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Opinion of the Scientific Committee on Food on a request to place genetically modified sweet maize line Bt11 on the market

(expressed on 17 April 2002)

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Opinion of the Scientific Committee on Food
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1. TERMS OF REFERENCE

The Committee is asked to assess the safety, from the point of view of consumer health, of the genetically modified sweet maize line Bt11. The Committee is also invited to focus its deliberations on the issues raised in the comments made by Member States' authorities.

2. BACKGROUND

The Commission has received a request for authorisation for consent to place genetically modified sweet maize line Bt11 on the market. During the consultation period foreseen in the Regulation, Member States' Authorities have submitted comments/objections.

This opinion is based on an application to market Bt11 sweet maize, as fresh vegetables or after processing. The sweet maize line is derived from Bt11 field maize, food products from which already have market consent in the European Union, following notification (30 January 1998) according to article 5 of Regulation 258/97/EC. Field maize of line Bt11 was transformed to produce a truncated form of the δ-endotoxin Cry1A(b) of *Bacillus thuringiensis* var. *kurstaki*. This trait confers resistance to several relevant insect pests of maize plants. As a selective marker for transformation, DNA encoding phosphinothricin acetyltransferase (PAT) was also introduced into line Bt11. This results in plants that detoxify the herbicide glufosinate ammonium and thus resist its action. The transgene cassette was transferred from field maize to sweet maize by traditional breeding methods. Since both lines are derived from one transformation event, the applicant has supplied data derived from experiments on both field- and sweet maize.

On 10 February 1998, the Scientific Committee on Plants published its opinion on hybrids derived from Bt11 field maize[1]. This opinion describes in detail the molecular genetics of the construction of the Bt11 line. Safety aspects were also considered, paying particular attention to the potential for gene flow and the safety of gene products and metabolites and the question of substantial equivalence. In addition, the environmental impact of growing hybrids from Bt11 field maize was considered. The opinion concludes that the use of imported genetically modified seed carrying the Bt11 event can be considered as safe as utilising seed from non-genetically modified plants. The European Commission subsequently published its decision to grant consent to the placing on the market of genetically modified maize of line Bt11 pursuant to Council Directive 90/220/EEC[2] to be used as any other maize grain but not for cultivation. A request for the cultivation of Bt11 maize including sweet maize under Directive 90/220/EEC was filed. The Scientific Committee on Plants is of the opinion that there is no evidence to indicate that the placing on the market for cultivation purposes of maize line Bt11 and varieties derived from this line by conventional crosses between Bt11 line and maize lines

other than genetically modified ones, is likely to cause adverse effects on human health and the environment[3].

In February 1999, the petitioner requested authorisation to place on the market food products (fresh and processed) derived from a genetically modified, insect protected sweet maize referred to as "Bt11 sweet maize", under Regulation 258/97/EC[4] of the European Parliament and of the Council. Subsequently, Member States have commented on this application, raising a number of issues, to which the applicant has responded in a second dossier, dated January 2001. The issues raised are summarised below:

- the scope of the request – does the application cover maize that may be eaten without processing of any kind?

- molecular characterisation regarding the site of insertion and whether extraneous fragments have been incorporated during the Bt11 transformation event;

- molecular characterisation regarding quantification of the *Bacillus thuringiensis* toxin, Cry1A(b), and PAT proteins in maize kernels;

- compositional analysis on Bt11 sweet maize;

- toxicity studies;

- labelling.

3. EVALUATION

The application presented by the petitioner follows the Scientific Committee on Food (SCF) recommendations 97/618/EC[5]. These concern the scientific aspects and the presentation of information necessary to support applications for placing of novel foods and novel food ingredients on the market. The maize plants that are the subject of this application fall into Class 3.1 of these guidelines, dedicated to GM plants and their products.

The present evaluation has taken the structured schemes that were previously provided by the SCF as a guide to identify the different aspects required to establish the safety of the novel food. In addition to the information submitted by the petitioner, the comments of the Member States on the initial assessment report made by the Competent Authority in The Netherlands have also been taken into account.

3.1 Specification of the novel food

3.1.1 Description of the novel food

Field maize was transformed to produce a truncated δ-endotoxin from *Bacillus thuringiensis* linked to the production of phosphinothricin acetyltransferase, an enzyme that confers resistance to glufosinate ammonium. The δ-endotoxin confers resistance to corn borer insect pests. Transgenic field maize from event Bt11 was used to introduce insect resistance and herbicide tolerance into sweet maize by conventional breeding.

Bt11 sweet maize produces a truncated form of the δ-endotoxin Cry1A(b) of *Bacillus thuringiensis* var. *kurstaki*. To aid selection of the original transformation event, the gene encoding this insecticidal protein was linked with a gene encoding production of phosphinothricin acetyltransferase, derived from the soil bacterium *Streptomyces viridochromogenes*. Both genes have been engineered to enhance expression in plant cells. Certified reference samples of Bt11 maize are available from the SIGMA company as products numbered 09754, 09757 and 17947.

3.2 Effect of the production process applied to the novel food

After harvesting, sweet maize may be sold unprocessed for domestic cooking, or may be canned or frozen or subjected to further processing. Sweet maize sold for consumption as a fresh vegetable is harvested at an immature stage. Some of the outer leaves of the cob are stripped by hand and the cobs trimmed for packaging. Processing for canning involves blanching by exposure to steam for a short period. During canning, maize is subjected to temperatures greater than 110^{o} C for more than ten minutes. Precise details of the processing vary by geographical location.

3.3 History of the organism used as the source of the novel food

Niebur (1993) has published a detailed description of maize (*Zea mays* ssp. *mays* L) plants[6]. Maize has been grown in Europe for about 500 years but consumption of sweet maize is a rather new behaviour for Western Europeans. Three main categories of sweet maize are currently available:

- the “sugary types”, also called “normal” or “standard”;
- the “sugary enhanced types”; and
- the “supersweet types”, also called “shrunken” or “supersweet”.

Field maize lacks the sugary gene, *su* that confers sweetness on the sugary type sweet maize varieties. Sugary enhanced lines have an additional gene, *se*, which modifies the action of the *su* gene product. The supersweet phenotype results from the expression of another gene, *shrunken-2*, independent of the *su* gene and inherited in a recessive fashion. Sugary and sugary enhanced varieties are grown mostly for canning, where the sweetness can be adjusted without the need for added sugar. Supersweet hybrids represent 75% of the fresh market and are also used in the preparation of frozen sweetcorn.

3.3.1 Proposed uses

Sweet maize is grown exclusively for human consumption although processing by-products are used as animal feedstuffs. It is eaten in different forms:

- as a fresh vegetable after steaming or barbecuing;
- as a frozen vegetable after cooking;
- as a canned product;
- dehydrated in powder form, for example as an ingredient of vegetable soup powders.

3.3.2 Labelling

The applicant proposes the following labelling for its products, in line with Commission Regulation 49/2000/EC[7]:

- fresh Bt11 sweet maize will be labelled;

- foods and food ingredients produced from sweet maize will not be labelled if they contain material derived from Bt11 sweet maize, which, together with any material placed on the market pursuant to Regulation 258/97/EC[4] derived from other genetically modified organisms, is present in a proportion no higher than 1% of the food ingredients individually considered or food comprising a single ingredient, provided this presence is adventitious;

- food or food ingredients produced from Bt11 sweet maize will be labelled if DNA or proteins resulting from the genetic modification are present; food and food ingredients produced from Bt11 sweet maize in which neither DNA nor proteins resulting from the genetic modification are present will not be labelled.

3.4 Effect of the genetic modification on the properties of the host organism

3.4.1 Intended effects

Maize of line Bt11 contains a modified *Btk* gene, encoding the δ-endotoxin Cry1A(b) of *Bacillus thuringiensis* var. *kurstaki* HD-1. The modifications include truncation and alterations in nucleotides. These modifications enhance gene expression in plants. Alterations in the nucleotide sequence do not lead to amino acid changes. The *pat* gene, encoding tolerance to the herbicide glufosinate ammonium, was derived from *Streptomyces viridochromogenes* Tu494. This gene was also altered to optimise expression in plants; in particular the G+C content of the bacterial gene was reduced. Again the amino acid sequence of the gene product was not altered.

The vector used for transformation was pZO1502, a commercially available derivative of plasmid pUC18. Transformation of plant cells was achieved using a linear fragment of DNA obtained by restriction endonuclease digestion of plasmid pZO1502 using *Not* I introduced into the plant by biolistics. The site of insertion maps on the long arm of Chromosome 8. The *Not* I fragment used for transformation was separated from a smaller *Not* I fragment of the bacterial vector. This smaller fragment carries a gene encoding a TEM β-lactamase and it was not used in transforming plant cells. Consequently, the plant does not contain a gene encoding ampicillin resistance. Zimmermann *et al.* (2000)[8] have reported a specific method for detecting Bt11 maize, based upon quantitative PCR at the site of integration.

As a consequence of the transformation, Bt11 maize resists attack from corn borers such as *Ostrinia nubilalis* the European corn borer, through expression of the *Btk* gene, encoding *Bacillus thuringiensis* δ-endotoxin. It is also tolerant of the herbicide glufosinate ammonium through expression of the *pat* gene of *Streptomyces viridochromogenes*, encoding phosphinothricin-N-acetyltransferase.

3.4.2 Compositional analysis

To identify unintended effects from the introduction a range of analyses were performed to assess the level of key nutrients and potential anti-nutrients in the genetically modified maize. These were compared with similar analyses on conventional maize plants.

Information has been supplied concerning kernels of Bt11 sweet maize based on data observed on 3 varieties with 2 replicates/variety in one growing season in comparison with the composition of kernels of isogenic lines. No differences have been detected in parameters concerning major and minor components. Parameters compared in this study included moisture, protein, fat, ash, sugars, total carbohydrates, calorific value, dietary fibre, vitamin A, vitamin C, sodium, potassium and iron. No gross differences between Bt11 sweet maize and its conventional counterparts were observed. In particular, the level of total and specific carbohydrates has been measured: the level of sugar amounted to 6.5% of the fresh weight and 9.4% of the dry matter, without any difference between the transformed and the isogenic, non-transformed lines.

Additional analyses conducted on Bt field maize kernel in two countries (France and the USA) have compared the composition of the kernels of:

- 2 varieties grown in greenhouses in France during 1999. Analysis was of dry matter, crude protein, starch, cellulose, fatty acids and amino acids;
- 2 varieties, grown in one location during 1995 for crude protein, oil, starch and fibre;
- 2 varieties, 2 replicates, 6 locations for crude protein, oil, starch and fibre;
- 20 samples from different varieties, grown in two different States in the USA, for amino acids and fatty acids;
- 2 varieties for minerals and vitamin determinations.

In general and with the exception of one case in which the content in crude protein of the transformed kernel was significantly lower than that of the isogenic control sample, no statistical differences have been observed.

On the basis of substantial equivalence established for the Bt field maize and additional data on Bt11 sweet maize produced by traditional breeding from Bt field maize it was concluded that Bt11 sweet maize kernels are substantially equivalent to non-transformed lines except for the new traits or proteins.

3.5 Genetic stability of the GM plant

Studies on the genetic stability of the insertion that led to the formation of the Bt11 line were carried out on Bt11 field maize. These demonstrated that insect resistance and herbicide tolerance demonstrate the Mendelian inheritance associated with a single dominant locus. The generation of Bt11 sweet maize further demonstrates the stability of the transformation event. Southern blot analysis shows that DNA isolated from plants after six backcrosses cannot be differentiated from plants only back-crossed for three generations, either by the mobility of

the inserted DNA or by the intensity of the signal. These data further support the genetic stability of plants generated by the transformation event denominated Bt11. The Southern blot data provided by the applicant to demonstrate the genetic stability of Bt11 plants shows a second faint band present in DNA from transformed plants that has been digested with *Eco*RV. This is probably due to contamination of the sample. One Member State raised the possibility that this was due to hybridisation with a small fragment of inserted DNA that had become incorporated at a different site from the site of insertion of the functional cassette. Other samples digested with this restriction endonuclease do not show the faint band that triggered the question from the Member State. Having examined further Southern blots, the Committee is content that the problem is due to contamination of the sample used in the original application.

3.6 Specificity of the expression of the novel genetic material

Measurements of the levels of introduced proteins reported in the original application were made on Bt11 field maize. Four hybrids were used for measurements and these were grown at two locations. These experiments showed that the highest level of δ-endotoxin was found in leaf tissue. As the plants mature, the presence of the δ-endotoxin drops in leaves, although the level of δ-endotoxin in the kernel, at approximately 3 micrograms Cry1A(b)/gram fresh weight, remains relatively constant. The kernels have significantly less δ-endotoxin present than does leaf tissue. Nanogram quantities per gram of the PAT protein were found in leaf and tassel tissue but this protein was not detected in other tissues including pollen and kernels.

Table 1 of this opinion provides a summary of the range of information provided in the original application to support the demonstration of a "substantial equivalence" (except for the new traits or proteins); Table 2 summarises additional studies undertaken by the applicant. On the basis of the data supplied in the original application dossier, no conclusion can be drawn from a comparison of Bt11 sweet maize with kernels from parental or isogenic lines. Subsequent to the applicant receiving comments from the Member States, a second dossier was submitted. This dossier included additional data, including replicates for the year 2000, on Cry1A(b) and PAT protein content in the kernels of Bt11 sweet maize in comparison with its isogenic parent. The Cry1A(b) protein levels in husks and kernels were found to vary, showing no consistent trend. No PAT protein was found in kernels, pollen or stalk material derived from transgenic plants. Data have been collected at different stages of harvesting and have also been measured on leaves (9.3 µg/g fresh weight for Cry1A(b) protein and <0.9 ng/g fw, for PAT, respectively). The Cry1A(b) protein has not been detected (<2 ng/g) after canning.

3.7 Considerations of gene transfer from the GM plant

The transformation event that generated Bt11 maize did not involve the use of a bacterial antibiotic resistance gene. The two bacterial genes present in Bt11 maize have been modified to optimise expression in plants rather than bacteria. It is expected that some introduced DNA will be present in food after cooking but in some foods the processing of maize in making the final product will have destroyed the introduced DNA.

3.8 Anticipated intake/extent of use of the novel food

Bt11 sweet maize will be eaten in the same manner as conventional maize, as fresh, cooked cobs, as canned sweetcorn, as a frozen food and as an ingredient of processed foods. Less than 1% of EU sweet maize production is used as an ingredient in processed foods. There are

45,000 tons of fresh maize eaten, as “corn on the cob”, with the British consuming approximately half of this crop. Maize for freezing amounts to 76,000 tons with the British consuming slightly more than half of this output. Germans, consuming 30% of the crop, are the largest consumers of the 298,000 tons of canned sweet maize, followed by the French who consume 27% of this crop, then the British who eat 22% of the canned sweet maize crop.

3.9 Information from previous human exposure to the novel food or its source

Maize has a long history of safe use within the European Community.

3.10 Nutritional evaluation

To evaluate the nutritional substantial equivalence between the Bt11 sweet maize and its non-transgenic comparators, the applicant has submitted four different studies to the Competent Authorities in The Netherlands. Based on questions raised by the Competent Authorities of four member countries, additional information was provided.

A range of components, summarised in Tables 1 and 2 of this opinion, has been measured in Bt11 maize compared with non-transgenic controls as well as maize varieties in commercial usage in different studies.

These measurements were executed with the use of various hybrids, different experimental designs and sometimes with different analytical methods. A total of ten studies was presented with different components analysed extracted from seven independent breeding studies in the USA and France over four seasons.

The Committee is of the opinion that despite the large number of studies, the company did not commission systematic information on the composition of the genetically modified or control plants. The Committee agrees, however, that the distinction between the results of the sweet maize and field maize is not relevant for the assessment as long as the appropriate corresponding non-modified maize is used as control.

The Committee does not consider the observed significant differences in some of the components of some studies, such as palmitic acid, stearic acid, cystine and arginine as relevant for the evaluation.

On the basis of the results presented, the Committee concludes that, with respect to the nutritional evaluation, the genetically modified Bt11 maize is substantially equivalent to non-modified maize hybrids.

3.11 Microbiological information on the novel food

The processing of Bt11 sweet maize will not differ from that for conventional maize regarding the presence of the associated microflora and thus food products derived from Bt11 sweet maize are not expected to differ from those derived from unmodified maize plants.

3.12 Toxicological information on the novel food

3.12.1 Toxicological data on the PAT protein

PAT from an *Escherichia coli* expression system was tested for digestibility and inactivation in a simulated mammalian gastric fluid (SGF) at pH 1-1.2. As visualised following SDS-polyacrylamide gel electrophoresis, the PAT protein degraded immediately when pepsin was present. Upon dilution of pepsin to 0.01x the standard concentration, degradation appeared to be complete by 2 minutes. Within 1 minute, the enzymatic activity of the PAT protein was lost in SGF, with or without pepsin. Data from literature provide additional support for the finding that PAT is rapidly digested in simulated gastric fluid[9].

The acute oral LD_{50} of PAT and heat-inactivated PAT expressed in recombinant *E. coli* was determined in groups of 5 male and 5 female albino mice. No mortality occurred at the administered dose of 5,050 mg/kg body weight.

The PAT protein produced through expression in *E. coli* was of the anticipated size (ca. 22,000 mol.wt.), was immunoreactive with PAT rabbit polyclonal antibodies and had the anticipated N-terminal amino acid sequence corresponding to the coding sequence introduced into another transgenic maize line (Ciba Seeds' Event 176) apart from the first 17 amino acids, which result from the expression of the *pat* gene in *E. coli*. A database search demonstrated that the PAT protein shares no significant sequence homology with any known protein toxins, bacterial endotoxins, allergens or venoms.

3.12.2 Toxicological data on the Cry1A(b) protein

Following incubation in gastric fluid for 2 minutes, more than 90% of the Cry1A(b) protein was broken down and the biological activity decreased by 74-90%. Incubation with intestinal fluid scarcely had any effect.

Single oral doses of Cry1A(b) protein up to 4,000 mg/kg body weight did not show signs of toxicity in groups of 10 male and 10 female albino mice.

The trypsin-resistant fragment of the full-length Cry1A(b) protein produced in *E. coli* was used in these studies. This was shown to be equivalent to the trypsin-resistant fragment of the truncated Cry1A(b) protein in transgenic maize with respect to the apparent molecular weight, antigen/antibody interaction, trypsin stability, N-terminal amino acid sequence, protein glycosylation and the biological activity against the European corn borer and the Corn Earworm (*Heliothis zea*).

In its evaluation of Bt11 maize, the Committee also considered ancillary information from animal feeding studies. These included a study in which laying hens were fed a diet containing 64% maize kernels and other studies in which ruminants were fed whole transgenic plant material and ensiled maize. No adverse effects were seen in these studies.

3.12.3 Comment

The toxicological information included data from digestibility studies with simulated gastric fluid and single dose oral toxicity studies with mice on the PAT and Cry1A(b) proteins. Such studies provide only limited evidence for safety.

The PAT protein is not expressed in Bt11 sweet maize grain. There are, nevertheless, additional toxicological data provided in the context of other applications confirming the safety of the PAT protein. The PAT protein belongs to the class of acetyltransferase enzymes common to all plant and animal cells and therefore occurring as natural components of the

human diet. No reports have been found of toxicity or allergenicity associated with acetyl transferases as a class.

In the case of the Cry1A(b) protein a number of feeding studies with Bt11 maize products, some of which also contain the PAT protein, in laying hens, lactating cows and steers over a few weeks confirmed the absence of visible adverse effects of either the Cry1A(b) protein or unexpected changes in the transgenic hybrids. Furthermore, it has been shown that there are no receptors for the protein δ-endotoxin of *Bacillus thuringiensis* on the surface of mammalian intestinal cells[10].

Additional data from literature corroborate the conclusion that consumption of transgenic Cry1A(b) protein poses no health concerns. Mice and rabbits, for example, received Cry1A(b) *ad libitum* through their drinking water during 28 days (1.5 and 15 µg/kg bw/day) and 31 days (60 µg/kg bw/day), respectively. No adverse effects were observed with regard to weights of body, -liver, and -kidney, in addition to haematology and histopathology of the GI tract[10]. Intravenous administration of a single dose of Cry1A(b) to mice (0.3 mg/kg bw) did not cause adverse effects in these animals during the subsequent 14 day-period with regard to mortality, body weight, and gross pathology[11].

In conclusion, there are no indications that the Bt11 sweet maize is less safe for human consumption than maize that has not been genetically modified.

3.12.4 Residue assessment

The safety of Bt11 maize varieties treated with the herbicide glufosinate ammonium has been considered by the Scientific Committee on Plants in its safety evaluations under the 90/220/EEC Directive[1,3].

4. CONCLUSIONS

The Committee has assessed the safety of Bt11 sweet maize modified to resist insect pests and to tolerate the herbicide glufosinate ammonium from the point of view of consumer health. The assessment has been made within the framework of the Regulation on Novel Foods 258/97/EC.

On the basis of the information supplied in the application and further material supplied by the applicant in response to queries raised by Member States and in the light of the published literature, the Committee concludes that Bt11 sweet maize is as safe for human food use as its conventional counterparts.

5. REFERENCES

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Table 1. Compositional analysis studies on Bt11 maize

Annex B. **Bt11 Sweet grain**	Measures on: Bt [Cry1A(b)] protein: 2.29 µg/g fresh and 0.8-1.46 µg/g (canned) PAT protein <9ng/g fw.
Annex D. **Bt11 Sweet grain**	Furfural, p-coumaric acid, ferulic acid, myo-inositol, raffinose.
Annex E. **Bt11 Sweet grain**, 2 sites, 1998 (France)	Energy, carbohydrates, crude protein, ether extract, amino acids, fatty acids, trypsin inhibitors, phytic acid.
Annex F. **Bt11 Sweet grain,** 2 sites, 1999 (USA)	Ca, Fe, vitamins A, E and C.
Annex G. Bt11 Field forage , 2 sites, 1995 (USA)	Dry matter, crude protein, ADF, NDF, TDN, Ca, P, K and Mg.
Annex H. Bt11 Field grain 3 sites, 1995 (USA)	Cu, Mg, Zn, Mn, folic acid, niacin, vitamins B_1 and B_2.

Table 2. Summary of supplementary studies used to compare Bt11 maize with non-transgenic maize comparators

Annex 0. **Bt11 Sweet grain, kernels**	Quantification of Bt [Cry1A(b)] and PAT protein at harvest Bt [Cry1A(b)]: (1999) = 1.97 (1.08-2.98); (2000) = 1.05 (0.73-1.46)µg/g fw. PAT <0.9ng/ in 1999 and 2000.
Appendix 5. **Bt11 Sweet grain,** 1998 (USA)	Level of Bt [Cry1A(b)] protein: Leaf: 56d. post planting: 9.34 µg; 105d: 8.25 µg/kernels; 105d +28d: 1.97 to 2.36 µg/g fw Bt [Cry1A(b)] protein not detected after canning.
Appendix 7-1. **Bt11 Sweet grain kernels** fresh and canned kernels, 3 varieties, 2 replicates (trans/isogenic), 1998 (USA)	No difference in the content of: Moisture, crude protein, fat, ash, sugars, CHO, calories, fibre, vitamins A and C, minerals Na, K, Ca, Fe. Canning decreases the sugar content from 6.5 to 1.85 % of fw and from 9.4 to 2.4 % of dw.
Appendix 7-2. Bt11 Field maize kernels, 2 varieties (trans/isogenic) Greenhouse, (France)	No difference in the content of: Moisture, crude protein, ash, starch, cellulose, xanthophyl, fatty acids and amino acids.
Appendix 7-3. Bt11 Field maize kernels 1995 (USA) a) 2 varieties, one location, 1995 (USA) b) 2 varieties, 2 replicates, 6 locations (id) c) 20 samples, trans/isogenic, 2 States (USA) d) 2 varieties	 No difference in protein, oil, starch and fibre; No difference in protein, oil, starch and fibre; No difference in fatty acids and amino acids; No difference in Cu, Mg, Mn, Zn, folic acid, niacin, vitamins B_1 and B_2.