of Microbial Enzyme Producers, 1980; Bureau of Foods, Food and Drug Administration, 1982; Food Additives and Contaminants Committee, 1982; Advisory Committee on Irradiated and Novel Foods, 1984; Blumenthal, 1984; Petricianni, 1984; Kahn and Gibbs, 1985).

## PROTEINS

Proteins are the primary products defined by the DNA code although they may be subsequently modified by a variety of means. These complex molecules (including enzymes, antigens, receptors and peptide hormones) can now be isolated and processed to provide highly purified products which can be subjected to tests of identity, purity and safety which previously could be applied only to simple chemical substances.

Genetically manipulated organisms offer great promise for production of specific proteins because insertion of the appropriate gene can provide the desired products in yields which would be impossible to achieve with the unmanipulated organism (Edge and Camble, 1984).

Biotechnological means are now available for production of many proteins for pharmaceutical and other specialized purposes but expense precludes their use in foods except as biocatalysts in food production, i.e. as enzymes (*see below*). Thus it is unlikely that any proteins will be produced in the foreseeable future as pure substances, either as functional components directly to provide textural qualities or as nutrients to supply essential and non-essential amino acids.

## ENZYMES AND BIOCATALYSTS

Enzymes, i.e. proteins with highly specific biocatalytic actions, are frequently used in the manufacture and processing of food and drink; some are derived from plant or animal sources but many more are now obtained from cultures of micro-organisms, fungi or bacteria, e.g. glucose oxidase (EC 1.1.3.4), α-amylase (EC 3.2.1.1), amyloglucosidase (glucan 1,4-α-glucosidase; EC 3.2.1.3) and various peptidases (serine proteinases; EC 3.4.21, cysteine proteinases; EC 3.4.22 and aspartic proteinases; EC 3.4.23).

Enzymes are very active substances: each enzyme molecule can rapidly induce the transformation of many substrate molecules. The number of molecules transformed per minute per enzyme molecule — the 'turnover number' — for many enzymes used in food processes ranges from about ten thousand to over one million: for instance, under optimum conditions, β-galactosidase (EC 3.2.1.23) and α-amylase can metabolize 12 500 and 1 100 000 substrate molecules per minute, respectively.

In practice, in the home or the food factory, enzymes are normally available in diluted forms, solid or liquid, and at various concentrations. Commonly the enzyme is diluted and standardized so that it can be used at the rate of about 1 part per thousand — about 1 kg per tonne of material to be treated. Such preparations frequently contain 10%, or less, of the enzyme itself. Because of the need for dilution the products used industrially, or in the kitchen, are crude or partially purified preparations adjusted to the required potency with an appropriate diluent.

In most applications, the enzyme is inactivated or removed from the product before it is consumed. The safety, or otherwise, of enzyme preparations may therefore depend not so much on the active principle itself as

on associated substances. For instance, although malt and rennet are crude extracts of sprouted barley and calves' stomachs, respectively, they are generally considered to be safe because they have been used for many years without causing any apparent harm. The same may be said of numerous enzyme preparations derived from microbial sources for use in the food industry, e.g. amylase, glucose oxidase, xylose isomerase (EC 5.3.1.5), pectin esterase (EC 3.1.1.11) and microbial rennin (EC 3.4.23.6). The production strains have often been derived from wild types by traditional methods of genetic recombination coupled with mutagenic and selection techniques. However, products from novel strains should not be used in food products or processes without assurance of absence of hazard to the consumer. If novel organisms are employed, then more intensive toxicological evaluation is needed, as suggested by the Advisory Committee on Irradiated and Novel Foods (1984) to ensure absence of risk from possible toxic metabolites .

## IMMOBILIZED ENZYMES

For economic reasons, enzymes immobilized in various ways (Poulsen, 1984; Powell, 1984) are being increasingly used in the processing of products for use in foods. Such preparations should also be much less hazardous because of the necessity for increased concentration and purity of the enzyme preparations used for immobilization compared with those used in the normal batch processes, and also because the immobilized enzyme is normally (but not invariably) removed from the product before consumption. Thus, provided that soluble and undesirable agents are removed from the immobilized preparation before use, and that enzymic contaminants do not lead to formation of toxic substances from the material during processing, immobilized enzymes should be relatively risk-free.

## IMMOBILIZED CELLS

Immobilized microbial cells may be used as biocatalysts (Karube, 1984; Akin, 1987), especially when the reaction entails the use of cofactors (such as NADP or ATP) which are expensive to provide but which can readily be regenerated in cells.

Immobilized organisms may, or may not, remain viable but even where viability is lost, care must be taken to ensure that the organisms do not contaminate the product with toxic metabolites. If non-viable cells are used there does not appear to be any objection to the use of genetically manipulated strains provided that these requirements, of non-viability and of safety in use, are met. If immobilized, but viable, cells are employed, special tests are essential to ensure that the organism used is not pathogenic and will not produce harmful metabolites to contaminate the product or the environment, if live organisms are released. Viable organisms which have been genetically manipulated should be shown not to cause any ill effects if they should contaminate the environment. Complete immobilization of viable organisms

is difficult to ensure because micro-organisms may continue to proliferate to such an extent, even when encapsulated, that the immobilizing agent (an alginate gel for instance) may rupture and so permit escape of the organisms.

## Use of genetically manipulated organisms

### MICRO-ORGANISMS

Bacteria, yeasts and fungi have been employed in foods and drinks for millenia, for the most part to yield secondary metabolites such as flavouring agents (the aromas of butter and cheese), desirable metabolites (ethanol and glutamic acid), preservatives (acetic, lactic and other acids in pickles and sauerkraut) or carbon dioxide produced by a microbial leavening agent. Although the direct contribution of these organisms to the nutrient content of the traditional diets of mankind has been small, indirectly — through fermentation and preservative actions — these agents permit use of much that would otherwise have been wasted. During the last 100 years, dried brewer's yeasts — mainly *Saccharomyces cerevisiae* — have been used in small amounts (up to 5% of the diet) to provide vitamins in animal feeding stuffs. Attempts have been made to use yeasts, notably *Torula edulis*, to provide protein in human foods but these efforts have failed because of the problems of cost and acceptability.

Over the past three decades a great deal of research and development has been applied in efforts to produce what has come to be known as single-cell protein (SCP), first from unicellular organisms (yeasts) but latterly from fungal hyphae as well.

### SINGLE-CELL PROTEIN (SCP)

This term is used to describe the biomass comprising the micro-organism itself. This material may be employed as a protein source, in animal feedstuffs or in human foods, to fill the roles normally played by animal or vegetable proteins, i.e. to contribute functional requirements such as emulsification, provision of texture, mechanical strength, 'mouth feel', viscosity and coagulability, as well as provision of the amino acids essential for adequate nutrition. In these ways SCP products can be used to replace products such as meat, milk, casein, egg white and wheat gluten.

Single-cell protein can be derived from a wide range of organisms cultivated on a variety of different substrates:

1. Bacteria grown on methanol (e.g. *Methylophilus methylotrophus*);
2. Yeasts, such as *Saccharomyces*, grown on substrates such as sugar, starch, whey and molasses;
3. Yeasts, such as *Candida*, grown on non-conventional substrates like *n*-alkanes;

4. Filamentous fungi grown on starchy substrates e.g. from cereals and cassava;
5. Photosynthetic algae such as *Chlorella*, *Scenedesmus* and *Spirulina*.

A very wide variety of micro-organisms grown on many substrates (often on waste products) have been used, or proposed, for SCP production (Ericsson, Ebbinghaus and Lindeblom, 1981). The problems of strain development have been discussed in detail by the latter authors and for 'Pruteen' production, from a bacterium, by Vasey and Powell (1984).

In the USSR and certain East European countries very large quantities of single-cell protein (millions of tonnes/year) are stated to be produced by fermentation of *Candida* sp. on petroleum (*n*-alkane) and, to a limited extent, on sulphite liquors. The product is used as a protein concentrate for animal feeding (Rimmington, 1985). In contrast, the use in feeding stuffs of protein products obtained from *Candida* yeasts cultivated on *n*-alkanes is prohibited in the European Community, under Council decision 85/382/EEC. The prohibition will remain until such time as it is established that these products are not a hazard to human health.

In the United Kingdom a mycoprotein product derived from culture of a fungal species has been approved for general use in foods (Edelman, Fewell and Solomons, 1983; Solomons, 1985). It is used as a food ingredient combined with others to yield products with textures and flavours comparable to those normally associated with animal flesh — in this way it is claimed that vegetarians can enjoy the pleasurable sensation of meat ingestion without the ethical or indeed microbial hazards of zoonoses associated with animal products!

## *Safety Testing of SCP*

The assessment of safety must be considered in relation to the process operator and the product consumer. The former is not the main subject of this review and will be dealt with only briefly.

Single-cell proteins (SCP) are intended to provide a major protein component of the food. As food ingredients, tests are essential to ensure compliance with regulatory needs for foods but other requirements must also be met.

*Pathogenicity* There must be adequate safeguards against carry-over of viable and potentially pathogenic organisms and against the possibility of contamination by other organisms. Viable cells released from the strain used for SCP manufacture and persisting in the product could proliferate in the environment.

Certain organisms are not normally pathogenic but, if present in sufficient numbers, or in susceptible hosts, may be harmful: for instance, some *Candida* yeasts can act in this way as opportunist pathogens (Silvano, 1984). Because it is not possible completely to eliminate contamination of the environment, or indeed of the product, such organisms should be avoided.

Limits have been suggested for pathogenic organisms, e.g. less than one *Salmonella* per 50 g SCP (Truhaut and Ferrando, 1976). Unfortunately, such tests cannot ensure microbiological safety when highly pathogenic organisms are concerned. The statistical probability of analytical tests detecting low-level contamination, say 50 infected particles (colony-forming units, cfu) per tonne of food, would be extremely small, whereas the probability of a person consuming a packet of food containing an infected granule would be extremely high. Microbial safety thus depends on scrupulous management and the application of appropriate microbial control rather than reliance on tests which can be applied to only a minute proportion of the output.

The productive micro-organism should be inactivated in the product before use in foods although complete elimination may not always be necessary or feasible. Plant personnel involved in SCP production may, on occasion, be exposed to viable cells and it is also difficult to prevent contamination of aerial effluent from large fermenters; thus, some environmental contamination may be unavoidable. Frequent monitoring will be required to detect contamination, no matter what precautions may be taken to avoid it.

*Chemical analysis* Typical analyses and data from regular surveys will be needed with regard to the content of nutrients and other major components, e.g. amino acids, nucleic acids, lipids, carbohydrate, fibre, vitamins and other micro-nutrients (PAG/UN Guidelines 1983). As far as possible, constituents that do not occur in normal foodstuffs should not be present. Where these do occur (e.g. branched-chain fatty acids), specific toxicity studies will be required to define permissible concentrations in the product.

### *Toxicology*

*Animal foods* SCP could find wide application in animal feedstuffs, and investigations of safety will be needed in the intended species (e.g. chickens, pigs, calves or fish). It is important to determine the amounts of toxicologically significant substances or metabolites that might be transferred from the SCP into the animal products, meat, fat, milk or eggs, and also any toxicologically significant chemical changes which may be induced in animal products by the use of SCP: for example, high levels of odd-number carbon atom fatty acids residues have been found in tissues of animals given diets based on *Candida* yeasts from *n*-alkane fermentation. Such fatty acids tested in rats have been reported to affect neurobehavioural parameters in offspring (Silvano, 1984).

There are various features which make the process of safety evaluation of single-cell protein and derived produce differ from that applied for the safety assessment of additives to be used in human or animal foods. The whole cell or a crude protein concentrate will normally be used, rather than the pure protein. This leads to several problems which concern (1) known toxicants which might be anticipated in cultures of the specific organism and which can be quantitatively assessed; (2) toxicity testing of various fractions

in attempts to ensure the absence of toxic agents for which no specific tests are available; (3) evaluation of any toxic hazard resulting from the consumption of the product in the way in which it will normally be used.

The testing for known toxicants should present no unusual problems, other than their quantitative estimation and the assessment of tolerable levels if such information is not already available.

The presence of unidentified toxins might be checked by toxicity tests on products derived by a variety of extraction or digestion procedures and in which toxins might be concentrated and thus be more readily detectable (Commoner *et al.*, 1978; Phillips, Kranz and Elias, 1980; Phillips *et al.*, 1980; Stich *et al.*, 1982). Such extracts could then be tested at levels very much higher than would be encountered on consumption of the SCP product. Such tests could lead to the identification and characterization of any toxins to determine the concentrations which could be tolerated in the SCP. However, these tests would be unable, by themselves, to ensure the safety of a product because of the number of fractions needed and the possibility that toxic agents could be lost, or destroyed, during the extraction processes.

Toxicological tests on food additives (which are usually required in very small amounts) can be carried out using concentrations many times greater than those normally employed in the diet. This is not possible in tests on SCP and SCP products which often provide 10–20% of the food. This is because there is a limit to the amount of food which can be ingested by laboratory animals and also because the growth and wellbeing of such test animals is reduced if the amount of any one nutrient consumed is greater, or less, than a certain limited range of concentrations characteristic of that particular nutrient. For some nutrients, e.g. vitamin A, vitamin D and selenium, this range is particularly small, while for major dietary components such as protein, fat and carbohydrate there is also the added difficulty that it is not possible to modify the concentration of one nutrient without affecting the amounts of the others. Toxicological studies should, therefore, be conducted at normal usage levels and concentrate on normal growth and reproductive ability as the main criteria.

Such tests require a detailed knowledge of the availability of nutrients in the test product and of the dietary needs of the test animal in order to avoid differences in behaviour which could arise from insufficient or excessive supplies of essential components in the test diet relative to the control. For instance, Silvano (1984) reported an adverse effect when poultry were fed *Candida* yeasts grown on *n*-alkanes. This resulted from a deficiency of methionine in the yeast protein and disappeared on supplementation of the diet with methionine. SCPs so far investigated contain relatively less methionine and lysine than animal or fish protein (Stringer, 1984). In addition, experimental rats should be tested at the usage level and at some multiple of this, subject to the difficulties of identifying specific adverse effects of high-protein diets, i.e. of diets containing more than the normal 15–20% protein commonly used in conventional diets (Stringer, 1984).

Suitable controls must be included in such studies. Simple adjustment of the control diet by, for instance, addition of casein or soya protein to match

the high protein content of the SCP diets may not suffice. Care should be taken to ensure that the content of amino acids and other nutrients — fat, carbohydrate, fibre and micro-nutrients — in the control approximate to those in the test diet and that all are appropriate for the test species, otherwise differences between the behaviour of the test and control animals may depend more on nutritional than on toxicological effects. The object of these tests is to determine whether overloading the diet with the SCP product induces specific adverse effects, tumour or teratological responses in particular. Conventional 2-year carcinogenicity, multi-generation and teratology studies should detect such responses.

Comparative analyses of excretory products should be undertaken because these may be the only way of detecting unusual constituents of the SCP. Such studies should be particularly concerned with risk assessments related to the usage concentration. If no adverse effects are observed from the studies with laboratory animals, the tested level could be considered as a 'no-effect' level. A safety factor need not be applied to this dose level if chemical analytical studies in all target species have shown the absence of toxicologically significant residues from the SCP. Such findings would indicate that the animal had completely transformed the SCP constituents. The concept of an acceptable daily intake (ADI) or a 'no-effect' level is not readily applicable to SCP. The value of tests with excessive amounts of the test material is to alert the toxicologist to effects that may be detected at lower concentrations if they are specifically investigated. Where the chemical composition data show the presence of residues or unusual substances in, for instance, fibre or cell-wall components, then feeding such SCP fractions or fractions of meat or fat from treated animals may give some indication of their toxicological significance.

*Human foods* When the SCP is to be used for human consumption as well as in animal feedstuffs, it will be necessary to conduct studies in human volunteers, utilizing the animal data as a guide to experimental objectives and design. In particular, these investigations should determine whether unusual constituents are handled in such a different manner by known human metabolic processes that the animal studies are inappropriate for the prediction of toxicity in man.

In addition, studies should be conducted to detect food intolerance or sensitization. There is, as yet, no suitable experimental model for such effects and the investigations must be conducted in human volunteers. If such effects occur, it may be possible to identify a specific component that provokes an immune response and to eliminate it from the product. Important leads may be provided by the immunological assays (*see later*) that will be mandatory for assessing industrial and environmental hazards.

No species exactly resembles another; thus, for the safety testing of drugs and of food additives, it is established practice to carry out tests on at least two species (one of which is not a rodent) to minimize the risk that a toxic hazard for man will be missed because the species chosen may not have been appropriate. When the SCP is intended for human use, tests on two

species would also probably be needed to comply with previously established toxicological practice.

Data on the total amounts and availability (at least for the test species) of essential nutrients in the SCP must be obtained to permit the design and interpretation of much of the safety and toxicological findings. Further information on availability of SCP components will be needed to assess the value of SCP products in the diet of man or animals so that any deficiencies can be rectified. If the food is intended for human use it may be necessary to consider whether the nutrient content is enough for the purposes intended and whether control of nutrient content is required before marketing can be permitted (Committee on the Medical Aspects of Food Policy, 1980). These topics are not discussed in detail here but clearly depend not only on the nutrient content (or lack of it) in the SCP but also on the amount which may be eaten and (because total food consumption will not be increased) the particular dietary items which may be displaced by the new SCP product. Whether or not legal controls, such as addition of nutrients to the food, may be desirable, could depend on estimates of consumption levels by various sectors of the population and whether or not they would significantly, and unfavourably, affect the nutrient status of the vulnerable groups, whether physiological (children and the elderly) or sociological (vegetarians). For instance, if SCP products were significantly to replace meat in the diet, then ferrokinetic and iron availability studies would be required in human subjects and, depending on the results, the addition of a suitable iron source to such products might become a mandatory requirement to safeguard the iron status of the population. In the United Kingdom, the Committee on the Medical Aspects of Food Policy (1980) enunciated that any substance which was to be an alternative to a common food should be the nutritional equivalent of the food it would simulate. The Advisory Committee on Irradiated and Novel Foods (ACINF) (1984) has published guidelines for safety testing of novel foods. These recommendations are summarized in *Table 1*. There are also guidelines from the Food Safety Council Scientific Committee (1978, 1980). Knudsen (1984) reported the main features of safety evaluations for large-scale industrial production of genetically engineered organisms as proposed by the Organization for Economic Co-operation and Development (OECD). These assessments should include evaluation of risks of pathogenicity, toxicity and oncogenicity in man and also tests for genetic stability, genetic transferability, infectivity/toxicity to other organisms and effects on ecosystems in the environment.

Steadman (1984) also suggests that the sequence of human studies (after other toxicology tests, at least analytical, mutagenicity and 90-day feeding trials, have been carried out) should be a preliminary palatability and feasibility type study at a single dose, followed by multiple-dose short-duration investigations and then further controlled studies with about 40 or 50 individuals per group to detect gastrointestinal upsets such as diarrhoea and vomiting. The duration of the study should be a minimum of 4 weeks in a blind crossover design, with standard biochemical, haematological and adverse-reaction monitoring. Nutrient balance and utilization studies would not be

**Table 1.** Safety evaluation of novel foods*

| | | |
|---|---|---|
| 1. | (a) | BACKGROUND INFORMATION<br>The nature of the novel food, its potential market, maximum levels of consumption; animal or population groups involved. |
| | (b) | Source of novel food, production process, presence of natural toxins or anti-nutritional factors such as unusual fatty acids, heavy metals, biotoxins, pathogenic microbes. |
| 2. | | SPECIFICATION OF PRODUCT<br>Provided product clearance is granted for novel food, pilot-scale specification will have exactly to match larger-scale production of food both nutritionally and toxicologically. |
| 3. | (a) | CHEMICAL COMPOSITION<br>If protein, fat or carbohydrate constitutes more than 10% of dry matter of novel food each may need to be investigated: |
| | (i) | Crude protein for true protein and non-protein, nitrogenous material (e.g. nucleic acids) and unusual toxic amino acids. |
| | (ii) | Total fat for saponifiable and non-saponifiable components, phospholipids, sterols, cyclic fatty acids, known toxic fatty acids, saturated, monosaturated and polyunsaturated fatty acids |
| | (iii) | Total carbohydrate for non-metabolizable fraction (fibre and chitin), tannins. Also novel food should be analysed for: |
| | (b) | Toxic metals (lead, arsenic) and nutritionally significant metals (iron, zinc, calcium) after ashing. |
| | (c) | Vitamin content. |
| | (d) | Anti-nutritional factors (phytate, trypsin inhibitors), toxins (haemagglutinins, mycotoxins). |
| 4. | | NUTRITIONAL STUDIES<br>Such studies can forecast impact of novel food on nutritional status of consumers. |
| | (a) | If novel food has a human dietary role, results in animals should be verified in humans. |
| | (b) | Intended uses are needed to judge nutritional consequences. |
| | (c) | If novel food is to replace a traditional food any consequential changes in dietary nutrient will need consideration. |
| | (d) | Replacement to a natural food should be its nutritional equivalent. |
| | (e) | Test diets must be correctly balanced for macro- and micronutrients. |
| | (f) | The influence (beneficial or detrimental) of novel food should be determined in the whole population including children and elderly. |
| | (g) | Effects on nutrients should be assessed after processing, storage and cooking. |
| | (h) | Animal studies may be needed to supplement chemical studies, e.g. when interaction of novel food with rest of diet may reduce nutritional value of whole diet. |
| | (i) | Results of animal studies can be extrapolated to humans by measurement of availability of nutrients to humans. |
| 5. | | TOXICOLOGICAL STUDIES<br>A one hundred-fold safety margin between the novel food level in human diet and the maximum non-toxic level in animals does not apply since it constitutes greater than 1% of the diet. |
| | (a) | *Studies in laboratory animals* |
| | (i) | *Palatability* studies with paired feeding techniques. |
| | (ii) | *Metabolic fate*<br>As foods are complex mixtures, studies on the metabolic fate of each constituent are impracticable. Metabolic fate of minor toxic components are appropriate. |
| | (iii) | *Acute toxicity studies*<br>Novel foods will consist of carbohydrates, lipid and proteins which are unlikely to produce acute toxic effects and such studies are inappropriate. |
| | (iv) | *Sub-acute toxicity tests*<br>These should be performed in a rodent species and one or more other species and started after weaning for at least 90 days. |

(*contd*)

**Table 1** (*contd*)

Novel foods should be fed to animals at two different levels and a control group must be included. Highest level should be in excess of the anticipated exposure level in man (or animals). The other level should be intermediate between high-dose-level and nil-dose-level of control. These high and low levels should determine toxic and non-specific effects.
The novel food should not distort the nutritional status of the test animal. Animals should be observed regularly for behavioural effects and health status (body weight, food and water consumption, haematology, ophthalmic examination, blood chemistry, urine and faeces analysis, hormone levels, vitamin and mineral excretory levels may be required). Organ weights, gross pathology, histopathology should be obtained for animals at the end of the study.

(v) *Long-term toxicity and carcinogenicity tests*
In many cases a long-term study can be omitted if the results of sub-acute toxicity and mutagenicity studies are satisfactory. However, if the process is particularly novel, a long-term study will be needed.
Such studies should be performed over a major part of the animals' lifetime where 50–80% will die from causes other than test material toxicity at study termination. This is about 24 months in rats and 18 in mice and hamsters. Strains should have a low incidence of spontaneous tumours and be susceptible to known carcinogens.
There should be 50 animals of each sex per group. Historical data should not substitute for adequate numbers in the control group.
The novel food should not distort the nutritional status of the test animal.

(vi) *Embryotoxicity*
Standard embryotoxicity and reproduction studies are required. If the process is particularly novel, a multigeneration study may be necessary consisting of a two-generation, two-litter design. Oogenesis, spermatogenesis, and physical and behavioural features of the offspring should be investigated.

(vii) *Mutagenicity tests*
Genetic damage to somatic and germ cells is measured and mutagenicity studies are required whether or not long-term toxicity studies are performed. Four categories of tests are proposed:
— bacterial mutation with and without metabolic activation (S9 mix)*
— *in vitro* cytogenetics ± S9 mix
— mammalian point mutation ± S9 mix or *Drosophila* mutation
— *in vivo* bone marrow cytogenetics or a dominant lethal study.

(b) *Studies in humans*

(i) Before carrying out human studies, a novel food must produce no adverse effects in animals. For very novel foods a minimum of sub-acute feeding studies in two species and relevant mutagenicity tests are required.
Before a novel food is used in a large-scale acceptability and marketing trial, reproductive studies must have been performed in animals. Ethical and legal considerations must be taken into account.
Urine and faecal analysis, clinical, chemical and haematological investigations and renal and hepatic function tests should be performed and other relevant variables monitored.
— Preliminary single-dose studies involving the feeding of a single meal to one volunteer at a time should be followed by:
— Four-week studies with follow-up studies of longer duration to different groups of volunteers with different levels of the novel food relating to anticipated human exposure.
— Concurrent controls should receive a similar diet without the novel food.
— Control and test groups should be of sufficient size for reliable statistical analysis of results and should be matched for age, sex, size, smoking and drinking habits.
— Blind crossover trials are most satisfactory. Sequential periods of novel food incorporation may be used so that a volunteer acts as his own control.

(*contd*)

**Table 1** (*contd*)

| | |
|---|---|
| | — If the novel food is intended for a particular group, e.g. diabetics, it should be tested in that group.<br>— If the novel food produces no allergic reactions it should be fed *ad libitum* and assessed for acceptability. |
| (ii) | *Worker health*<br>The health of workers in contact with the novel food should be monitored because they are in contact with an untested substance and allergic responses may be identified. It should be noted whether manufacturing or laboratory staff are exposed to the novel food in the raw or cooked state. |
| (iii) | *Allergenicity studies*<br>If allergenicity is suspected, types of immunological tests should be established in consultation with relevant specialists and through an allergy clinic — a standardized antigen made from a component of the novel food may be included in the screening profile. |
| (iv) | *Marketing and acceptability trials*<br>One medical practitioner should be given overall responsibility for health monitoring and it would be useful to restrict the trial to a defined geographical region. Adverse reactions not noticed earlier might be determined. |
| (v) | *Special groups*<br>The elderly, children, pregnant women, diabetics, and groups with congenital metabolic defects should be considered and protected by appropriate measures such as labelling. Those groups taking commonly used drugs should also be monitored for adverse effects. |

* After guidelines for testing by the Advisory Committee on Irradiated and Novel Foods, Memorandum December, 1984.
† *See* Maron and Ames (1983).
Details of tests can be found in DHSS (1981, 1982a,b).

included in a toxicological analysis but would be an essential part of the nutritional evaluations. There would be little place for pharmocokinetic and metabolic studies as part of the toxicological investigations unless adverse effects could be associated with a particular component. In such an event, further studies on that component would be required and these could involve investigation of biochemical mechanisms.

Nucleic acids are well recognized as constituents that are present in rapidly growing cells of both prokaryotes and eukaryotes, often reaching concentrations of up to 15% of dry solids content of microbial and other cell cultures (Stringer, 1984). Man, in distinction to other species, is defective in metabolizing the uric acid that occurs as a major metabolite of nucleic acids and an excess intake is associated with gout. It has been advised that the nucleic acid content of SCP should be reduced below 2% to avoid this syndrome, and methods are now available to achieve this.

Diamino acids are also present in bacteria as structural constituents of the cell wall. Studies carried out, mainly in chickens, show that large amounts of diamino acids may be consumed without any adverse effect (Stringer, 1984).

## TOXINS

It is now well established that under certain conditions of culture and storage, various bacteria and fungi can produce proteins and secondary metabolites

that can have very serious adverse biological effects at very small dosage. There is now a substantial literature on the mycotoxins, which can induce disease in man and animals and many of which appear capable of surviving the various forms of food processing to exert their effects (*see* reviews by Austwick (1984), Butler (1984), Moss (1984), Neale (1984), Smith (1984) and Watson (1984) presented at a symposium on mycotoxins). It is thus mandatory that the products of biotechnical processes do not include potential toxins of this nature. As is so often the case in toxicology, the problem is one of detecting the presence of potent toxins at very small concentrations when not knowing what biological activity they might exhibit.

Knowledge of metabolites produced by parent and other species related to the production strain, coupled with toxicological tests on extracts of the SCP, may offer the best means of determining whether potent mycotoxins are likely to be a cause for concern.

Further research may be required and recent proposals in this area warrant serious consideration (*Table 2* and Heathcote (1984)).

## IMMUNOLOGY

Given that the human immune system appears to be more highly developed than that of some other species, it is conceivable that conventional test systems involving experimental animals may not be able to detect materials capable of sensitizing man or of immunosuppression in man. Tests of allergic potential are likely to be required for industrial and environmental toxicity and these may suffice to safeguard the consumer in respect of the products of large-scale manufacture. Where a product to be used in small amounts is intended for direct human consumption, however, it may be advisable to ensure the absence of excess sensitizing potential by using human volunteers. Information on health of production staff may give an indication of allergic responses, although exposure in the case of process workers is more likely to be by inhalation and dermally rather than by ingestion.

Stringer (1984) reported a human trial where individuals were fed SCP-constituted soups versus soya-constituted soups and monitored for nausea, vomiting and diarrhoea. There was no difference between the two groups. Furthermore, a double-blind trial of SCP-constituted and soya-constituted 'ginger cookies' indicated certain problems which may be met in attempting double-blind nutritional trials in man because some participants did not like eating excessive numbers of biscuits and some did not like the taste of ginger!

There is a need for an *in vitro* system to determine allergenic potential. Such a system might conceivably be based on the ability of human macrophages to take up and process the material so that it becomes antigenic to human lymphocytes, but no such test is yet available. Steadman (1984) proposes that one way to obtain information of the frequency of induced allergy to SCP would be to prepare standardized antigens from SCPs which could be used as part of a standard batch of antigens in allergy clinics which are investigating allergic responses in the general population. Volunteer studies will be necessary until suitable *in vitro* methods are developed.

**Table 2.** A proposal to develop a general non-specific screening test for the presence of toxins*

| 1<br>Organisms† | 2<br>Criteria of effect | 3<br>Basis of evaluation | 4<br>Further studies to include novel foods |
|---|---|---|---|
| Bacteria‡<br>*Bacillus megaterium*<br>*Bacillus stearothermophilus*<br>*Escherichia coli* — repair proficient and deficient strains to give an indication of genotoxic potential | As appropriate to the various organisms the methods will include:<br>— failure of multiplication<br>— death<br>— failure of development<br>— exclusion of vital dyes<br>— leakage of cytoplasmic enzymes<br>— morphology | Dose response studies will be carried out with the various organisms using a range of toxins of microbial origin These will include:<br>— aflatoxin $B_1$<br>— ochratoxin A<br>— Fusarium-derived toxins<br>— Zearalenone (a mycotoxin)<br>— a staphylocal exotoxin<br>— a bacterial endotoxin | Dose-response studies with extraction, concentration and digestion procedures of enzyme products or other food bases. |
| Mammalian cells<br>Cell Lines‡<br>Baby hamster kidney (BHK) cells<br>Chinese hamster ovary cells<br>Primary Culture<br>Rat hepatocytes | | | |
| Protozoa‡<br>*Tetrahymena pyriformis*<br>*Paramecium* species | | | |
| Crustacea<br>Brine shrimp | | | |
| Plants<br>Germinating pea seeds | | | |

1, 2 and 3 will be considered in the first instance. If the evaluations in 3 are satisfactory, the testing of novel foods for toxins in 4 might prove suitable.

* In the report of the Food Additives and Contaminants Committee (FACC) 1982 on the Review of Enzyme Preparations, a need was identified for 'Rapid non-specific biological and/or chemical screening tests capable of detecting a wide range of toxins' (Appendix IV para. 12).

† Organisms chosen are sensitive to at least one microbial toxin.

‡ A metabolizing system will also be incorporated (*see* Maron and Ames, 1983).

Many assessments will be essential before a novel food product can be released to the general market. The evaluation appropriate for a particular novel food will depend on its source, composition, processing and proposed level in the diet. Thus it will be the responsibility of the particular organization, intending to make the novel food, in light of its experience and

knowledge of the product, to make the initial assessment as to which tests may be needed (Advisory Committee on Irradiated and Novel Foods, 1984).

### MANUFACTURING RISKS

The production of large volumes (in excess of 20 litres) of genetically manipulated or natural organisms or their products can present hazards to the health of workers, similar to those from laboratory experiments. However, according to Collins (1984), larger-scale production is probably safer because hazards associated with the organism, its mutants and products, will have been assessed and controlled or eliminated during research and development before full-scale production, or the organism may have already been used in an industrial process without producing any ill effects (even quite large volumes of hazardous organisms can be grown safely (Harris-Smith and Evans, 1968)). The experience gained by chemical, mechanical and hydraulic engineers in fermentation and similar industries has facilitated prevention of contact between processors and product and provision of environmental protection. Safety measures in downstream processing have been described by Turner (1982). The possible effects, on plant and animal life, of effluents discharged into rivers or the sea, and of bacterial masses disposed of on land, could adversely affect the environment because other undesirable organisms may flourish on their living or dead cells and their products. Such hazards are carefully monitored by such bodies as trade unions and conservationists, and the health and safety authorities.

### MICROBIAL INFECTION OF NOVEL FOODS

The problem of food, novel or otherwise, which has been infected by bacteria is an everyday concern. The food industry is well aware of the dangers of contamination and the need for its control, which will be as important for SCP as for any other food component; however, the dangers should be reduced in that successful SCP production depends on strict control of microbial growth, so that risk of contamination of the product will be minimized and, indeed, should be far less than that incurred when dealing with animal carcasses.

## Possible applications of genetically manipulated higher plant and animal cultivars intended for food

### PLANTS

It is now possible to transfer DNA into the nuclear (Holsters *et al.*, 1982; Leemans *et al.*, 1982; Chilton, 1983; Watson, 1984) and chloroplast (de Block, Schell and Van Montagu, 1985) genomes of certain higher plants, as well as into cytoplasmic particles. Further progress is hindered by (1) lack of suitable vector systems, especially for major monocotyledonous crop plants; (2) insufficient information on means of ensuring appropriate expression of transferred genes; (3) difficulty in producing protoplasts of

certain species and regenerating plants from them; (4) paucity of information on the molecules affecting many features (growth, maturation, product quality, hardiness, heat resistance and so forth) which might be advantageously modified by genetic manipulation. No legal requirements (other than those applying to all foods) have yet been promulgated concerning the use of genetically manipulated crop plants (or animals). The provisions of the UK Food Act (1984) may well suffice to ensure that safety is established before any novel food, no matter how it is derived, may be offered for sale.

Genetic manipulation of a food-plant genome may be no more (and perhaps much less) likely to produce a novel weed or poisonous cultivar accidentally than are traditional mutation and breeding/selection procedures. Nevertheless, there is rapid progress in the genetic manipulation of plants (Nijkamp, 1986). Some dangers are apparent and some form of control may be advisable to allay the public concern at growth, on the large scale, of plants and animals derived by hitherto untested procedures.

Certain potential hazards, at least to the environment, can be readily foreseen: for example, several laboratories are using genetic manipulation to develop plants resistant to herbicides, by inducing formation in the crop plant of key enzymes resistant to the action of the herbicide (Comai *et al.*, 1985). Crops made resistant in this way to a broad-spectrum herbicide such as glyphosate could be grown free from all weeds in fields treated with this non-persistent herbicide. However, the use of such genetically manipulated plants could involve certain dangers: if the crop were made resistant to persistent herbicides, the increased use of such agents would be encouraged, with possible risks to the environment, while unplanned transfer of resistance from the crop plant to related species might lead to novel strains of crop or weed resistant to the herbicide and thus difficult to eradicate. The same problems could also arise if the resistance were developed in other ways, e.g. by permitting production, in the crop, of enzymes to destroy the relevant herbicide.

Resistance to insect pests may also be a possibility, for instance by transfer and expression of genes coding for *Bacillus thuringiensis* toxins (Dean, 1984) or similar agents known to have little effect on stock animals or man but lethal on ingestion by insects. However, although these substances are harmless to normal persons, they may be toxic to man and higher animals under certain physiological conditions; it would be most irresponsible, therefore, to permit the presence of any such agents in foods until their mode of action, absorption, excretion and metabolism had been thoroughly investigated.

Viral, fungal and bacterial infections might also possibly be controlled by gene transfer to permit production of inhibitory agents. Viral infections, for instance, might be controlled by introducing genes for viral coat proteins into the plant genome, thereby protecting the plant against naked viral DNA, as shown for tobacco mosaic virus by Abell *et al.* (1986).

In many plants, mechanical injury or microbial invasion induces defensive reactions such as the formation of protective agents — the phytoalexins, for instance. It is conceivable that genetic manipulation could endow food plants with similar defensive mechanisms dependent on production of novel and

powerful antimicrobial or insecticidal agents. The safety of these crops could be assured only after the determination of safe levels of consumption of these induced substances, by methods such as those applied to food additives. The safety of the food could then be assessed from this information, coupled with data on the concentrations to be found in the crop and probable consumption levels in the diet.

Breeding for disease resistance, even by traditional methods, has led to toxicity in the crop, e.g. in potato (*Solanum tuberosum*) cultivars (Maga, 1980; Curtis, 1986). Thus some assurance of safety will almost certainly be a requirement for novel crops made pest resistant by genetic manipulation.

There is a possibility, not yet commercially realized, that plants could be manipulated to produce valuable but potentially toxic substances. These could be primary gene products (antigens, peptide hormones, or enzymes) or even secondary metabolites (antibiotics, steroid hormones and other pharmaceutically active agents). Crop plants would be especially attractive for such purposes because they are already adapted for farm use and much is known of their agronomic and genetic characteristics. The development of such modified cultivars would demand investigations to ensure safety during their growth, propagation, harvesting and disposal. Care would be essential to assess and to control any danger there might be of these plants contaminating food crops, either as weeds or through uncontrolled cross-breeding. In almost all instances, the novel agent induced by genetic manipulation will be a known substance. Safety assessments, in these circumstances, will thus depend on the toxicity of the agent and its concentration in the food.

## ANIMALS

Genetic manipulation is already possible in higher vertebrates either by direct microinjection of DNA into fertilized eggs or by the use of retroviruses (Miller *et al.*, 1984). Such introduced genes can be expressed and are heritable. The application of genetic manipulation to farm food animals is unlikely to be as rapid as applications in plants for several reasons:

1. It is not yet possible easily to clone or to multiply vegetatively higher vertebrates, although it is already possible to divide early 8–16-cell embryos and to derive viable embryos from the separated cells;
2. With the exception of certain fish it is not possible to multiply stocks quickly because generation times are long — about 12 months — and numbers of offspring are small (even poultry cannot provide more than 200–300 offspring in a year);
3. The ova are difficult to handle and embryos of many species cannot yet be brought to independent existence by *in vitro* methods.

It is not possible to predict the rate or type of exploitation which may occur but the possibilities are being investigated intensively (e.g. AFRC, 1986; Nijkamp, 1986) so that controls will be needed. Because of the similarity of metabolism of man and higher animals, toxicity of meat (muscle) may not

easily be induced in farm stock, although variations (possibly harmful) in the hormone content of certain edible organs (such as thyroid, liver and kidney) might well be induced. Of special concern could be genetic modifications, already being investigated (Nijkamp, 1986) of animals specifically designed to express high concentrations of pharmacologically active agents (such as interferon, somatomedin and gonadotrophins) in edible animal products such as milk or eggs. The safety or otherwise of by-products and carcasses would depend on the oral toxicity of the agent and its activity and concentration in the food as consumed.

## Control of genetically manipulated plants and animals

### CONTROL OF CULTIVARS INTENDED FOR FOOD

Appraisal of new cultivars and novel food plants may now be needed to safeguard the consumer (the safety of the husbandman, grower, breeder and processor is not considered here). Numerous cultivars of our food plants and animals have been developed over many years to provide strains which, almost without exception, have yielded wholesome and nutritious foods when prepared in the customary ways. Many of these foods (beans, potatoes, wheat and yams) contain substances which are highly toxic but not normally present in sufficient quantity to cause detectable adverse effects in the consumer. There is now the possibility that new, more powerful genetic procedures may increase the concentration of toxic agents in our food or may induce the production of novel poisonous agents. It is for such reasons that many consider it advisable to carry out appropriate tests on new breeds of food plants and animals before granting permission for their general use and consumption.

### CULTIVARS PRODUCED BY TRADITIONAL BREEDING METHODS

Traditionally, modification of organisms by genetic means has depended on selection of recombinants with desirable phenotypes by recombination and/or mutagenesis of pre-existing genes within a pool confined to those organisms capable of sexual interaction to produce recombinants. Because of the limited size of the gene pool there is a very low probability that adverse characteristics (other than those present in the species contributing to the gene pool) will be demonstrable in cultivars developed from such traditional breeding projects. Thus it is not considered necessary to test novel cultivars for toxicants other than those produced by the parent species, e.g. solanine alkaloids in *Solanum tuberosum* cultivars or glucosinolates and goitrogens in cultivars derived from *Brassica* species.

### CULTIVARS DERIVED BY GENETIC MANIPULATION

Genetic manipulation now permits transfer of DNA, no matter what its origin—natural or synthetic—into the genomes of plants and animals. Thus

the opportunity to transfer both harmful and beneficial activities is now greatly increased.

*Tissue culture procedures*

Various techniques may be applied to plant tissue cultures to modify the DNA complement and then to regenerate plants from the resultant cultures. In this way DNA in viroids or plasmids may be eliminated from the cytoplasm. Cell ploidy and chromosomal characteristics may be manipulated and chimaeras can also be constructed in which the several cell layers of the plant and the organs formed from them may be of different genetic constitutions. All these different processes ultimately produce variation by deletion, modification or recombination of pre-existing DNA. There will thus be little probability of formation of toxicants qualitatively different from those produced by the parent plant or in closely related species. Hence, the safety of new varieties derived in these ways may depend only on the concentration, in the edible moiety, of toxic substances identical with, or closely related to, those formed in the parent species.

*Unselective ('shot gun') DNA transfer*

Techniques are now available (protoplast fusion, electroporation, plasmid and cosmid vectors and a variety of 'shot gun' techniques) whereby unselected genes in chromosomes, or DNA in chromosomal fragments or in plasmids, may be incorporated into the genome to provide a much wider range of recombinants than is possible by traditional breeding procedures. These novel methods enormously increase the possible number of both desirable and undesirable phenotypes. The potential for variation in the progeny strains may be much greater than in traditional breeding but there will still only be a low probability of formation of primary or secondary gene products other than those capable of synthesis by paths available to one or other of the donor or recipient organisms. However, much less may be known of the synthetic potential of the donor organism than of the recipient food plant so that there may be a much higher probability of the appearance of a novel or unanticipated toxicant in cultivars developed by these means than in those created by traditional breeding techniques. As a result of these considerations it would appear advisable that release of such cultivars should not be permitted before detailed investigation for possible toxic components. The type of examination proposed by the Advisory Committee on Irradiated and Novel Foods (1984) would seem appropriate, together with analyses for any toxicants of types produced by the parent strains.

*Selective DNA transfer*

Particular genes can be deleted or inactivated and purified specific genes, either from other organisms or derived by synthesis, together with suitable promoters and terminator sequences, may be introduced by various means

into the plant or animal genome. Provided that insertions are made at redundant loci they will be most unlikely to facilitate production of primary or secondary metabolites other than those which can be produced in the parent organism and modified by the introduced gene. There would thus appear to be little reason to test such manipulated cultivars, except for potentially toxic substances known to be capable of synthesis by the parental and closely related species.

Genes to confer resistance to pests and herbicides will probably be introduced into food organisms by specific gene transfer. Toxicological investigations such as those applied to food additives could establish whether these agents, at the concentrations found in the crop, would be likely to be harmful to the consumer (man or animal) but further considerations — on persistence and environmental impact for instance — might well be mandatory before general release of such cultivars.

## Possible means of controlling genetically manipulated food plants and animals intended to serve uses other than food

The modification of food plants and animals to produce valuable proteins and powerful pharmacological agents is now a distinct possibility and could lead to serious control problems. These genetically manipulated strains might (1) accidentally be used as food, or (2) the productive strains might interbreed with cultivars used for food. This could lead to the proliferation of dangerous or undesirable hybrids difficult to distinguish from the wholesome types. Some control would thus appear to be essential before commercial production of such strains could be tolerated.

This problem may not be restricted to cultivars derived by genetic manipulation. Some food plants, certain yams (*Dioscorea* sp.) for instance, produce valuable, but toxic, steroid substances in concentrations normally too low to produce adverse effects in the consumer. If the concentration of these substances were to be increased by any means (genetic or otherwise), there would be a risk that such poisonous yams would be eaten, with dire consequences.

Several methods of control could be employed:

1. *Total prohibition of the use of food plants or animals for non-food uses.* Edicts of this type could cause serious problems because little is known of the genetics and husbandry of all but a few inedible plants (such as cotton (*Gossypium* spp.), tobacco (*Nicotiana tabacum*) and *Petunia*) and animals. Some plants grown as ornamentals, e.g. varieties of *Narcissus*, *Dahlia* and *Tulipa* sp., might usefully be employed in this way but (except for the rat, mouse, fruit fly (*Drosophila* sp.) and the silk moth (*Bombyx mori*)), little is known of the genetics of inedible animals. However, the use of inedible species should be encouraged — at least until more experience is gained.
2. *Quarantine.* Isolation would be possible (although expensive and difficult) for farm animals, e.g. cows producing specific antigens or other

peptides in their milk, but there would be severe problems in preventing wind or insect pollination of food plants outside the quarantine area, or the accidental transfer of vegetative propagules or seed in soil from the quarantine sites.

3. *Use of infertile strains (sterility).* There is a possibility that plants or animals displaying the desired synthetic abilities could also be made genetically sterile. Synthesis of the desired product could then be realized in toxic quantities, only in the sterile $F_1$ hybrid progeny derived from a limited number of parents (which could be quarantined if necessary). This method of control would prevent the accidental dissemination of potentially harmful characteristics to other strains of the species but could not eliminate the risk of accidental entry of the crop, or its products, into the food chain or being propagated, accidentally or otherwise, by vegetative or cloning procedures.
4. *Use of genetic markers other than sterility.* Linkages between the productive gene complex and other genes controlling features such as distinctive morphology, colour, smell or taste of the productive organism or of the product (seed, root, milk or eggs) might provide effective ways of rapidly identifying the potentially harmful cultivars and their progeny.

The precise procedure used to control the plants and animals will depend on assessments of the nature of any hazard and the benefits which may accrue from the use of the manipulated species for any purpose.

## Conclusions

Many substances traditionally used in foods are manufactured on a commerical scale by physicochemical procedures or by biotechnology using viable organisms or catalytic systems derived from them, while the biomass from some micro-organisms is already used as food for man and animals. The techniques of genetic manipulation permit the introduction and expression of genes, not only in microbes but also in higher plants and animals, including vertebrates.

Undoubtedly there will be continuing research to identify, isolate and synthesize genes controlling desired characteristics. As these genes are recognized, so efforts will be made to insert them into the genome and to ensure their expression to best advantage. In this way biotechnology holds great promise for the improvement of cultural and agronomic features, of 'quality', of nutritional value and of other attributes of traditional foods and food components.

The introduction of complex mixtures of DNA from a vast range of sources may now be achieved by a number of 'shot gun' procedures to form novel variant strains unobtainable by other means. Among the progeny there may be some with desirable features but others may acquire the ability to show unsuspected harmful characteristics.

Genetic manipulation also offers the possibility of direct introduction of precisely defined synthetic or highly purified DNA into the genome. One

would expect that strains derived in this way would be less likely to display unforeseen harmful activity than might other strains, even those derived by traditional recombination and mutation selection methods.

The consequences of genomic modification cannot yet be precisely foretold and thus safety evaluation should be applied before novel strains, or their products, are permitted for general use in foods. The assessment of strains and products from less specific ways of modification should be more rigorous than those demanded of strains derived by specific gene transfer or modification.

Controls should not be so strict that they inhibit development of novelties, but general applications should not be permissible without a sufficient assurance of safety.

Direct legislation defining methods of production and safety tests would be difficult to devise because the technology of strain production and safety test methodology may develop in unpredictable ways. However, these problems may be met by an overall requirement (as in almost all countries) that nothing may be offered for sale as food, or a food component, that has not been shown to be, or is not known to be, safe in use. To this could be added a further requirement, that no novel food, food constituent or food process should be employed, or offered for sale, until shown to be safe. In the United Kingdom two bodies have been appointed to determine the safety of new foods and food processes: the Advisory Committee on Irradiated and Novel Foods (1984) has the task of assessing the safety of novel foods and food processes, while the Committee on the Medical Aspects of Food Policy (1980) is concerned, *inter alia*, with the safety of substances used in food processing and possible effects on health of the introduction of novel foods and processes. These Committees assess the available evidence and may suggest further investigations relating to safety assessment of the process or products under consideration. The members are appointed by the Ministries responsible for food and health, and comprise independent experts in toxicology; representatives from industry and other groups concerned may present evidence. These Committees have the power to recommend modifications to the safety test procedures in accord with scientific developments. This system has advantages in that it is capable of rapid response to changing circumstances and research findings, while directly consulting those concerned.

In other countries with different legal and executive systems, other types of control might be more appropriate, but whatever the procedures employed, they should be capable of responding to scientific advances, to consumer needs and to any international agreements on food safety.

**Acknowledgement**

While taking full responsibility for the views expressed in this survey we wish to express our gratitude for the generous help and critical comment provided by Dr K.J. Dale, Dr H.J. Dunn, Dr D.G. Lindsay and Dr M.E. Knowles (of the Ministry of Agriculture, Fisheries and Food), by Dr H.J. Steadman (Department of Health and Social Security), and Professor D.M.

Conning (British Nutrition Foundation) for his advice in the early stages of this work.

## References


ABELL, P.O., NELSON, R.S., DE.B., HOFFMANN, N., ROGERS, S.G., FRALEY, R.T. AND BEACHEY, R.T. (1986). Delay of disease development in transgenic plants that express the Tobacco Mosaic Virus Protein. *Science* **232**, 738–743.

ADVISORY COMMITTEE ON IRRADIATED AND NOVEL FOODS (1984). *Memorandum on the Testing of Novel Foods Incorporating Guidelines for Testing*. MAFF, London.

AGRICULTURAL AND FOOD RESEARCH COUNCIL (1985–86). *Annual Report*. AFRC, London.

AKIN, C. (1987). Biocatalysis with immobilized cells. In *Biotechnology and Genetic Engineering Reviews* (G.E. Russell, Ed.), volume 5, pp. 319–367. Intercept, Wimborne, Dorset.

AMES, B.N. (1983). Dietary carcinogens and anticarcinogens. *Science* **221**, 1256–1264.

ASSOCIATION OF MICROBIAL FOOD ENZYME PRODUCERS (1980). *General Standards for Food Enzyme Regulation*. Brussels.

AUSTWICK, P.K.C. (1984). Human mycotoxicosis — past, present and future. *Chemistry and Industry*, 547–550.

BENDER, A. E. (1987). Effects on nutritional balance: micronutrients. In *Natural Toxicants in Foods. Progress and Prospects* (D.H. Watson, Ed.), pp. 110–124. Ellis Horwood, Chichester.

BLOCK, M. DE., SCHELL, J. AND VAN MONTAGU, M. (1985). Chloroplast transformation by *Agrobacterium tumefaciens*. *EMBO Journal* **4**, 1367–1372.

BLUMENTHAL, H. (1984). Overview of the work of the FDA in the area of biotechnology. In *Proceedings of the European Meeting of the Toxicology Forum, Geneva, Switzerland, 18–20 Sept.* pp. 411–412. Toxicology Forum, Geneva.

BUREAU OF FOODS, FOOD AND DRUG ADMINISTRATION (1982). *Insights on Food Safety Evaluation. Department of Health and Human Services Contract Number FDA 223–81–2394*. Life Sciences Research Office, Washington.

BUTLER, W.H. (1984). Pathological aspects of mycotoxicoses. *Chemistry and Industry*, 541–544.

CHILTON, M.D. (1983). A vector for introducing new genes into plants. *Scientific American* **248**, 36–45.

COLLINS, C.H. (1984). Safety in microbiology: a review. In *Biotechnology and Genetic Engineering Reviews* (G.E. Russell, Ed.), volume 1, pp. 141–165. Intercept, Ponteland, Newcastle upon Tyne.

COMAI, L., FACCIOTTI, D., HIATT, W.R., THOMPSON, G. AND STALKER, D.M. (1985). Expression in plants of a mutant *aro*A gene from *Salmonella typhimurium* confers tolerance to glyphosate. *Nature* **317**, 741–744.

COMMITTEE ON THE MEDICAL ASPECTS OF FOOD POLICY (1980). *Foods which Simulate Meat. The Nutritional Aspects of Vegetable Protein Foods which are Meat Analogues*. HMSO, London.

COMMONER, B., VITHAYATHIL, A.J., DOLARA, P., NAIR, S., MADAYASTHA, P. AND CUCA, G.C. (1978). Formation of mutagens in beef and beef extract during cooking. *Science* **201**, 913.

CURTIS, R.F. (1986). Food science, food quality and food standards. *Institute of Food Science and Technology Proceedings* **19**, 151–164.

DEAN, D.H. (1984). Biochemical genetics of the bacterial insect-control agent *Bacillus thuringiensis*: basic principles and prospects for genetic engineering. In *Biotechnology and Genetic Engineering Reviews* (G.E. Russell, Ed.), volume 2, pp. 341–363. Intercept, Ponteland, Newcastle upon Tyne.

DHSS (1981). *Guidelines for the Testing of Chemicals for Mutagenicity* HMSO, London.

DHSS (1982a). *Guidelines for the Testing of Chemicals for Carcinogenicity*. HMSO, London.

DHSS (1982b). *Guidelines for the Testing of Chemicals for Toxicity*. HMSO, London.

EDELMAN, J.A., FEWELL, C.H.A. AND SOLOMONS, G.L. (1983). Myco-protein : a new food. *Nutrition Abstracts and Reviews. Reviews in Clinical Nutrition*, volume 53, pp. 471–480. Commonwealth Agricultural Bureaux, Slough.

EDGE, M.D. AND CAMBLE, R. (1984). Interferon synthesis by micro-organisms. In *Biotechnology and Genetic Engineering Reviews* (G.E. Russell, Ed), volume 2, pp. 215–252. Intercept, Ponteland, Newcastle upon Tyne.

EFIMOV, V.A., CHAKHMAKHCHEVA, O.G. AND OVCHINNIKOV, YU.A. (1986). Synthetic DNA fragments as useful tools in genetic and protein engineering. In *Biotechnology and Genetic Engineering Reviews* (G.E. Russell, Ed.), volume 4, pp. 79–116. Intercept, Ponteland, Newcastle upon Tyne.

ERICSSON, M., EBBINGHAUS, L. AND LINDEBLOM, M. (1981). Single cell protein from methanol: Economic aspects of the Norprotein process. *Journal of Chemical Technology and Biotechnology* **31**, 33–43.

FOOD ACT (1984). Chapter 30. HMSO, London.

FOOD ADDITIVES AND CONTAMINANTS COMMITTEE (1982). *Review of Remaining Classes of Food Additives used as Ingredients in Food. Report on the Review of Enzyme Preparations. FAC/REP/35*. HMSO, London.

FOOD SAFETY COUNCIL SCIENTIFIC COMMITTEE (1978). Proposed system for food safety assessment. *Food and Cosmetics Toxicology* **16**, Suppl. 2, pp. xi, 136.

FOOD SAFETY COUNCIL SCIENTIFIC COMMITTEE (1980). *Proposed System for Food Safety Assessment. Final Report*. Food Safety Council, Washington. 160 pp.

HARRIS-SMITH, R. AND EVANS, C.G.T. (1968). The Porton mobile enclosed chemostat (POMEC). In *Continuous Cultivation of Microorganisms. Proceedings of the 4th Symposium held in Prague, 17–21 June 1968* (I. Malek *et al.*, Eds), pp. 391–410. Academia, Prague

HEANEY, R.K. AND FENWICK, G.R. (1987). Identifying toxins and their effects: glucosinolates. In *Natural Toxicants in Foods. Progress and Prospects* (D.H. Watson, Ed.), pp. 76–109. Ellis Horwood, Chichester.

HEATHCOTE, J.G. (1984). Introduction and review of the development of research activity into mycotoxins. *Chemistry and Industry*, 530–532.

HOLSTERS, M., HERNALSTEENS, J.P., VAN MONTAGU, M. AND SCHELL, J. (1982). Ti plasmids of *Agrobacterium tumefaciens*: The nature of the TiP. In *Molecular Biology of Plant Tumours* (G. Kahl and J. Schell, Eds), pp. 269–298. Academic Press, New York.

KAHN, J.S. AND GIBBS, J.N. (1985). Food and Drug Administration Regulations of Medical Devices. *Biotechnology and Food and Additive Biotechnology* **11**, 507–516.

KARUBE, I. (1984). Possible developments in microbial and other sensors for fermentation control. In *Biotechnology and Genetic Engineering Reviews* (G.E. Russell, Ed), volume 2, pp. 313–339. Intercept, Ponteland, Newcastle upon Tyne.

KNUDSEN, I.B. (1984). Potential food safety problems in genetic engineering. In *Proceedings of the European Meeting of the Toxicology Forum, Geneva, Switzerland, 11–22, Sept.* pp. 424–427. Toxicology Forum, Geneva.

LEEMANS, J., GREVE, H. DE, HERNALSTEENS, J.P., SHAW, C., WILMITZER, L., OTTEN, L., VAN MONTAGU, M. AND SCHELL, J. (1982). Ti plasmids and directed genetic engineering. In *Molecular Biology of Plant Tumours* (G. Kahl and J. Schell, Eds), pp. 537–545. Academic Press, New York.

MAGA, J.A. (1980). Potato (*Solanum tuberosum*) glyco-alkaloids. *CRC Critical Reviews in Food Science and Nutrition*. **12**, 371–405.

MARON, D.M. AND AMES, B.N. (1983). Revised methods for the *Salmonella* mutagenicity tests. *Mutation Research* **113**, 173–215.

MILLER, A.D., ONG, E.S., ROSENDFELD, M.G., VERMA, I.M. AND EVANS, R.M. (1984). Infectious and selectable retrovirus containing an inducible rat growth hormone minigene. *Science* **225**, 993–998.

MOSS, M.O. (1984). Conditions and factors influencing mycotoxin formation in the field and during storage of food. *Chemistry and Industry*, 533–536.

NEALE, G.E. (1984). Involvement of mycotoxins in acute and chronic animal disease — metabolic aspects. *Chemistry and Industry*, pp. 542–546.

NIJKAMP, H.J.J. (1986a). Genetic engineering of virus and cellular genes important in animal husbandry. *BTF-Biotech-Forum* **3**, 115–116.

NIJKAMP, H.J.J. (1986b). Genetic engineering of plants and microorganisms important for agriculture. *BTF-Biotech-Forum* **3**, 116–117.

PETRICIANNI, J. (1984). FDA views on safety issues. In *Proceedings of the European Meeting of the Toxicology Forum, Geneva, Switzerland, 18–22 Sept.*, pp. 413–418. Toxicology Forum, Geneva.

PHILLIPS, B.J., KRANZ, E. AND ELIAS, P.S. (1980). An investigation of the genetic toxicology of irradiated foodstuffs using short-term test systems. II. Sister chromatid exchange and mutation assays in cultured Chinese hamster ovary cells. *Food and Cosmetics Toxicology* **18**, 471–475.

PHILLIPS, B.J., KRANZ, E., ELIAS, P.S. AND MUNZER, R. (1980). An investigation of the genetic toxicology of irradiated foodstuffs using short-term test systems. I. Digestion *in vitro* and the testing of digests in the *Salmonella typhimurium* reverse mutation test. *Food and Cosmetics Toxicology* **18**, 371–375.

POULSEN, P.B. (1984). Current applications of immobilized enzymes for manufacturing purposes. *Biotechnology and Genetic Engineering Reviews* (G.E. Russell, Ed), volume 1, pp. 121–140. Intercept, Ponteland, Newcastle upon Tyne.

POWELL, L.W. (1984). Developments in immobilized-enzyme technology. In *Biotechnology and Genetic Engineering Reviews* (G.E. Russell, Ed.), volume 2, pp. 409–438. Intercept, Ponteland, Newcastle upon Tyne.

PROTEIN ADVISORY GROUP OF THE UNITED NATIONS (1983). Nutrition and Safety Aspects of Protein Sources for Animal feeding. PAG/UN N15. *Food Nutrition Bulletin* **5**, 67–70.

RIMMINGTON, A. (1985). Single cell protein : the Soviet revolution. *New Scientist* **1462**, 12–15.

SILVANO, V. (1984). Single cell proteins as animal feeds : criteria for safety assessment. In *Proceedings of the European Meeting of the Toxicology Forum, Geneva, Switzerland, 18–22 Sept.*, pp. 355–361. Toxicology Forum, Geneva.

SMITH, J.E. (1984). Summary and thoughts on possible direction of future research effort. *Chemistry and Industry*, 551–553.

SOLOMONS, G.L. (1985). Production of biomass by filamentous fungi. In *Comprehensive Biotechnology* (C.L. Cooney and A.E. Humphrey, Eds.), volume 3, pp. 483–505. Pergamon Press, Oxford.

STEADMAN, J. (1984). Government viewpoint on single cell proteins. In *Proceedings of the European Meeting of the Toxicology Forum, Geneva, Switzerland, 18–22 Sept.*, pp. 362–366. Toxicology Forum, Geneva.

STICH, H.F., ROSIN, M.P., WU, C.H. AND POWRIE, W.D. (1982). The use of mutagenicity testing to evalate food products. In *Mutagenicity — New Horizons in Genetic Toxicology* (J.A. Heddle, Ed.), p. 177–142. Academic Press, New York.

STRINGER, D. (1984). Industry viewpoint — illustrated by 'Pruteen'. In *Proceedings of the European Meeting of the Toxicology Forum, Geneva, Switzerland, 18–22 Sept.*, pp. 366–374. Toxicology Forum, Geneva.

TRUHAUT, R. AND FERRANDO, R. (1976). Remarks on nutritional and toxicological evaluation of non-conventional sources of proteins. *Folia Veterinaria Latina* **6**, Suppl. 1, 109–123.

TURNER, M.K. (1982). Downstream processing : hygiene or containment. *Chemistry and Industry*, p. 876.

VASEY, R.B. AND POWELL, K.A. (1984). Single-cell protein. In *Biotechnology and Genetic Engineering Reviews* (G.E. Russell, Ed), volume 2, pp. 285–311. Intercept, Ponteland, Newcastle upon Tyne.

WATSON, D.H. (1984). Survey and control of mycotoxins in human and animal food. *Chemistry and Industry*, 536–540.

WATSON, D.H. (ED.) (1987). *Natural Toxicants in Food Progress and Prospects*. Ellis Horwood, Chichester.