REPORT ON PROCESSED PRODUCTS FROM GENETICALLY MODIFIED (GM) INSECT PROTECTED MAIZE

EXECUTIVE SUMMARY

This report summarises the safety review carried out by the Advisory Committee on Novel Foods and Processes (ACNFP) on processed products from genetically modified (GM) maize, *Zea mays* L. The GM maize line, MON 810 - developed by Monsanto - and the subject of this review is resistant to a lepidopteran insect pest, the European corn borer. The GM maize will not be grown in the UK, but the grain is expected to be imported from continental Europe for processing in the UK.

The GM maize contains a bacterial gene, *cry1A(b)*, which codes for a protein which is toxic to certain insect species.

The ACNFP concentrated its evaluation on the food safety issues of the processed products from the GM maize, but also considered other factors which could be pertinent to their safety. These factors included the identification of any unintentional changes which may have occurred as a result of the genetic modification procedure and confirmation of whether or not the introduced genes were stable when inherited by later generations.

The Committee was content with the data on the composition of the unprocessed grain, and that there were no compositional differences between the grain of the GM maize and that of the conventionally-bred maize. The product of the *cry1A(b)* gene was detected at levels of up to 0.0009% (fresh weight tissue) in certain parts of the maize plant. However, the Committee noted that the processing procedures would destroy the functionality of the protein product of the gene.

Detailed data on the genetic modification procedure satisfied the ACNFP that no unintentional changes had taken place at a molecular level and the Committee was further reassured that no unintentional changes had taken place by the results of morphological and agronomic studies which compared lines derived from the GM maize to those of conventionally-bred varieties. Data from genetic segregation studies also satisfied the ACNFP that the introduced genes were inherited in a stable manner.

The ACNFP was able to conclude from the information provided, that the processed products obtained from the GM maize and its progeny would be substantially equivalent to, and as safe for food use as, those obtained from conventional maize.

The Food Advisory Committee (FAC) has examined the labelling implications of the products, taking into account the proposed EU Regulation on Novel Foods and Novel Food Ingredients agreed between Member States. It has concluded that no special labelling should be required for any of the processed products, but expressed a wish to encourage the voluntary provision of information in response to public interest.

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

REPORT ON PROCESSED PRODUCTS FROM GENETICALLY MODIFIED (GM) INSECT PROTECTED 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, truncated 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 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.

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* 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.

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)*.

8. The transformation was achieved by the introduction of a plasmid containing a synthetic, truncated 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 35 S 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 - NOS 3^1 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, and in 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, µg/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, 4,000 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 3[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, and 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 Food Advisory Committee (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. 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 XXXX.

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 harmless soil-living bacterium.
biolistic method:	method of transferring DNA into cells by the use 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.
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 gene.
δ-endotoxin:	protein toxic to certain insects
ELISA assay:	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 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, eg, 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, eg 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 blot analysis:	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.
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