Remit

The Advisory Committee on Novel Foods and Processes is an independent body of experts whose remit is:

> 'to advise Health and Agriculture Ministers of Great Britain and the Heads of the Departments of Health and Social Services and Agriculture for Northern Ireland on any matters relating to the irradiation of food or to the manufacture of novel foods or foods produced by novel processes having regard where appropriate to the views of relevant expert bodies'.

The Secretariat is provided jointly by officials of the Department of Health and the Ministry of Agriculture, Fisheries and Food. As well as formal meetings, the Committee organises workshops on specific topics related to its remit.

The interaction between the ACNFP and other independent advisory committees is outlined in Figures 1 and 2.

Membership and Members' Interests

The membership of the Committee provides a wide range of expertise in fields of relevance in the assessment of novel foods and processes. A list of the membership during 1996, together with the names of assessors and the secretariat may be found overleaf.

In common with other independent advisory committees, the ACNFP is publishing a list of its members' commercial interests. These have been divided into different categories relating to the type of interest:

Personal:	a)	direct employment or consultancy;
	b)	occasional commissions;
	c)	share holdings.
Non-personal:	a)	fellowships;
	b)	support which does not benefit the member directly e.g. studentships.

Details of the interests held by members during 1996 can be found on pages 28–30.

A copy of the code of conduct for ACNFP members can be found on pages 31–35.

MEMBERSHIP OF THE COMMITTEE DURING 1996

Chairman

Professor Derek C Burke, CBE, BSc, PhD, HonLLD, HonScD, DL
Former Vice-Chancellor, University of East Anglia

Members

Professor G E Adams, BSc, PhD, DSc, FACR
Director of the Medical Research Council Radiobiology Unit, Chilton, Oxfordshire (until March 1996)

Professor P J Aggett, MSc, ChB, FRCP (Lond, Edin & Glasg), DCH
Assistant Director, Institute of Food Research, Norwich

Professor, T Atkinson, BSc, PhD
Former Deputy Director of PHLS Centre for Applied Microbiology and Research, Porton Down, Wiltshire

Dr M J Gasson, BSc, PhD
Head of the Department of Genetics and Microbiology,
Institute of Food Research, Norwich

Professor W P T James, CBE, MA, MD, DSc, FRCP, FRCP (Edin), FRSE
Director of the Rowett Research Institute, Aberdeen

Professor D A Ledward, MSc, PhD, FIFST
Professor of Food Science, University of Reading

Professor B J Miflin, BSc, MS, PhD
Director of Research, Institute of Arable Crops Research,
Rothamsted Experimental Station

Mrs H Millar, MA, FRSA
Formerly of the Adult Education Department,
University of Glasgow

Professor B E B Moseley, OBE, BSc, PhD
Retired Head of the Institute of Food Research,
Reading Laboratory

Reverend J C Polkinghorne, MA, PhD, ScD, FRS
Former President of Queens' College Cambridge

Dr P J Rodgers, MA, DPhil
Formerly of Zeneca BioProducts,
Billingham, Cleveland

Professor T Sanders, BSc, PhD, DSc
Head of the Department of Nutrition and Dietetics,
Kings College, London

Dr N A Simmons FRC Path, FIFST
Emeritus Consultant in Microbiology,
Guy's & St Thomas' Hospital Trust,
London

Professor J E Smith, BSc, MSc, PhD, DSc, FIBiol, FRSE
Head of the Applied Microbiology Division,
Department of Bioscience and Biotechnology, University of Strathclyde

Professor R Walker PhD, CChem, FRSC, FIFST
Professor of Food Science, University of Surrey

Professor H F Woods BSc, BM, BCh, DPhil, Hon. FFOM,
FIFST, FFPM, FRCP (Lond & Edin)
Head of the Department of Medicine and Pharmacology,
Royal Hallamshire Hospital, Sheffield

Assessors

Dr F Amijee	Department of the Environment
Dr P Baker	Department of Trade and Industry
Dr J Bell	Ministry of Agriculture, Fisheries and Food
Dr J Furlong	Health and Safety Executive
Professor A Gilmour	Department of Agriculture, Northern Ireland
Mr I Jackson	Welsh Office
Mr I Strachen	Scottish Office, Agriculture, Environment and Fisheries Department

	Personal Interest		Non-Personal Interest	
Member	**Company**	**Interest**	**Company**	**Interest**
Prof G E Adams	None		None	
Prof P J Aggett	None		Nestec, Milupa, Nutricia, Wyeth FDF Unilever	Departmental Commissioned Research
Prof T Atkinson	None		None	
Prof D C Burke	None		None	
Dr M J Gasson	None		Various	Departmental Research
Prof W P T James	None		Palm Oil Inst. of Malaysia	Studentships
Prof D A Ledward	None		Dalgety plc Various	Chair at Reading part funded by Dalgety (until mid–1996) Departmental teaching and research funded by various food companies
Prof B J Miflin	AGC Limited CIBA Seeds (Novartis)	Non-executive Director Ex-employee, occasional contact maintained, shareholder		
Mrs H Millar	Unilever	Shareholder	None	
Prof B E B Moseley	None		None	

	Personal Interest		Non-Personal Interest	
Member	**Company**	**Interest**	**Company**	**Interest**
Rev J Polkinghorne	None		None	
Dr P J Rodgers	Zeneca Ltd Marlow Foods Ltd F. Hoffmann-La Roche Ltd	Shareholder, former employee Consultancy Consultancy	None	
Prof T Sanders	Nutrasweet Seven Seas Ltd Coca-cola ILSI Europe	Consultancy Consultancy Consultancy Lapsed Consultancy	Unilever	Free supply of oils and fats for research purposes
Dr N A Simmons	Food Micro Ltd Infection Management Ltd Marks and Spencer plc McDonalds Restaurants Ltd PPP/Columbia Healthcare Ltd Waitrose Ltd Worshipful Company of Fishmongers	Director and Shareholder Advisor and Shareholder Consultant and Adviser Consultant and Adviser Consultant Consultant and Adviser Bacteriologist	None	
Prof J E Smith	Nestlé (Switzerland), Rhône Poulénc Diagnostics	Consultancy Royalty Agreement	Robertson Trust, Cow & Gate, Rhône Poulénc Diagnostics	Staff Support Research Research
Prof R Walker	Cadbury Beverages, Proctor and Gamble, IDV Ltd, Food Safety Advisory Centre, Coffee News Information Service, RHM, Tate and Lyle	Consultancy Consultancy Consultancy Consultancy Consultancy Consultancy Lapsed Consultancy Lapsed	Nestlé Ltd	Research

	Personal Interest		Non-Personal Interest	
Member	**Company**	**Interest**	**Company**	**Interest**
Prof H F Woods	None		Wide range of national and international food and chemical companies	Dean of the University of Sheffield Faculty of Medicine which has extensive activity in teaching and research in nutrition and toxicology and in topics related to, and supported by, many companies in the food and chemical industry. Harry Bottom Charitable Trust and Special Trustees for the former United Sheffield Hospitals

REGISTER OF INTERESTS: A CODE OF CONDUCT FOR MEMBERS OF THE ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

Introduction

1. This code of conduct guides members of the Advisory Committee on Novel Foods and Processes as to the circumstances in which they should declare an interest in the food industry.

2. The advice of the Advisory Committee concerns matters which are connected with the food industry and it is therefore desirable that its members and those of its support groups should have a good understanding of the work of the industry. It is also desirable that some members should have practical experience of the scientific problems of product development and safety evaluation. The food industry relies heavily on the advice of a wide range of specialists including scientists outside the industry in, for example, the universities. To avoid any public concern that commercial interests might affect the advice of the Committee, Ministers have decided that the arrangements which govern relationships between members and the food industry and information on significant and relevant interests should be on public record.

Definitions

3. In this code, 'food industry' means:

- companies, partnerships or individuals who are involved with the production, manufacture, packaging, sale or supply of food or food processes, subject to the Food Safety Act 1990;
- trade associations representing companies involved with some products;
- companies, partnerships or individuals who are directly concerned with research, development or marketing of a food product which is being considered by the ACNFP.

4. In this code 'the Secretariat' means the Secretariat of the ACNFP.

Different types of interest

5. The following is intended as a guide to the kinds of interests which should be declared. Where a member is uncertain as to whether an interest should be declared he should seek guidance from the Committee's Secretariat or, where it may concern a particular product which is to be considered at a meeting, from the Chairman at that meeting. **If a member has an interest not specified in these notes but which be believes could be regarded as influencing his advice, he should declare it.** However, neither the members nor the Secretariat are under an obligation to search out links between one company and another, for example where a company with which the member is connected has an interest in a food industry company of which the member is not aware and could not reasonably be expected to be aware.

Personal Interests

6. A personal interest involves payment to the member personally. The main examples are:

- *Consultancies:* any consultancy, directorship, position in or work for the food industry which attracts regular or occasional payments in cash or kind.

- *Fee-Paid Work:* any work commissioned by the food industry for which the member is paid in cash or kind.

- *Shareholdings:* any shareholding in or other beneficial interest in shares of the food industry. This does not include shareholdings through unit trusts or similar arrangements where the member has no influence on financial management.

Non-Personal Interests

7. A non-personal interest involves payment which benefits an organisation or department for which a member is responsible, but is not received by the member personally. The main examples are:

(i) *Fellowships:* the holding of a fellowship endowed by the food industry.

(ii) *Support by the Food Industry:* any payment, other support or sponsorship by the food industry which does not convey any pecuniary or material benefit to a member personally but which does benefit his position or department, e.g.:

- a grant from a company for the running of a unit or department for which a member is responsible;

- a grant or fellowship or other payment to sponsor a post or a member of staff in the unit for which a member is responsible. This does not include financial assistance for students;

- the commissioning of research or other work by, or advice from, staff who work in a unit for which a member is responsible.

Members are under no obligation to seek out knowledge of work done for or on behalf of the food industry by departments for which they are responsible, if they would not normally expect to be informed. Where members are responsible for organisations which receive funds from a very large number of companies involved in the food industry, the Secretariat can agree with them a summary of non-personal interests rather than draw up a long list of companies.

(iii) *Trusteeships:* any investment in the food industry held by a charity for which an ACNFP member is a trustee.

Where a member is a trustee of a charity with investments in the food industry, the Secretariat can agree with the member a general declaration to cover this interest rather than draw up a detailed portfolio.

Contractual obligations of confidentiality

8. Some members of the Committee may, **at the time of adoption of this Code** or (in the case of new members) on their joining the Committee, be bound by the terms of a contract which requires them to keep the fact of the contractual arrangement confidential. As a transitional measure any member so affected shall seek to agree an entry for the public register with the other party. If such agreement does not prove possible, the member shall seek a waiver permitting him to disclose his interest, in confidence, to the Chairman and the Secretariat. The Secretariat will maintain a confidential register of such disclosures which will not form party of the public record.

9. On adoption of this Code members shall not enter into new contractual obligations which would inhibit their ability to declare a relevant interest.

Declaration of interests to the Secretariat

10. Members of the Committee, should inform the Secretariat **in writing** when they are appointed of their **current personal and non-personal** interests, including the principal position held. Only the name of the company and the nature of the interest is required, the amount of any salary, fees, shareholding, etc need not be disclosed to the Secretariat. An interest is current if the member has an on-going financial involvement with the food industry e.g. if he holds shares in a food company, if he is in the consultancy contract with the food industry, or if he is in the process of carrying out work for the food industry. Members are asked to inform the Secretariat at the time of any change of their **personal** interest, and will be invited to complete a declaration form once a year. It would be sufficient if changes in non-personal interests are reported in the annual declaration form following the change. (Non-personal interests involving less than £1000 from a particular company in the previous year need not be declared to the Secretariat.)

Special position of Chairman

11. It is not appropriate for the Chairman of the Advisory Committee on Novel Foods and Processes to have any current personal interest in the food industry.

Declaration of interests at meetings and participation by members

12. Members are required to declare relevant interests at Committee meetings, and to state whether they are personal or non-personal interests and whether they are specific to the product under consideration or non-specific:

(i) A member must declare a **personal specific** interest if he has **at any time** worked on the product or process under consideration and has personally received payment for that work, in any form, from the food industry. The member shall take no part in the proceedings as they relate to the product or process, except that he may at the Chairman's discretion answer questions from other members. If the interest is no longer current, the member should declare it as a **lapsed personal specific** interest;

(ii) A member must declare a **personal non-specific interest** if he has a **current** personal interest in the food company concerned which does not relate specifically to the product under discussion. The member shall take no part in the proceedings as they relate to the product, except that he may at the Chairman's discretion answer questions from other members;

(iii) A member must declare a **non-personal specific interest** if he is aware that the department for which he is responsible has at any time worked on the product or process but the member has not personally received payment in any form from the food industry for the work done. The member may take part in the proceedings unless he has personal knowledge of the product or process through his own work or through direct supervision of other people's work, in which case he should declare this and not take part in the proceedings (except to answer questions);

(iv) There is no need for members to declare **non-personal non-specific** interests (i.e. if a member is aware that the department for which he/she is responsible is currently receiving payment from the food industry company concerned and which does not relate specifically to the product or process under discussion). If, exceptionally, a member feels such an interest might be thought to influence his advice, he should seek guidance from the Chairman on whether to draw the facts to the attention of other members.

13. The examples, of personal, non-personal and current interests given in the previous paragraphs should be read in the context of paragraphs 6, 7 and 10. 'Taking part in the proceedings' includes both speaking and, if necessary, voting. A member who is in any doubt as to whether he has an interest which should be declared, or whether he should take part in the proceedings, should ask the Chairman for guidance. The Chairman has the power to determine whether or not a member with an interest shall take part in the proceedings.

14. If a member is aware that a product or process under consideration is or may become a competitor of a product or process manufactured, sold or supplied by a company in which the member has a **current personal** interest, he should declare his interest in the company marketing the rival product or process. The member should seek the Chairman's guidance on whether he should take part in the proceedings.

Register of interests

15. A record is kept by the Secretariat of:

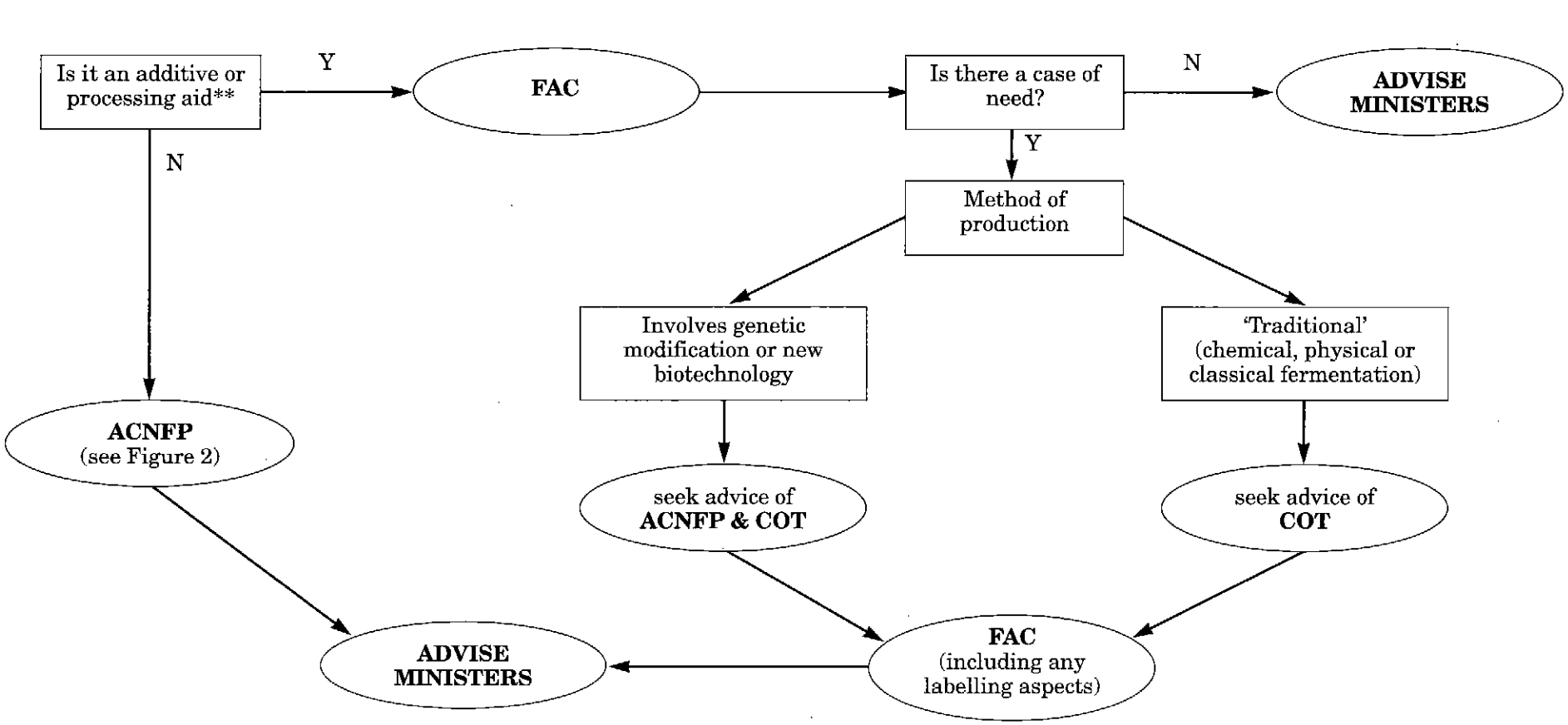
- names of members who have declared interests to the Secretariat on appointment, as an interest first arises or through the annual declaration, and the nature of the interest;

- names of members who have declared interests at meetings of the Committee, giving dates, names of relevant products and companies, details of the interest declared and whether the member took part in the proceedings.

Publication

16. Information about interests declared by members to the Secretariat will be published each year in the Annual Report of the ACNFP.

Figure 1: Decision Tree for allocation of Submissions to ACNFP or FAC.

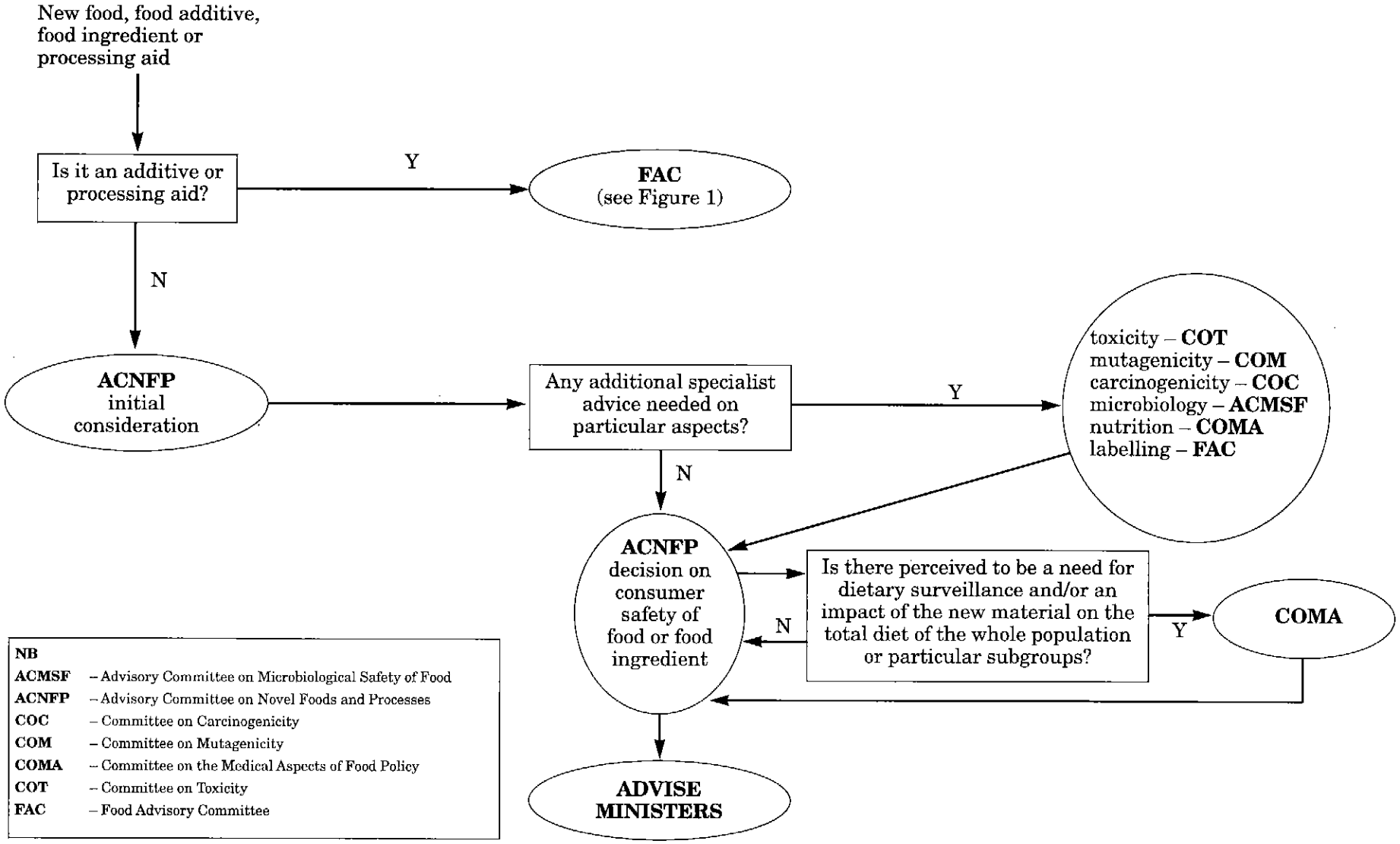


* If a medicinal licence has been applied for, or any medical claims are made for the product, then the Medicines Control Agency will need to be consulted.

** Using definitions from Council Directive 89/107/EEC of 21/12/88 – Food Additives Authorised for Use in Foodstuffs Intended for Human Consumption.

Use of this decision tree will involve consideration of the extent of use (with implications for dietary intake), whether other uses are likely to result in a changed classification giving a different route of evaluation and the status of the substance with respect to existing additive regulations.

Figure 2: Relationship of ACNFP with other Expert Committees involved in the assessment of food safety.



APPENDIX II

ACNFP REPORT ON PROCESSED PRODUCTS FROM GM GLUFOSINATE-AMMONIUM TOLERANT MAIZE

INTRODUCTION

1. In February and May 1996, the ACNFP considered an application from AgrEvo USA Company[1], for food safety clearance of a line of genetically modified (GM) yellow dent maize. The GM maize had been genetically modified for tolerance to the glufosinate-ammonium based herbicides. The application sought clearance of products of the seeds from the conventionally-bred inbreds* and hybrids of the GM line, T25.

2. It is not intended that the GM maize will be grown in the UK, but it is likely that the processed grain will be used in food products sold in the UK. The Company has applied for a consent to market the maize throught the EU under the Deliberate Release Directive, 90/220/EEC[2], which is administered in the UK by the Department of the Environment.

3. The GM maize line was transformed with a plasmid which contains one copy of a synthetic, truncated form of a *pat* gene which encodes an enzyme, phosphinothricin-acetyl-transferase (PAT), which confers tolerance to the glufosinate-ammonium (GA) based herbicides, and one copy of an *ampR* antibiotic resistance selectable marker gene which encodes an enzyme, β-lactamase, which confers tolerance to the antibiotic ampicillin. The *pat* gene is expressed in the maize, but the *ampR* gene is disrupted and is not expressed. The GM maize was produced through genetic modification of the conventionally bred AgrEvo tissue culture line He/89.

4. In its evaluation of the application, the ACNFP focused on the food safety aspects of the processed products which would be derived from the maize. The Committee compared the compositional data of the unprocessed grain of the GM maize line with those of conventionally bred varieties. The Committee also used a similar comparative approach in its consideration of supporting information on plant morphology and agronomic performance.

5. The Food Advisory Committee (FAC) has examined the labelling implications of the product and concluded that no special labelling should be required for the product.

BACKGROUND

6. Maize *(Zea mays)* has been cultivated for several thousands of years in the Americas and has been an important crop in continental Europe for the past 500 years. In the UK most maize is grown for silage, but imported products derived from maize enjoy widespread application and are used for the production of starch, flour, breakfast cereals, brewing ingredients, syrup and oil.

7. The purpose of the transformation was to provide maize which was tolerant to GA based non-selective, post emergent herbicides, e.g. Basta, Ignite, etc. GA is one of the phosphinothricin (PPT) based herbicides, it controls weeds through the inhibition of glutamine synthetase (GS), an important plant enzyme. Inhibition of GS leads to an accumulation of phytotoxic levels of ammonia in plants as it is the only enzyme which can detoxify ammonia, a compound which is released by photorespiration, nitrate reduction and amino acid degradation.

*Technical terms not explained in the body of the report are underlined where they appear for the first time and are explained in the glossary; explanations are used in the context of the report and should not be taken as general definitions.

8. The transformation was achieved by the introduction of a synthetic, truncated form of a *pat* gene which is naturally found in the soil bacterium *Streptomyces viridochromogenes*. A disrupted *ampR* selective marker gene from the bacterium *Escherichia coli* is also present. Marker genes are used to aid in the earlier part of the genetic transformation process when conditions are arranged to specifically select the genetically modified organism.

PRODUCTION OF THE GENETICALLY MODIFIED LINE

9. The GM transformant was obtained by the introduction of a copy of a synthetic *pat* gene, from *S. viridochromogenes* strain TÜ 494, and a copy of an *ampR* antibiotic resistance marker gene, from *E. coli*, into the maize tissue culture line He/89 by a direct uptake technique.

10. The synthetic *pat* gene encodes an enzyme, PAT, which catalyses the conversion of PPT, the active ingredient in GA based herbicides, to an inactive form thus conferring tolerance to it.

11. The *pat* gene is regulated by a constitutive 35 S transcript of a cauliflower mosaic virus (promoter), which results in its expression throughout the plant, and a 35 S 3' non-translated region of a cauliflower mosaic virus (terminator). The *ampR* gene is under the control of bacterial regulatory sequences, but in this application the gene was found to be disrupted and non-functional after transfer to the maize line.

12. A synthetic *pat* gene is used to allow optimal expression, because bacterial genes differ from those of plants.

PROCESS DESCRIPTION AND USE

Processing

13. It is intended that the grain of the GM maize will undergo one of two types of conventional processing, either wet milling or dry milling. Wet milling, which involves steeping before milling, is used to produce starch, glucose syrup and oil. Following wet milling the oil is processed by conventional means, utilising the application of pressure, heat and solvent extraction. Dry milling is used to produce maize meal and flour.

Use

14. Products derived from maize, in particular modified starch and glucose syrup, are ubiquitous in the UK diet where they are found in processed foods, confectionery and soft drinks. Maize flour is used for breakfast cereals, snack foods, bakery products and in brewing; while maize oil is used in margarine, frying and mayonnaise/salad dressing oils. Products from the processed GM maize would be expected to replace those from conventionally-bred maize, no new uses or markets are foreseen.

Specification

15. There are no toxic or anti-nutritional factors present in maize which would need to be controlled by a specification.

SAFETY ASSESSMENT

16. As part of the safety assessment of the GM maize, the Committee compared the data of the GM maize to that of non-GM maize lines. The Committee considered:

- the safety of the intentional changes;
- whether or not any unintentional or secondary changes arising from the modification had taken place;
- the stability of the genetic change; and
- the possibility of the transfer of the genetic material from the processed maize products.

Intentional changes

17. The Committee was satisfied with the data pertaining to the safety of the intentional changes. The intentional changes to the GM maize resulted in the introduction of one intact copy of a synthetic *pat* gene and one disrupted copy of an *ampR* gene plus bacterial and plant specific viral regulatory sequences. The intentional effect of the genetic modification was to provide tolerance to the GA based herbicides.

18. The Company analysed whole plants, leaves, roots, seeds, and mature pollen from the GM maize line and its non-GM counterparts grown in Europe for PAT protein using ELISA, HPLC, enzyme activity assays and Western blot analyses. PAT protein was detected in the seeds, leaves, stems and roots, but not in the pollen. In studies from the US field grown maize, the Company found PAT protein to be absent from maize oil and starch, but it was detected at approximately, 0.00002% of the crude protein of wet and dry milled hulls, grits, meal and flour.

19. The Committee concluded that the disrupted *ampR* gene product would not be functional in or expressed in the GM maize as the bacterial regulatory sequences controlling it do not allow expression in plants.

20. The Committee noted that processing the GM maize for human food use would denature the genetic material present and would denature any gene products present in the grain.

Unintentional changes

21. The Committee examined data on the gene constructs used, including the expected effect, site of expression of the introduced genes and the method used for the genetic modification.

22. A plasmid vector was introduced into the maize protoplasts by a direct uptake technique. Buffered protoplasts and plasmids were intimately mixed together and polyethylene glycol solution was added 'a drop at a time'. The plasmid contained one copy of a *pat* gene, and one copy of an *ampR* antibiotic resistance marker gene (see paragraph 11). The putative GM micro-colonies which resulted from the transformation process were grown on a selective medium containing GA. Transformation event T25 was chosen for commercial exploitation.

23. Southern blot and PCR analyses confirmed that one intact copy of a *pat* gene and one disrupted copy of an *ampR* gene were present in the GM line.

24. The Committee was able to conclude that no unintentional changes or effects had taken place during the genetic modification procedure.

25. It also considered whether or not there had been any unintentional changes in the composition of the grain from the GM plants or in the plants themselves as a result of the genetic modification. The Company provided data on compositional analyses, morphological studies and agronomic field tests conducted in the USA and Europe. The compositional analyses included: fatty acid profile, protein, amino acid, crude fibre, ash, phytate and moisture contents, determined for grain and silage of GM and non-GM maize. The agronomic characteristics included: data, yield, vigour, disease and insect susceptibility. Although there was some variation for some parameters between the GM and non-GM lines, the Company was able to satisfy the ACNFP that there had been no unintentional changes in the composition of the grain from the GM plants nor in the plants themselves as a result of the genetic modification.

26. As ELISA analyses had detected small amounts of the PAT protein in the grain and silage from the GM line its allergenic potential was investigated. Searches of the EMBL and the SwissProt databases revealed no significant sequence homology between the PAT protein and a range of known allergens. The Committee was satisfied that there was no evidence to suggest that there was allergenic potential in the PAT protein.

Stability of the genetically modified organism

27. The Company was able to present evidence for a normal Mendelian inheritance of the new genes over several generations. This satisfied the Committee that there had been stable integration of the introduced genes into the genome of the GM maize.

Genetic transfer

28. The Committee evaluated the risk of genetic transfer of novel genes present in the GM maize to consumers or to gut micro-organisms through consumption of products made from the processed grain. It concluded that since processing would destroy any DNA present, the risk of genetic transfer from processed products could be discounted. Because the *ampR* gene was disrupted, the Committee had no concerns about the use of unprocessed GM maize in animal feed.

DISCUSSION

29. A number of issues were taken into account by the Committee when it evaluated the safety of the processed food products derived from the GM maize. These issues included the analytical composition of the grain and silage from the GM maize, the analytical composition or characterisation of the transferred DNA, the search for any unintentional or pleiotropic changes which may have occurred as a result of the genetic modification, confirmation that the introduced genes were inherited stably, the evaluation of the GM maize's agronomic performance and the effect of processing on the transferred DNA and its product.

30. The data submitted on the composition of the grain and silage reassured the Committee that the composition of the GM maize does not differ from that of conventionally-bred maize.

31. Consideration of the data on the genetic modification procedure satisfied the Committee that no unintentional changes had taken place at the molecular level and this was further confirmed by the data provided on the morphology at the macroscopic level.

32. Data from the genetic segregation studies demonstrated that the introduced genes were stably inherited into the GM maize genome. However, as there is little experience in predicting the effect of genetic drift on the metabolism of any line of plants, whether genetically modified or conventionally-bred, the Committee has asked that the seed composition, including the amino acid profile, and the fatty acid profile of the oil from T25 and the lines derived from it using conventional plant breeding techniques should be monitored over time. The Company has agreed to provide such information.

33. The Committee was satisfied that the disrupted *ampR* antibiotic resistance gene present did not raise any concerns over the consumption of unprocessed GM maize.

CONCLUSIONS

34. The Committee concluded that the processed products obtained from the GM maize line T25 and the inbred and hybrid lines derived from it through conventional plant breeding techniques were safe to use in food and would not differ in composition from those of conventionally-bred maize. Because the *ampR* gene was disrupted, it caused the Committee no concerns.

35. The FAC has examined the labelling implications of the GM maize line T25, its derivatives and the processed products obtained from them. The FAC has concluded that no special labelling should be required for any of the products. However, the FAC encourages the voluntary provision of information in response to public interest.

REFERENCES

1. Submission from AgrEvo USA Company dated 10 November 1995. This submission will be deposited in the British Library, identified as BL SUP 11113.

2. EC Council Directive of 23 April 1990 on the deliberate release into the environment of genetically modified organisms (90/220/EEC) OJ L117 8 May 1990 page 15. Implemented by the Genetically Modified Organisms (Deliberate Release) Regulations 1992. SI 1992 No 3280. HMSO, London: ISBN: 011 033152 4 and the Genetically Modified Organisms (Deliberate Release) Regulations 1995. SI 1995 No 304. HMSO, London: ISBN: 011 0524330.

GLOSSARY

antibiotic: a substance, derived from micro-organisms (e.g. bacteria) that destroys or inhibits the growth of other micro-organisms. Many antibiotics are used as drugs in treating disease.

β-lactamase (antibiotics): antibiotics, e.g., ampicillin, which contain the cyclic B-amide molecule β-lactam.

buffered: a solution resisting pH change on addition of acid or alkali.

constitutive promoter: promoter which enables a gene to be expressed throughout a plant.

denature: to irreversibly alter the structure of a protein by chemical or physical means.

direct uptake technique: method of transferring DNA into cells by mixing protoplasts with polyethylene glycol and buffered DNA.

DNA: deoxyribonucleic acid which is found in all living cells and contains the information for cellular structure, organisation and function.

ELISA: enzyme linked immunosorbent assay.

Escherichia coli: a bacterium found in animal intestines; certain types have been used widely in microbiological studies and in biotechnology research and development and production.

expression: manifestation of the genetic material of an organism.

functionality: ability to perform a function or job.

gene: a unit of inheritance, usually understood to mean a region of DNA which encodes one function.

gene construct: gene sequence made *in vitro* containing trait genes and associated regulatory sequences.

genome: a complete ensemble of the genes in a cell, e.g. chromosomal and plasmid elements.

HPLC: high performance liquid chromatography.

hybrid lines: line of plants produced from a cross between genetically dissimilar parents.

inbreds: plants which have been self-pollinated over several generations and are nearly genetically uniform.

Mendelian inheritance: the inheritance of trait genes from one generation to the next in accordance with a pattern first described by Gregor Mendel.

morphological: form or appearance of an organism.

PCR: polymerase chain reaction – a sensitive method used to amplify a specific region of DNA.

plasmid: loop of DNA found in bacteria and some other organisms, e.g., yeast, that replicates independently of the chromosomes.

pleiotropic: the effect of a single gene addition/substitution or mutation on a number of phenotypic characteristics in an organism.

polyethylene glycol: chemical which will form pores or channels in protoplasm.

promoter: the key control element that enables a gene to be expressed by transcription into mRNA.

protoplast: plant protoplasm with cell wall removed.

selectable marker gene: genes with a phenotype that can be selected for in gene transfer experiments. Selectable marker genes are used to enable the selection/deletion of neighbouring sequences in a gene construct.

Southern blot analysis: a technique for detecting the presence of specific DNA.

Streptomyces viridochromogenes: a harmless soil-living bacterium.

synthetic: formed artificially by chemical synthesis.

terminator: DNA sequence which terminates the synthesis of mRNA.

transformant: a plant derived from a cell in which the genetic modification has been successful and which contains the introduced gene construct.

truncated gene: part of a gene.

vector: DNA segment which can incorporate foreign DNA and transfer it between organisms.

Western blot analysis: a technique for detecting the presence of specific protein.

APPENDIX III

ACNFP REPORT ON OIL FROM GM BROMOXYNIL–TOLERANT COTTONSEED

INTRODUCTION

1. In September 1996, the Committee considered a request from Calgene Inc.[1] for food safety clearance of oil from the seed of genetically modified (GM) cotton (*Gossypium hirsutum* L.) The cotton has been modified for tolerance to the broadleaf herbicide bromoxynil (Buctrilâ-manufactured by Rhône-Poulénc) and is termed BXN cotton. The Company requested clearance for oil derived from BXN cotton. The ACNFP operates on a 'case-by-case' basis and at present is willing to recommend clearances only for products that it has evaluated and their descendants produced through conventional plant breeding.

2. The BXN cotton contains the BXN gene for tolerance to bromoxynil. It also contains the *kan*^{r} gene for resistance to the antibiotics kanamycin, neomycin and geneticin.

3. In its evaluation of the submission, the Committee focused on the consumer safety aspects of the oil extracted from the GM cotton. The ACNFP compared the analytical data on the oil from the GM lines with that on oil from conventionally-bred varieties and with the Codex standard for edible cottonseed oil. The ACNFP also used a comparative approach during its consideration of supporting information on compositional analyses of whole seed and agronomic performance.

4. The Food Advisory Committee has examined the labelling implications and has concluded that no special labelling should be required for the product, as the GM cotton does not contain any ethically sensitive genes and the refined oil that would be used in food contains neither genetic material nor the products of the introduced genes.

5. The GM cotton will not be grown in the UK. The lines have been developed and will be grown in the USA. Cottonseed oil would be consumed as a component of processed foods imported into this country from the USA.

BACKGROUND

6. Cultivated cotton is grown extensively across the southern states of America, primarily for the fibre (lint) produced on its seedcoat. Cottonseed is an important edible vegetable oil crop in the USA, which is one of the major producers. The meal and hulls are mainly used in animal feeds, though processed products of cottonseed are approved for human consumption by the US FDA with the specification that free gossypol, a natural toxicant, should not exceed 450 ppm.

7. Though cottonseed oil is not, as far as can be determined from the UK trade (the Seed Crushers and Oil Processors Association), used in food produced in the UK it would be consumed as a component of foods imported into this country from the USA, where the oil is used as an ingredient for margarine, shortenings, salad and cooking oil.

8. The U.S. Department of Agriculture (USDA) made a 'Determination of Non-regulated Status' for BXN cotton in February 1994, concluding that it was neither a plant pest nor deleterious to the environment and could therefore be grown in the same manner as any other cotton variety. BXN cottonseed was first sold to farmers in the USA in spring 1995. Deregulation requests have also been made to Health Canada and Agriculture and Agri-foods Canada.

PRODUCTION OF THE GENETICALLY MODIFIED LINES

9. The BXN cotton lines were produced through the introduction of the BXN gene from *Klebsiella pneumoniae* subsp. *ozaenae (K.ozaenae)* which encodes an enzyme (nitrilase) that degrades the herbicide bromoxynil. *Klebsiella* species are common micro-organisms found in the human gastrointestinal tract, in soil and water, in grain and other widely distributed locations in nature. *K. pneumoniae* subsp. *ozaenae* is commonly consumed unintentionally.

10. The *kan*^{r} gene (also known as *neo* and *nptII,* from *Escherichia coli)* was introduced as an antibiotic resistance marker gene* to enable transformed cotton plant cells to be identified. It encodes the enzyme aminoglycoside 3'-phosphotransferase II (APH(3')II) which inactivates the antibiotics kanamycin, neomycin and geneticin. The safety of this gene and its product has previously been considered by the ACNFP in its assessment of the FLAVR SAVR tomato.

PROCESS DESCRIPTION AND USE

Processing

11. BXN cottonseed would be processed for oil production in the same way as non-GM cottonseed. Oil is removed from the seed primarily by mechanical screw presses, by solvent extraction or by a combination of both. In both processes the seed kernels are crushed to thin flakes by heavy iron rollers.

12. For screw pressing, the flakes move from the roller to a cooker or conditioner where they are 'cooked' and the moisture reduced to a low level. From the cooker they flow directly into the screw press where flakes are exposed to high pressure. Oil is forced from the meals and filtered.

13. In direct solvent extraction, the flaked and cooked kernels are exposed to hexane, which dissolves out the oil. The mixture of oil and solvent is then put through a series of evaporators and stills to remove all solvent from the oil.

14. Crude cottonseed oil from the mill requires further processing to remove impurities before it is used in food. The refining process also removes the darker colouring materials present, leaving a clear yellow oil. Cottonseed oil is finally deodorised by exposing it to steam under a partial vacuum to remove unwanted flavours and to further purify it before use.

* Technical terms not explained in the body of the report are underlined where they appear for the first time and are explained in the glossary; explanations are used in the context of the report and should not be taken as general definitions.

Use

15. The oil from the BXN cottonseed is intended to replace some of the oil currently obtained from non-GM cottonseed and would be used for the same purposes as oil from non-GM cottonseed. No new uses or increases in consumption of cottonseed oil are expected as a result of the introduction of the oil from the BXN cottonseed.

16. It has not been possible to provide data on consumption of cottonseed oil in the UK as this is present only in imported food products and is labelled as vegetable oil. The Company calculates cottonseed oil consumption in the USA to be approximately 4.2 gm/person/day.

16. The use of cottonseed oil in infant formulae is specifically prohibited in the UK on account of the potential presence of cyclopropenoid fatty acids which may be detrimental to health.

Product specification

18. The fatty acid composition of cottonseed oil from the BXN cotton lines cleared by the Committee is comparable to that from the non-GM parental variety and falls within the Codex standard 22-1981 for edible cottonseed oil.

SAFETY ASSESSMENT

19. In assessing the safety of the oil from the BXN cotton, the Committee compared it with oil from conventionally-bred cotton cultivars which complies with the specifications in Codex standard 22–1981 for edible cottonseed oil. In particular, the Committee considered:

- the safety implications of the intentional changes;
- whether any unintentional changes have occurred as a result of the genetic modification;
- the genetic stability of the BXN cotton; and
- the likelihood of transfer of modified genetic material from the oil to the human consumer.

Intentional changes

20. Data reviewed by the Committee indicated that the only intentional change to the cotton was the presence of the introduced genes *BXN* and *kan*^{r}, leading to the intentional effects of tolerance to the herbicide bromoxynil and resistance to the antibiotics kanamycin, neomycin and geneticin.

21. The Committee examined the results of tests to demonstrate that the *BXN* and *kan*^{r} genes had been inserted as intended in the five lines cleared. Southern blot analysis had established that homozygous plants of these lines contain two complete copies of the introduced genes at a single functional locus. The refined oil contains neither genetic material nor the products of the introduced genes.

Unintentional changes

22. The Company considered the possibility of untoward secondary effects as a result of the genetic modification and has provided evidence to the Committee to show that these have not occurred. The evidence included the levels of fibre and gossypol in the cottonseed, the fatty acid composition of and levels of cyclopropenoid fatty acids in the oil and the agronomic performance and characteristics of the cotton plants.

Stability of the genetically modified organism

23. The Committee was given data to show that the *BXN* gene was integrated in a stable manner, based on bromoxynil spray survival tests. Genetic stability in the BXN cotton lines was demonstrated by maintenance of the bromoxynil tolerance trait over at least six seed generations (selfed) and at least five generations of backcrossing with commercial varieties. Molecular analysis of DNA from these lines also indicated stable integration of the introduced genes into the plant genome. Southern blot analyses to compare plants of several generations confirmed that the inserted DNA had not been significantly rearranged over generations and was inherited in a stable manner. Further Southern blot analyses of later generations of pre-commercial lines confirmed that stability was maintained in subsequent generations.

Genetic transfer

24. The Committee considered the risk of genetic transfer of novel genes present in the BXN cotton to human consumers or their gut micro-organisms through consumption of oil from the seed. It concluded that this was not an issue since the refined oil contains neither genetic material nor the products of the introduced genes.

DISCUSSION

25. Although the focus of the ACNFP's evaluation of the oil from BXN cotton was on its composition, other factors which could affect its safety in use were also considered. These included the identification of any unintentional changes from the genetic modification and confirmation of the stability of the introduced genes.

26. The data submitted on the composition of the cotton seed and oil and the description of the processing procedure satisfied the Committee that the composition of the oil from five numbered lines (10109, 10211, 10215, 10222 and 10224) of BXN cotton does not differ from conventionally-bred cotton varieties and that none of the products from the introduced genes are present in the fully processed oil.

27. From the detailed information given on the genetic modification procedure, the Committee was satisfied that no unintentional changes had taken place at the molecular level. Similarly, evidence provided on agronomic traits demonstrated that no unintentional changes in phenotypic characteristics had taken place.

CONCLUSIONS

28. The Committee concluded that oil from lines 10109, 10211, 10215, 10222 and 10224 of BXN cotton, and from lines derived from these by conventional breeding, was safe for use in food and that it did not differ from oil from conventionally-bred cotton seed.

29. The Committee recommended food safety clearance of oil from BXN cotton lines 10109, 10211, 10215, 10222 and 10224, and from lines derived from these by conventional breeding, provided:

- the oil complies with internationally agreed standards; including Codex and those used in the trade of oils and fats, with due regard to whether the oil is crude or fully processed;

- the procedures established by the ACNFP for monitoring compositional characteristics of the seed, the crude and refined oil from the BXN cotton, paying particular attention to gossypol and cyclopropenoid fatty acid levels and the fatty acid profile of the oil, are observed, with particular attention to a periodic review of levels of gossypol and cyclopropenoid fatty acids in the oil; and

- its exclusion from infant formulae.

REFERENCES

1. Submission from Calgene dated 9 August 1996. This submission has been deposited in the British Library, identified as BL SUP no 11108.

GLOSSARY

antibiotic resistance marker gene: antibiotic resistance gene which is engineered into a vector to enable selection of genetically modified cells containing the introduced trait genes.

DNA: deoxyribonucleic acid which is found in all living cells and contains the information for all cellular structure, organisation and function.

enzyme: a naturally-occurring protein capable of facilitating or instigating a biochemical reaction without being either consumed or destroyed in it.

homologous chromosomes: pairs of chromosomes capable of pairing during meiosis and having approximately or exactly the same order of loci.

homozygous: having the identical gene situated at the same site on each of a pair of chromosomes, coding for the same version of a given characteristic.

locus: position on homologous chromosomes occupied by a gene.

meiosis: process (reduction division) whereby a cell nucleus divides by two divisions in four nuclei, each containing half the original number of chromosomes (one from each pair of homologous chromosomes).

pleiotropic effects: effects due to the ability of genes or gene products to have, or be involved in, more than one effect e.g.: through an enzyme or regulatory protein involved in several different metabolic pathways.

secondary effects: effects other than those intended that might arise from the insertion of novel DNA e.g.: due to the disruption of adjacent DNA or unexpected effects on metabolic pathways and including pleiotropic effects.

selfed: the fertilisation of a flower using pollen from the same flower.

Southern blot analysis (Southern hybridisation) – a technique which uses probes consisting of complementary nucleic acid to detect the presence of specific segments of DNA that have been isolated from an organism.

trait: observable characteristic.

transformed cells: cells in which genetic modification has been successful and which contain the introduced genes.

vector: DNA segment which can incorporate foreign DNA and transfer it between organisms.

APPENDIX IV

ACNFP REPORT ON PROCESSED PRODUCTS FROM GM INSECT-RESISTANT MAIZE

INTRODUCTION

1. In May 1996, the ACNFP considered an application from Monsanto Europe SA, via the European Commission[1], for food safety clearance of a line of genetically modified (GM) yellow dent maize. Food safety clearance is necessary in pursuance of the obligations to gain a consent to market the maize throughout the EU under the Deliberate Release Directive 90/220/EEC[2]. It is not intended that the GM maize will be grown in the UK, but it is likely that the processed grain will be used in food and feed products sold in the UK.

2. The GM maize had been genetically modified for resistance to lepidopteran insect pests, principally, the European corn borer. The application sought clearance of products of the seeds from the conventionally-bred inbreds* and hybrids of the GM line, MON 810.

3. The GM maize was produced through genetic modification of the tissue culture line Hi-II by transformation with a plasmid which contains one copy of a synthetic form of a *cry1A(b)* gene which encodes a protein toxin.

4. In its evaluation of the application, the ACNFP focused on the food safety aspects of the processed products which would be derived from the maize. The Committee compared the compositional data of the unprocessed grain of the GM maize line with those of conventionally-bred varieties. The Committee also used a similar comparative approach in its consideration of supporting information on plant morphology and agronomic performance. It concluded that the GM maize was substantially equivalent to the non-GM maize.

5. The Food Advisory Committee (FAC) has examined the labelling implications of the processed products derived from the GM maize and concluded that no special labelling should be required as there was no viable DNA remaining in the processed maize products destined for human consumption.

BACKGROUND

6. Maize *(Zea mays)* has been cultivated for several thousands of years in the Americas and has been an important crop in continental Europe for the past 500 years. In the UK most maize is grown for silage, but imported products derived from maize enjoy widespread application and are used for the production of starch, flour, breakfast cereals, brewing ingredients, syrup and oil.

7. The purpose of the transformation was to provide maize which was resistant to the lepidopteran insect pest, the European corn borer, *Ostrinia nubilalis (O. nubilalis),* and certain other lepidopteran pests such as the pink borer, *Sesamia cretica (S. cretica).*

*Technical terms not explained in the body of the report are underlined where they appear for the first time and are explained in the glossary; explanations are used in the context of the report and should not be taken as general definitions.

8. The transformation was achieved by the introduction of a plasmid containing a synthetic form of a *cry1A(b)* gene from *Bacillus thuringiensis* subsp. *kurstaki*. The plasmid also contained one copy of an *nptII* antibiotic resistance selectable marker gene which encodes an enzyme, neomycin phosphotransferase, which confers tolerance to the antibiotic neomycin, but this gene was not transferred to the maize.

9. A second plasmid was also used in the transformation, but it failed to integrate into the maize chromosome. This plasmid contained two genes encoding for tolerance to the glyphosate based herbicides, 5-enol-pyruvyl-shikimate-3-phosphate synthase (CP4 EPSPS) and glyphosate oxidoreductase (GOX), and the antibiotic resistance marker gene, *nptII*. These genes are not present in the GM maize.

PRODUCTION OF THE GENETICALLY MODIFIED LINE

10. The GM transformant was obtained by the introduction of a modified *cry1A(b)* gene from B. *thuringiensis* subsp. *kurstaki* into the maize tissue culture line Hi-II, a derivative of the inbred breeders' lines A188 and B73, by a biolistic or particle acceleration method. A synthetic *cry1A(b)* gene was used to allow optimal expression, because bacterial genes differ from those of plants.

11. The synthetic *cry1A(b)* gene encodes a gene product, a δ-endotoxin, which is toxic to *O. nubilalis,* and S. *cretica.*

12. The *cry1A(b)* gene is regulated by a constitutive 35S transcript of a cauliflower mosaic virus (promoter) with a duplicated enhancer region a maize intron for the heat shock protein 70 – (*hsp 70) and a 3 [1] non-translated region of the *nopaline synthase* gene – *nos3* [1] moiety (terminator).

PROCESS DESCRIPTION AND USE

Processing

13. It is intended that the grain of the GM maize will undergo one of two types of conventional processing, either wet milling or dry milling. Dry milling is used to produce maize meal and flour, while wet milling, which involves steeping before milling, is used to produce starch, glucose syrup and oil. Following wet milling the grain is processed by conventional means with the oil fraction undergoing the application of pressure, heat and solvent extraction.

Use

14. Products derived from maize, in particular modified starch and glucose syrup, are ubiquitous in the UK diet where they are found in processed foods, confectionery and soft drinks. Maize flour is used for breakfast cereals, snack foods, bakery products and in brewing; while maize oil is used in margarine, frying and mayonnaise/salad dressing oils. Products from the processed GM maize would be expected to replace those from conventionally-bred maize, no new uses or markets are envisaged.

Specification

15. There are no toxic or anti-nutritional factors present in maize which would need to be controlled by a specification.

SAFETY ASSESSMENT

16. As part of the safety assessment of the GM maize, the Committee compared the data of the GM maize to that of the non-GM maize lines. The Committee considered:

- the safety of the intentional changes;
- whether or not any unintentional or secondary changes arising from the modification had taken place;
- the stability of the genetic change; and
- the possibility of the transfer of the genetic material of the processed maize products to the human consumer.

Intentional changes

17. The Committee was content with the data pertaining to the safety of the intentional changes. The intentional changes to the GM maize resulted in the introduction of one intact copy of a synthetic *cry1A(b)* gene, maize intron – #hsp 70, and plant specific viral regulatory sequences. The intentional effect of the genetic modification was to provide resistance to *O. nubilalis* and certain other lepidopteran pests.

18. The Company analysed whole plants, leaves and grain from the GM maize line, its progeny and their non-GM counterparts grown in Europe and the USA for the *cry1A(b)* gene product using ELISA. The *cry1A(b)* gene product was detected at the following levels (mean weight, mg/g fresh weight tissue): whole plant, mean, 4.15; grain, mean, 0.53; and leaf, mean,-9.26. The *cry1A(b)* gene product was monitored in the leaf during the growing season and at the last reported analysis, six weeks from the start of monitoring, the level had fallen to a mean of 4.91. The use of the Western blot technique and insect bioassay confirmed the presence of the *cry1A(b)* gene product.

19. The tests were repeated for the CP4 EPSPS and GOX proteins, which would have been produced had the second plasmid integrated into the maize genome, but none were found in any of the plants as the genes were not transferred. No test was performed for the NPTII protein because, again, the gene was not transferred.

20. The Committee noted that processing the GM maize for human food use would denature the genetic material and any gene products present in the grain.

21. There are no receptors for the δ-endotoxin *cry1A(b)* gene product) in mammalian intestinal cells and it, therefore, does not pose a safety issue. However, its safety was confirmed in an acute, single dose mouse gavage study with the trypsin resistant core of the *cry1A(b)* gene product. No adverse effects were found at the maximum oral dose administered, 4000 mg/kg.

Unintentional changes

22. The Committee examined data for the gene constructs used, including the expected effect, site of expression of the introduced genes and the method used for the genetic modification.

23. The plasmid vector was introduced into the maize tissue culture line Hi-II by a biolistic method. Buffered plasmids were precipitated onto a gold or titanium carrier and 'shot' into the target maize tissue. One plasmid contained one copy of a *cry1A(b)* gene, and one copy of an *nptII* antibiotic resistance marker gene. The other plasmid, which did not integrate, contained one copy of a *cp4 epsps* gene, one copy of a *gox* gene, and an *nptII* antibiotic resistance marker gene.

24. The putative GM micro-colonies which resulted from the transformation process were grown on a medium in the absence of glyphosate. Transformation event MON 810 was chosen for commercial exploitation.

25. Southern hybridisation analyses were used to determine the nature, number and molecular stability of the inserts of transformation event MON 810. The analyses confirmed that there is only one intact copy of the *cry1A(b)* gene together with the #hsp 70, E35 S and *nos* [1] sequences present.

26. The Committee was able to conclude that no unintentional changes or effects had taken place during the genetic modification procedure.

27. It also considered whether or not there had been any unintentional changes in the composition of the grain from the GM plants or in the plants themselves as a result of the genetic modification. The Company provided data on compositional analyses, morphological studies and agronomic field tests conducted in the USA and Europe. The compositional analyses included, fatty acid profile, protein, amino acid, crude fibre, ash and moisture contents, determined for grain and forage of GM and non-GM maize. The agronomic characteristics data included yield, vigour, disease and insect susceptibility. The Company was able to satisfy the ACNFP that there had been no unintentional changes in the composition of the grain from the GM plants nor in the plants themselves as a result of the genetic modification.

28. As ELISA analyses had shown the detection of small amounts of the *cry1A(b)* gene product in the whole plant, grain, forage and leaf of the GM line, its allergenic potential was investigated. Searches of the GenBank, EMBL, PIR and the SwissProt databases using the FASTA program did not show any homology of the *cry1A(b)* gene product with known allergens.

Stability of the genetically modified organism

29. The Company presented evidence for a normal Mendelian inheritance of the new gene over seven generations. This satisfied the Committee that there had been stable integration of the introduced genes into the genome of the GM maize.

Genetic transfer

30. The Committee evaluated the risk of genetic transfer of the novel gene present in the GM maize to consumers or to their gut micro-organisms through consumption of products made from the processed grain. It concluded that there could be no hazard caused by genetic transfer from processed products because the transferred genes were not controlled by bacterial regulators and that processing would destroy the function of any DNA present.

DISCUSSION

31. A number of issues were taken into account by the Committee when it evaluated the safety of the processed food products derived from the GM maize. These issues included the analytical composition of the grain and plant material from the GM maize, the analytical composition or characterisation of the transferred DNA, the search for any unintentional or pleiotropic changes which may have occurred as a result of the genetic modification, confirmation that the introduced gene was inherited in a stable manner, the evaluation of the GM maize's agronomic performance and the effect of processing on the transferred DNA and its product.

32. The data submitted on the composition of the grain and plant material reassured the Committee that the composition of the GM maize does not differ from that of conventionally-bred maize.

33. Consideration of the data on the genetic modification procedure satisfied the Committee that no unintentional changes had taken place at a molecular level, as did the data provided on the morphology at a macroscopic level.

34. Data from the genetic segregation studies demonstrated that the introduced gene was inherited into the GM maize genome in a stable manner. However, as there is little experience in predicting the effect of genetic drift on the metabolism of any line of plants, whether genetically modified or conventionally-bred, the Committee has asked that the seed composition, including the amino acid profile, the fatty acid profile of the oil from MON 810 and the lines derived from it using conventional plant breeding techniques should be monitored over time. The Company has agreed to provide such information.

CONCLUSIONS

35. The Committee concluded that the processed products obtained from the GM maize, line MON 810 and the inbred and hybrid lines derived from it through conventional plant breeding techniques, were substantially equivalent to and safe to use in food and would not differ in composition from those obtained from conventionally-bred maize.

36. The FAC has examined the labelling implications of the GM maize line, MON 810, its derivatives and the processed products obtained from them. The FAC has concluded that no special labelling should be required for any of the products, but wished to encourage the voluntary provision of information in response to public interest.

REFERENCES

1. Application, dated 20 May 1996, from Monsanto Europe SA, via European Commission, to obtain a consent to market the maize throughout the EU under the Deliberate Release Directive 90/220/EEC OJ L117 8 May 1990 page 15. The Deliberate Release Directive is administered in the UK by the Department of the Environment. This submission will be deposited in the British Library, identified as BL SUP 11112.

2. The Genetically Modified Organisms (Deliberate Release) Regulations 1992. SI 1992 No 3280. HMSO, London: ISBN: 011 033152 4 and the Genetically Modified Organisms (Deliberate Release) Regulations 1995. SI 1995 No 304. HMSO, London: ISBN: 011 052433 0.

GLOSSARY

Bacillus thuringiensis: a soil-living bacterium.

biolistic method: method of transferring DNA into cells by the use of an explosive charge.

buffered: a solution resisting pH change on addition of acid or alkali.

constitutive promoter: promoter which enables a gene to be expressed throughout a plant.

denature: to irreversibly alter the structure of a protein by chemical or physical means.

DNA: deoxyribonucleic acid which is found in all living cells and contains the information for cellular structure, organisation and function.

δ-endotoxin: protein toxic to certain insects.

enhancer region: site of eukariotic (organisms more complex than bacteria and blue-green algae) DNA which a protein may bind to and turn on transcription of a particular R gene.

ELISA: enzyme-linked immunosorbent assay.

expression: manifestation of the genetic material of an organism.

functionality: ability to perform a function or job.

gene construct: gene sequence made *in vitro*, containing trait genes and associated regulatory sequences.

gene: a unit of inheritance, usually understood to mean a region of DNA which encodes one function.

genome: a complete ensemble of the genes in a cell, e.g., chromosomal and plasmid elements.

hybrid: offspring of a cross between two genetically dissimilar parents.

inbreds: plants which have been self-pollinated over several generations and are nearly genetically uniform.

intron: an apparently non-functional segment of DNA.

Mendelian inheritance: the inheritance of trait genes from one generation to the next in accordance with a pattern first described by Gregor Mendel.

morphological: form or appearance of an organism.

plasmid: loop of DNA found in bacteria and some other organisms, e.g. yeast, that replicates independently of the chromosomes.

pleiotropic: the effect of a single gene addition/substitution or mutation on a number of phenotypic characteristics in an organism.

promoter: the key control element that enables a gene to be expressed by transcription into mRNA.

selectable marker gene: genes with a phenotype that can be selected for in gene transfer experiments. Selectable marker genes are used to enable the selection/deletion of neighbouring sequences in a gene construct.

Southern hybridisation analysis (Southern blot): a technique for detecting the presence of specific DNA.

synthetic: formed artificially by chemical synthesis.

terminator: DNA sequence which terminates the synthesis of mRNA.

transformant: a plant derived from a cell in which the genetic modification has been successful and which contains the introduced gene construct.

vector: DNA segment which can incorporate foreign DNA and transfer it between organisms.

Western blot analysis: a technique for detecting the presence of specific protein.

APPENDIX V

ACNFP REPORT ON GRAIN FROM MAIZE GENETICALLY MODIFIED FOR INSECT RESISTANCE

INTRODUCTION

1. In July 1996, the ACNFP considered an application from the Northrup King Company[1] (the company) for food safety clearance of a genetically modified (GM) maize, *Zea mays L.* The maize has been genetically modified for resistance to the larvae of lepidopteran* insect pests, including those of the European corn borer *(Ostrinia nubilalis)*, South-western corn borer *(Diatraea grandlosella)*, fall army worm *(Spodoptera frugiperda)* and corn earworm *(Helicoverpa zea)* and for tolerance to the herbicide glufosinate-ammonium. The submission sought clearance of grain from the original transformant Bt11 crossed with the Northrup King Company inbred line #2044, and any inbred and hybrid lines derived from it which contained the introduced genes.

2. The GM maize contains a synthetic, truncated form of the *cry1A(b)* gene from *Bacillus thuringiensis* subsp. *kurstaki* strain HD-1, which is also referred to as the Btk gene, for resistance to lepidopteran insect pests and the *pat* selectable marker gene for tolerance to the herbicide glufosinate-ammonium.

3. In its evaluation of the submission, the Committee focused on the consumer safety aspects of the processed products from the GM maize. The ACNFP compared data on the composition of the unprocessed grain from the GM maize with isogenic conventionally-bred lines. The Committee also used a comparative approach in its consideration of supporting information on plant morphology and on agronomic performance.

4. The Food Advisory Committee (FAC) has examined the labelling implications of the product and has concluded that in accordance with the proposed EC Regulation on Novel Foods and Food Ingredients[2], no special labelling should be required for this product. The GM maize contains no ethically sensitive copy genes, and the introduced genes would be destroyed during processing of the grain.

5. The Company has applied through the UK for a consent to market, the maize throughout the EU under the Deliberate Release Directive 90/220/EEC[3], which is administered in the UK by the Department of the Environment. It is not intended that the GM maize be grown in the UK, but it is likely that if this consent to market is granted, the unprocessed grain may be imported, and the processed grain will be used in food and feed products sold in Britain.

BACKGROUND

6. Maize has a long history of use as a human food and more recently as an animal feed. It has been cultivated for several thousand years in the Americas and in the last 500 years has become a major food and feed crop elsewhere, including Europe.

7. Maize is susceptible to a range of fungal diseases and insect pests, as well as competition from weeds. The European corn borer, *O. nubilalis*, is a major insect pest of economic importance affecting maize in Europe and in North America.

*Technical terms not explained in the body of the report are underlined where they appear for the first time and are explained in the glossary; explanations are used in the context of the report and should not be taken as general definitions.

Several generations of moths are produced during the growing season and, during the course of development, the larvae tunnel into the plant, often into the stalk near the maturing ear, resulting in severe yield loss in the crop either through stalk breakage or through the ear dropping to the ground. There is also a correlation between infestation by lepidopteran larvae of maize stalks and ears and fungal diseases of these plant parts, as it is believed that the feeding cavities created by the insect larvae provide entry points for fungi.

8. The soil bacterium *Bacillus thuringiensis* produces crystalline proteins, δ-endotoxins. which when broken down in the insect gut, produce core fragments which exhibit highly specific insecticidal activity. The *cry1A(b)* gene in the GM maize is a synthetic, truncated form of the naturally occurring gene in *B. thuringiensis* var. *kurstaki* strain HD1, and codes for a CRY 1 protein which is particularly effective against the larvae of lepidopteran insect pests, in particular the European corn borer.

9. In addition to the *cry1A(b)* gene, the GM maize also contains the *pat* gene from the soil micro-organism *Streptomyces viridochromogenes* which codes for the enzyme phosphinothricin-N-acetyl transferase (PAT). This enzyme confers tolerance to the herbicide glufosinate-ammonium, and acts as a selectable marker enabling selection of plants which contain the introduced genes.

PRODUCTION OF GENETICALLY MODIFIED LINES

10. The Company used a standard transformation system, to introduce the plasmid vector from which the *ampR* gene had been excised, into the maize. The plasmid contained one copy of a synthetic, truncated *cry1A(b)* gene and one copy of the pat gene. After selection of the transformants by growing the tissue on a medium containing L-glufosinate, the surviving tissue was regenerated and the pollen from these GM plants (Bt11) used to pollinate the conventionally bred line #2044. The progeny from this cross were used in conventional backcross and hybrid breeding programmes to develop the GM hybrid lines.

11. The plasmid vector used to introduce the gene constructs into the maize contained the synthetic, modified *cry1A(b)* gene from *B. thuringiensis* subsp. *kurstaki* HD-1 (Btk) regulated by the 35S CaMV promoter and the *nos 3'* terminator from *Agrobacterium tumefaciens*. The IVS6 intron from *Z. mays* is fused between the 35S promoter and the *cry1A(b)* gene to enhance expression of the *cry1A(b)* gene. It also contains the phosphinothricin – acetyl transferase *(pat)* gene from *S. viridochromogenes* regulated by the 35S CaMV promoter and the *nos 3'* terminator from *A. tumefaciens*. The IVS2 intron from *Z. mays* is fused between the 35S promoter and the *pat* gene to enhance expression of the *pat* gene.

12. The wild-type *cry1A(b)* gene has been modified to enhance expression in plants by truncation and DNA sequence changes. This modification did not result in any amino acid sequence changes to the δ-endotoxin protein encoded by the gene. The native DNA sequence of the *pat* gene was modified to optimise expression in plants. This modification was designed to lower the GC content of the DNA, but to leave the amino acid sequence of the encoded protein unaltered.

13. The *ampR* gene which confers resistance to the antibiotic ampicillin was also inserted into the plasmid vector, and was used as a selectable marker when the plasmid was being generated in *Escherichia coli*. This gene was excised from the plasmid prior to transformation. In addition, the gene is under the control of a bacterial promoter and would not be expressed in plant cells. Thus, neither the *ampR* gene or the gene product are present in the GM maize.

PROCESS DESCRIPTION AND USE

Processing

14. The grain from the GM maize is intended to undergo conventional processing, either wet milling or dry milling. Wet milling, used to produce starch, glucose syrup and oil involves steeping the grain before milling. The maize germ separated out after the wet milling contains 40-50% oil which is recovered by mechanical and solvent processing. Dry milling is used to produce maize meal and flour for use in brewing, breakfast cereals, snack foods and bakery products.

Use

15. Products derived from maize, in particular starch and glucose syrup, are ubiquitous in the UK diet where they are found in processed foods, soft drinks and confectionery. The maize flour is used in brewing, breakfast cereals, snack foods and bakery products; the oil is used in frying and salad oils and in margarine. Products from the GM maize would be expected to replace those from conventionally-bred maize, no new uses or markets are envisaged.

Specification

16. There are no inherent toxic or antinutritional factors present in maize which would need to be controlled by a specification.

SAFETY ASSESSMENT

17. In assessing the safety of the GM maize, the Committee compared data on the GM maize with data from conventionally bred maize. The Committee considered:

- the safety of the intentional changes;
- whether any unintentional changes had occurred as a result of the genetic modification;
- the genetic stability of the GM maize; and
- the possibility of transfer of genetic material from the processed maize products to the human consumer.

Intentional changes

18. Data reviewed by the Committee indicated that the only changes were the presence of the intentionally introduced genes: the synthetic, truncated *cry1A(b)* and *pat* genes. The intentional effects of the genetic modification were to provide resistance to certain lepidopteran pests, in particular the European corn borer, and provide tolerance to the herbicide glufosinate-ammonium.

19. Using the sensitive ELISA test, the Company has analysed field grown GM maize for the presence of the products of the introduced genes. The leaf husk stalk and kernels were analysed for the presence of the *cry1A(b)* gene product. The highest levels were found in the leaves, with the other plant tissues having significantly lower levels of the protein. The *pat* gene product was found at significant levels in the leaf, silk and tassel, but was below the level of detection (1ng/ml) in the stalks, roots, pollen and kernels.

20. The Committee noted that the processing of the GM maize would destroy any gene products present in the grain.

21. The mammalian gut does not possess receptors for the δ-endotoxin, therefore the presence of this protein does not pose a safety issue. However, the safety of this gene product was confirmed by an acute oral toxicity study in mice, using the trypsin-resistant core protein produced in *E. coli*. There were no adverse effects observed at the maximum oral dose administered of 4000 mg/kg.

Unintentional changes

22. Data on the genetic modification procedure used to produce the GM maize was presented for assessment by the Committee. This included information on the gene construct used, including the expected effect, site of expression of the introduced genes and the method used for the genetic modification.

23. Southern blot analyses of the F1 plants from the initial #2044 x Bt11 cross showed that a single copy of each gene is present in the GM line. Further genetic molecular studies indicated that the insertion site is on the long arm of maize chromosome 8.

24. The Committee was satisfied that no unintentional changes had taken place during the genetic modification procedure. It then considered whether there had been any unintentional changes in the GM plants themselves or the grain produced from them due to the genetic modification.

25. The Company presented results from compositional analyses on grain harvested from four hybrid lines derived from Bt11 and four conventionally-bred hybrid lines with the same genetic background but lacking the introduced genes. These lines were chosen to represent middle and late maturity groups. Starch, protein, oil and fibre content was analysed using near infra-red spectroscopy (NIR). Statistically significant differences were found between the middle maturity GM hybrids and the corresponding conventionally bred lines for protein content. This was the result of the conventional backcross breeding that had been carried out. Values for all the parameters measured fell within the ranges cited in published literature.

26. Fatty acid and amino acid analyses were performed on twenty grain samples and the results presented to the Committee. The Committee was satisfied that, apart from normal variations, there were no significant differences in composition.

27. Field trials were conducted in the USA and Canada to assess any differences in the morphological and agronomic characteristics between the GM and the non-GM maize. The Company provided data to satisfy the ACNFP that no statistical differences were found between the GM and conventionally bred hybrids for characteristics considered to be unrelated to the *cry1A(b)* function. This information provided further evidence that no unintentional changes had taken place.

28. Although the gene product of the *cry1A(b)* gene is within the size range of known allergens, it does not show any other characteristics common to allergens. Database searches for homology to known allergens were carried out for the *cry1A(b)* gene product and the *pat* gene product. No homology with known allergens was found. The Committee was satisfied that there was no evidence to suggest that either gene product had any allergenic potential.

Stability of the genetically modified organism

29. The ACNFP was provided with data from genetic segregation studies to demonstrate that the GM maize was genetically stable. Mendelian inheritance patterns, characteristic of dominant genes, were observed for the introduced genes. The absence of segregation of the genes indicated that the genes are located at a single locus.

Genetic transfer

30. The Committee considered the risk of genetic transfer to the human consumer of the novel genes present in the GM maize from consumption of products made from the processed maize. It concluded that since processing would destroy any DNA present, the risk of genetic transfer from processed products could be discounted.

DISCUSSION

31. The Committee based its safety assessment of the processed food products derived from GM maize on a number of factors. This involved looking for any unintentional changes that may have occurred due to the genetic modification, confirmation that the introduced genes were inherited stably and that there was no risk of genetic transfer to the human consumer. However, as there is little experience in predicting the effect of genetic drift on the metabolism of any kind of plants, whether genetically modified or conventionally-bred, the Committee has asked that the seed composition, including the amino acid profile, and the fatty acid profile of the oil from the Bt11-#2044 cross and the lines derived from it using conventional plant breeding techniques should be monitored over time. The Company has agreed to provide such information. The Committee also considered information on the composition of the grain.

32. The information provided on the genetic modification procedure satisfied the Committee that no unintentional changes had taken place at the molecular level. The Committee particularly noted that the *ampR* gene had been excised from the plasmid prior to transformation and, therefore, was not present in the GM maize.

33. Data from field trials to assess the morphology and agronomic performance of the GM maize in comparison with conventionally-bred lines, provided evidence to reassure the ACNFP that no changes had taken place other than the intentional changes due to the introduced genes.

34. Consideration of the analytical data on the composition of the GM grain compared with non-GM grain satisfied the Committee that no changes had taken place that were not attributable to the backcross breeding process.

CONCLUSIONS

35. The Committee concluded that the processed products from the inbred and hybrid GM maize lines derived from Bt11 were safe for use in food and would not differ in composition from those from conventionally-bred maize.

36. The Food Advisory Committee (FAC) has examined the labelling implications of the GM maize and the processed products derived from the grain. It has concluded that no compulsory labelling would be required. However, the FAC encourages companies to provide voluntary information in response to public interest.

REFERENCES

1. Submission from Northrup King Company dated July 1996. This submission will be deposited in the British Library, identified as BL SUP 11111.

2. European Commission. Proposal for a Council Regulation (EEC) on Novel Foods and Novel Food Ingredients. OJ C190 27 July 1992 page 3. And subsequently amended.

3. EC Council Directive of 23 April 1990 on the deliberate release into the environment of genetically modified organisms (90/220/EEC) OJ L117 8 May 1990 page 15. Implemented in the UK by the Genetically Modified Organisms (Deliberate Release) Regulations 1992. SI 1992 No 3280. HMSO, London: ISBN: 011 033152 4 and the Genetically Modified Organisms (Deliberate Release) Regulations 1995. SI 1995 No 304. HMSO, London: ISBN: 011 0524330.

GLOSSARY

amino acid: building blocks of proteins.

DNA: deoxyribonucleic acid which is found in all living cells and contains the information for cellular structure, organisation and function.

δ-endotoxin: crystalline, protein toxic to certain insects.

ELISA: enzyme linked immunosorbent assay.

F1 plants: the first progeny of a cross between plant lines.

GC: guanine, cytosine are bases found in DNA.

gene construct: gene sequence made *in vitro* containing trait genes and associated regulatory sequences.

genotype: the genetic makeup of a cell or organism.

homology: having identical or similar structure.

hybrid lines: plant lines produced from a cross between genetically dissimilar parents.

inbred: plant that has been self pollinated over several generations and is nearly genetically uniform.

intron: an apparently non-functional segment of DNA.

isogenic: having the same genotype.

lepidopteran: part of the insect order *Lepidoptera*, including butterflies and moths, having scaled wings.

(gene) locus: position of a gene on a chromosome.

Mendelian inheritance: the normal inheritance of trait genes from one generation to the next in accordance with a pattern first described by Gregor Mendel.

plasmid: loop of DNA found in bacteria and some other organisms, e.g. yeast, that replicates independently of the chromosomes.

promoter: the key control element that enables a gene to be expressed by transcription into mRNA.

selectable marker gene: genes with a phenotype that can be selected for in gene transfer experiments. Selectable marker genes are used to enable the selection/deletion of neighbouring sequences in a gene construct.

Southern blot: a technique for detecting the presence of specific DNA.

synthetic: formed artificially e.g. by chemical synthesis.

terminator: DNA sequence which terminates the synthesis of mRNA.

transformant: a plant derived from a cell in which the genetic modification has been successful and which contains the introduced gene construct.

truncated gene: part of a gene.

trypsin-resistant: resistant to enzymic digestion by the enzyme trypsin.

wild-type: the standard gene, or the standard genotype or phenotype of an individual, that is found in a wild or natural population.