

Opinion On The Potential For Adverse Health Effects From The Consumption Of Genetically Modified Maize (*Zea Mays L*) (Expressed On 13 December 1996)

N.B. Updated to include references 21 February 1997

Terms of Reference

To consider whether there is reason to believe that the genetic modification of the maize lines of *Zea mays L.* will have adverse effects on the health of human consumers of the maize. The Committee is asked to give particular attention to the concerns raised by certain Member States with respect to any potential toxic or allergenic effects associated with the introduced genes and any potential adverse effects from the non-expressed β -lactamase gene.

Background

The Commission has submitted a proposal for a Council Decision concerning the placing on the market of genetically modified maize (*Zea mays L.*) with the combined modification for insecticidal properties conferred by the Bt-endotoxin gene and increased tolerance to the herbicide glufosinate ammonium pursuant to Council Directive 90/220/EEC.

Member States have expressed a variety of concerns which have led the Commission to request the opinions of the Scientific Committee for Food, the Scientific Committee on Animal Nutrition (SCAN) and the Scientific Committee on Pesticides to examine the dossier as concerns safety matters within their remits.

Evaluation

This evaluation addresses transgenic maize CG-00526-176. The submission included the administrative data necessary for its unique identification and for record keeping purposes ⁽¹⁾.

1. Characterisation of the inserted genes and their expression

On the basis of the information provided, the inserted genes (CRYIA(b), bar and bla) and their expression are characterised as follows:

- two plasmids have been inserted into the same locus in two to five gene copies. Their presence has been demonstrated by southern blotting.
- the product of the inserted genes, the CRYIA(b) protein (Bt-delta-endotoxin) from the two genes is expressed in the leaves and in pollen respectively but its concentration is below 5 ppb. However the toxin is apparently expressed in the kernel since in a bioassay study with the European corn borer, insecticidal activity was observed in fresh, but not in dried or re-hydrated kernels. Phosphinothricin acetyl transferase (PAT), responsible for the increased tolerance to the herbicide glufosinate ammonium, was not detectable in kernels, but traces were found in the plant. On the basis of current knowledge the prokaryotic *bla* gene construct (β -lactam antibiotic resistance) would not be expected to be expressed in the maize plant.

2. Toxicological assessment

2.1 Products of the *cryIA(b)* gene encoding Bt-delta-endotoxin:

Both the truncated maize *cryIA(b)* gene and the native *cryIA(b)* gene produce protoxins which undergo proteolytic cleavage in the mid-gut of insects resulting in the same active toxin. The native CRYIA(b) protein and the corresponding protein from transgenic maize, have similar target range effects thereby demonstrating the likelihood of similar biological properties for the two proteins.

The native CRYIA(b) protein (65% purity) has been tested for acute toxicity in mice and no mortality has been reported at a dose of 5 g per kg body weight. Furthermore, reports in the literature ⁽²⁾ of a 28 day study with mice on CRYIA(b) protein, did not reveal any mammalian toxicity at 1.5 g per kg body weight, the only dose level tested. Moreover, it was demonstrated that CRYIA(b) was rapidly degraded *in vitro* in simulated gastric fluid containing pepsin at pH 1-1.2. Since the CRYIA(b) product level in kernels is below 5 ppb, dietary exposure to CRYIA(b) from maize kernels is expected to be very low.

2.2 Products of the *bar* gene encoding phosphinothricin acetyl transferase (PAT)

The enzyme phosphinothricin acetyl transferase (PAT) is not likely to present safety problems. The quantitative level of PAT in kernels is very low. Its enzymatic function is specific to a substrate which is not naturally present in humans, namely phosphinothricin, and furthermore, it is degraded and inactivated in simulated gastric fluid containing pepsin at pH 1-1.2. It is therefore unlikely to retain any enzymatic activity *in vivo*. Furthermore, no sequence homology between the PAT protein and known toxins has been found. The native PAT protein (51% purity) has been tested for acute toxicity in mice and no toxicity has been reported at a dose of 5 g per kg body weight.

3. Nutritional assessment

The newly expressed proteins have no nutritional significance and the composition of the transgenic maize is within the known biological variation of the composition of the host plant.

It is concluded that transgenic maize (event 176) is substantially equivalent to the corresponding non-transgenic maize from a nutritional point of view.

4. Allergenicity

The Committee expressed an opinion covering general aspects of food intolerance including allergenicity at its 98th Meeting ON 21/22 September 1995 ⁽³⁾.

The amino acid sequences of the proteins CRYIA(b) and PAT do not show any homology with proteins of known allergenic potential. Moreover, the new gene products appear to be readily degraded by simulated gastric fluid *in vitro* ^(1,4). Comparison of the protein profiles of the transgenic maize and the native maize by SDS gel electrophoresis and iso-electric focusing give no indication that the maize protein have been changed. There are no indications that the prolamine proteins have been altered which is of relevance for patients with coeliac disease, but this possibility cannot be excluded. It is therefore concluded that it is unlikely that the genetic modification changes the potential for allergenicity in the kernel of the transgenic maize. This does not exclude the possibility that there will be individuals allergic to this variant of maize, just as there are individuals who are allergic to traditionally produced variants of maize.

5. Horizontal gene transfer

Studies of the transfer of intact genes from plant materials to micro-organisms have demonstrated an extremely low likelihood of transfer, suggesting that the probability of this event occurring in practice is very small ⁽⁵⁾. There is no evidence that genes from plants have ever been transferred under natural conditions to bacteria. In addition, the degradation of DNA occurring during processing of maize and its intestinal passage reduces this possibility even further. Bacteria with natural ampicillin resistance exist in the environment as well as in human intestines. Nevertheless, in the view of the SCF, the acquisition of additional resistance from this transgenic maize by intestinal

bacteria needed special attention ^(6,7,8). The Commission convened an expert consultation on the subject, where SCF and SCAN together posed a number of questions to the specialised experts ⁽⁹⁾. From this consultation it is confirmed that the degeneration of DNA through processing of maize and its products and the enzymatic decomposition of DNA in the gastrointestinal tract of man and animal makes the residual amount of intact DNA which could contain a gene very small. Furthermore, the probability for transfer of plant DNA by transformation to bacteria is small, as is the chance for the transformed DNA to become functional in the bacteria. Even if this unlikely sequence of events, each of which has a very low probability, were to take place, it would have no detectable additional effect as the *bla* gene is already widely spread in nature including human and animal gastrointestinal tracts. Should transformed bacteria harbouring the high copy plasmid pUC18 of the transgenic maize arise, they would not have a competitive advantage and therefore would not lead to their spread and interference with therapy by beta-lactam antibiotics.

6. Assessment of secondary changes

In addition to the products of the inserted genes, a number of comparisons between the transgenic maize plant and the equivalent non-transgenic plant have been performed. A series of morphological parameters were examined as well as yield. DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one), one of the natural defence compounds of the maize plant towards, for example, the European corn borer was also examined. No significant differences in morphology, yield or DIMBOA content were observed. Differences between the concentrations of other components of toxicological or nutritional relevance in the genetically modified plant and the parent plant were statistically significant in some instances but, even so, the measured levels were still within the published reference biological variation for maize. Animal feeding studies with the genetically modified maize supported its substantial equivalence

Conclusions

On the basis of the information provided the Committee draws the following conclusions:

- The transgenic maize is, except for the inserted traits, substantially equivalent to maize presently on the market.
- Animal feeding studies with the genetically modified maize support its substantial equivalence to the parent plant.
- No nutritional concerns are associated with the use of this transgenic maize.
- It is unlikely that the genetic changes introduce any new potential for allergenicity.
- No human toxicological concerns arise regarding the inserted traits based upon the toxicological and degradation data considered.
- The possibility that the product would add significantly to the already widespread occurrence of ampicillin resistant bacteria in animals and man is remote.

The latter conclusion was based on the balance of evidence available at this time to the Committee, which derived from theoretical considerations and laboratory studies. A stepwise assessment regarding the gene construct itself, its distribution and persistence in maize and its products, the possibility of its transfer from maize to gram negative bacteria, and the possibility that it would function in such bacteria led to the conclusion that the risk of bacterial transformation is extremely low.

The Committee was conscious of the general question of the use of genes coding for antibiotic resistance in marker gene constructs in the development of novel foods and proposes to scrutinise the future needs and application of marker genes.

References:

1. Full dossier submitted by Ciba Geigy in two volumes (Volume 1 consisting of parts A, B1-9 and C1-11; Volume 2 consisting of parts C12-18, D and E1-15) [CS/NF/MAIZE/1]
Maize genetically modified to protect itself against corn borers and containing an ampicillin resistance marker gene with a bacterial promoter. Information by Ciba Geigy Limited. [CS/NF/MAIZE/9]
Ciba Seed's responses to scientific committee's requests (dated September 19 and 20, 1996) for additional

- information re: event 176 "BT" maize, prepared by D. Vlachos.
2. Noteborn HUB, Kuiper HA 1995: Safety evaluation of transgenic tomatoes expressing *Bt* endotoxin. In: Application of the principles of substantial equivalence to the safety evaluation of foods or food components from plants derived by modern technology. Report of a WHO workshop, Copenhagen, World Health Organisation, Food Safety Unit.
 3. Scientific Committee for Food (98th Meeting); Report on adverse reactions to foods and food ingredients. Opinion expressed on the 22 Sept 1995. European Commission, Brussels.
 4. Metcalf DD, Astwood JD, Townsend R, Sampson HA, Taylor SL, and Fuchs RL 1996: Assessment of the Allergenic Potential of Foods Derived from Genetically Engineered Crop Plants. *Crit Rev. Food Sci. Nutr.* 36(S):S165-S186.
 5. Schlüter K, Fütterer J, Potrykus I 1995: "Horizontal" gene transfer from a transgenic potato line to a bacterial pathogen (*Erwina chrysanthemi*) occurs - if at all - at an extremely low frequency. *Biotechnology* 13: 1094-1098
 6. Schubert R, Lettmann C, Doerfler W 1994: Ingested foreign (phage M13) DNA survives transiently in the gastrointestinal tract and enters the bloodstream of mice. *Mol. Gen. Genet.* 242:495-504
 7. Webb V, Davies J 1994: Accidental release of antibiotic-resistance genes. *Trends Biotechnol.* 12(3):74-75
 8. Ciba-Geigy genetically modified maize. Additional information from MAFF, UK concerning the risk of transfer of the intact *bla* gene. [CS/NF/MAIZE/14]
 9. Report of an expert hearing on questions identified by the Scientific Committee for Food and Scientific Committee for Animal Nutrition (SCAN) on the evaluation of the potential for increased ampicillin resistance from GMO Maize (CG-00526-176). Brussels 6. December 1996 [CS/NF/MAIZE/20]

Further references:

- SCF opinions on the assessment of novel foods I. Recommendations concerning the scientific aspects of information necessary to support applications for placing on the market of novel foods and novel food ingredients. [CS/NF/GUID/1-FINAL]
- SCF opinions on the assessment of novel foods II. Recommendations concerning the scientific aspects of the presentation of information necessary to support applications for placing on the market of novel foods and novel food ingredients. [CS/NF/GUID/3]
- SCF opinions on the assessment of novel foods III. Recommendations concerning the scientific aspects of the preparation of the initial assessment reports on applications for placing on the market of novel foods and novel food ingredients [CS/NF/GUID/4 Rev.2]
- The scientific basis for the classification of the PUC-plasmids according to Directive 90/219/EEC. [CS/NF/MAIZE/18]