EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Scientific Opinions
C3 - Management of scientific committees II; scientific co-operation and networks

SCIENTIFIC COMMITTEE ON PLANTS

SCP/GMO/232-Final
22 September 2000

OPINION OF THE SCIENTIFIC COMMITTEE ON PLANTS ON THE SUBMISSION FOR PLACING ON THE MARKET OF GENETICALLY MODIFIED MAIZE (ZEA MAYS) LINE GA21 WITH TOLERANCE TO GLYPHOSATE HERBICIDE NOTIFIED BY MONSANTO

(NOTIFICATION C/ES/98/01)

(Opinion adopted by the Scientific Committee on Plants on 22 September 2000).

Rue de la Loi 200, B-1049 Bruxelles/Wetstraat 200, B-1049 Brussel - Belgium - Office: B232 6/18.
Telephone: direct line (+32-2)296.58.91, switchboard 299.11.11. Fax: 295.73.32.

Internet: Jean.Ferriere@cec.eu.int
F:\WebDev\4299.doc

1. TITLE

OPINION OF THE SCIENTIFIC COMMITTEE ON PLANTS ON THE SUBMISSION FOR PLACING ON THE MARKET OF GENETICALLY MODIFIED MAIZE (ZEA MAYS) LINE GA21 WITH TOLERANCE TO GLYPHOSATE HERBICIDE NOTIFIED BY MONSANTO (NOTIFICATION C/ES/98/01).

[Application for consent to market maize (*Zea mays*) line GA21 tolerant to glyphosate herbicide and the seed of any progeny (inbred lines or hybrids) derived from crosses of the product with any traditionally bred maize.]

2. TERMS OF REFERENCE

The Scientific Committee on Plants is asked to consider whether there is any scientific reason to believe that the placing on the market of genetically modified maize (*Zea mays*) line GA21 tolerant to glyphosate herbicide with the purpose to be used as any other maize is likely to cause any adverse effects on human health and the environment within the scope of Directive 90/220/EEC.

3. BACKGROUND

Directive 90/220/EEC[1] requires an assessment to be carried out before a product containing or consisting of genetically modified organisms (GMOs) can be placed on the market. The aim of the assessment is to evaluate any risks to human health and the environment connected with the release of the GMOs. For genetically modified plants, the assessment must be based on the information outlined in Annex II B of Directive 90/220/EEC and take account of the proposed uses of the product.

Following the entry into force of the Regulation on novel foods and novel food ingredients (EC No. 258/97) on 15 May 1997[2], in order for this maize and its derived products to be placed on the market for food purposes, the requirements of the Regulation will have to be satisfied. Such a regulation does not exist on novel feeds and novel feed ingredients.

The evaluation of the herbicide glyphosate and its metabolite AMPA[3] is in progress under Directive 91/414/EEC[4]. Maximum residue levels (MRLs) for residues of glyphosate were already set in the Council Directive(s) 98/82/EEC[5]. These MRLs and the possible effect of herbicide residues on human and animal health have to be reviewed in the framework of the mentioned evaluation under Directive 91/414/EEC.

---

1 OJ N° L 117 of 08.05.1990 p. 15.
2 OJ N° L 43 of 14.02.1997 p. 1.
3 Aminomethylphosphonic acid.
4 OJ N° L 230 of 19.08.1991 p. 1.
5 OJ N° L 290 of 29.10.1998 p. 25, amending Annex II to Directive 76/895/EEC and the Annex to Directive 90/642/EEC.

4. OPINION

Question

The Scientific Committee on Plants (SCP) is asked to consider whether there is any scientific reason to believe that the placing on the market of genetically modified maize (*Zea mays*) line GA21 tolerant to glyphosate herbicide with the purpose to be used as any other maize is likely to cause any adverse effects on human health and the environment within the scope of Directive 90/220/EEC.

Opinion of the Committee

The Committee, after examining the information and data provided in the dossier and using available background knowledge underpinning the areas concerned, considers that there is no evidence to indicate that the placing on the market of the modified maize *(Zea mays)* line GA21 with tolerance to glyphosate herbicide is likely to cause any adverse effects on human health and the environment.

Scientific background on which the opinion is based

4.1 Proposed uses

The notification covers production and commercialisation of glyphosate tolerant maize (line GA21) and the seeds of any progeny (inbred or hybrid lines) derived from this line by conventional breeding methods. The grain and derived products from this line will be distributed by traders and processors and the proposed uses will be the same as for any other maize. However, the use of this modified maize for human food is not considered in this notification.

4.2 Description of the product

Maize line GA21 has been developed by Monsanto and the DEKALB Genetics Corporation to have tolerance to glyphosate (Roundup®) herbicide. Maize line GA21 was produced by the introduction of a modified 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) gene from maize. The insertion of the genetic material was performed by particle acceleration technology with a DNA fragment containing a rice-actin promoter and intron sequence, the modified EPSPS gene, fused to an optimised transit peptide sequence and the termination signal NOS 3' from *Agrobacterium tumefaciens*.

4.3 Molecular/Genetic Aspects

4.3.1. *Transformation technique*

Based on the information provided, maize line GA21 was produced by particle acceleration technology. A DNA fragment, as detailed below, was introduced into embryogenic maize cells. The maize plant tissue that was the recipient of the introduced DNA was a cell culture designated AT224 initiated from immature embryos of an inbred maize line (AT). Transformants were selected by their ability to survive and grow in the presence of glyphosate.

4.3.2. *Vector constructs*

Information provided in the dossier provides a detailed description of both the transformation fragment and the plasmid vector from which it was isolated. Agarose gel isolated *Not*1 restriction fragment of plasmid vector pDPG434 was isolated from agarose gel and utilised for transformation of maize line GA21.

The donor genes in this restriction fragment of plasmid pDPG434 used for transformation have been well characterized and contained the following components:

(i) *The modified maize EPSPS gene:* The modified maize EPSPS (mEPSPS) gene was used to provide tolerance to glyphosate. The mEPSPS gene was produced by cloning the wild-type EPSPS gene from maize and introducing two mutations by *in vitro* mutagenesis which have been characterised. The deduced amino acid sequence identity between the mEPSPS protein and the wild-type maize EPSPS is greater than 99.3%.

(ii) *Chloroplast transit peptide sequence:* The mEPSPS gene was fused to chloroplast transit peptide (CTP) sequences based on sequences isolated from maize and sunflower ribulose l,5-bisphosphate carboxylase oxygenase (RuBisCo) genes to produce an optimised transit peptide (OTP). The deduced amino acid sequence of the mEPSPS protein with the OTP was determined and resulted in an additional methionine at the N-terminal end of the mEPSPS protein.

Based on kinetic analysis the dossier states that the mEPSPS enzyme purified from *Escherichia coli* interacts with its substrates similarly to the wild-type maize EPSPS enzyme.

(iii) The promoter used was from the rice-actin 1 gene contained on a 1.37 kb fragment that also contained the first intron. The terminator region used was contained on a 0.24 kb fragment of the 3' non-translated region of the nopaline synthase gene from the T_1 plasmid of *Agrobacterium*.

Genes present in the pDPG434 plasmid backbone but not present in the *Not*1 restriction fragment used for transformation included *lac*Z, ColE1(ori) and the *bla* gene (ß-lactamase gene from *E. coli* plasmid pBR322).

4.3.3 *Transgenic construct in the genetically modified plant*

Data presented in the dossier to characterise the transgene in the plant is rigorous and included Southern blotting, genomic clone isolations, DNA sequencing, PCR[6], western blotting and bioinformatics to confirm the following points:

1. A single plasmid insert has occurred containing an 18.5 kb fragment.

[6] Polymerase chain reaction.

2. None of the plasmid backbone has been inserted.

3. Genetic elements inserted are linked in succession from 5' to 3'.

4. The mEPSPS cassette begins with the last 148 bp of the 3' end of the rice-actin promoter plus the complete rice intron followed by the OTP, full length mEPSPS gene and NOS 3' terminator sequence.

5. It appears that three complete copies of the mEPSPS cassettes are inserted which are functional (northern and western blots positive).

6. One mEPSPS insert is a partial cassette containing the full length actin promoter and its intron, a full length OTP but a truncated mEPSPS gene. Expression of this truncated form cannot be precluded but western blots show no protein of the correct size that could arise from the translation of truncated mEPSPS mRNA.

7. One partial mEPSPS cassette is present containing only the rice-actin promoter but truncating before the start of the actin intron.

8. Two putative ORFs[7] were identified proximal to the insert both derived from maize sequences. Sequence analysis indicates no homologies with known toxins or allergens. Northern blot and the correct controls were used to show that transcription of these ORFs does not occur in maize line GA21.

9. Traits are stably inherited in a Mendelian fashion.

4.4 Safety Aspects

4.4.1 *Potential of gene transfer to (pathogenic) micro-organisms*

The *bla*-gene coding for ampicillin resistance present in the original vector pDPG434, has been eliminated in the actual transformation process of GA21. Consequently there is not even a theoretical risk of this gene being transferred from the plant material to micro-organisms.

Regarding the EPSPS gene present in GA21, which is under the control of a plant promoter (from rice-actin 1 gene), the possibility of its expression in the very unlikely event of its being transformed to a bacterium, would be slight. Even if some recombination event brought the gene under the control of a bacterial promoter, there would not be any foreseeable harmful consequences since the gene product is non-toxic and would not interfere with any therapeutic measures.

[7] Open reading frames.

4.4.2 *Safety of gene products/metabolites*

Intended use of GA21 maize as animal feed:

Grains, forage and by-products from GA21 maize are intended for the same uses as conventional maize, the main use being animal feed. This includes by-products from wet milling (e.g. corn gluten feed and meal) and dry milling (e.g. maize grits, maize meal, maize flours).

Toxicity and degradation of the novel gene product in animals and humans:

The modified maize EPSPS gene encodes a 47.4 kDa protein consisting of 445 amino acids. The sequence is 99.3% similar to the endogenous maize EPSPS and shows high similarity (>80%) to EPSPS from other food crops. The mEPSPS does not share similarity in its amino acid sequence with known toxins and allergens (PIR, SwissProt, Genbank databases). EPSPS isolated from leaves of GA21 maize, including mEPSPS (70% of activity), is rapidly degraded *in vitro* in artificial human gastric and intestinal fluids (<15 seconds and <1 minute, respectively). The applicant states that ruminants have a gut environment with a bacterial proteolytic system, which is more hostile to proteins than in non-ruminants. An acute oral toxicity study has been carried out in mice, with a single oral application of maximally 45.6 mg recombinant mEPSPS (*E. coli*)/kg bodyweight. No effects on bodyweight, feed consumption, and clinical signs were observed. At sacrifice, gross pathology was examined. No adverse effects were found.

The applicant calculates that a dairy cow fed a diet with maximum maize forage, will ingest 4.9 mg mEPSPS/kg bodyweight at most. This is by a factor 9 lower than the highest dose applied in the acute oral mice toxicity study. The highest possible dose ("worst case") for animals fed maize grains (0.23 mg/kg) is 200 times lower than the highest dose for mice, according to the applicant.

Studies on the equivalence of the *E. coli* mEPSPS and the plant mEPSPS have been described. These studies involved the analysis of N-terminal amino acid sequences, molecular weight (SDS-PAGE), densitometry, western blot analysis, enzyme activity, and protein glycosylation. It could be concluded that *E. coli* mEPSPS used for toxicity tests was comparable to plant mEPSPS. The mEPSPS enzyme was more efficiently inhibited by glyphosate than the CP4 EPSPS enzyme in kinetic investigations. Affinities for the substrate PEP, on the other hand, were comparable. The expression profile of mEPSPS in GA21 maize by employing the rice-actin promoter would account for the high glyphosate resistance of GA21 maize in the field. The immunoreactivity (ELISA, western blot) of *E. coli* mEPSPS and plant EPSPS were comparable.

In addition, a feeding study has been performed on chickens. Seven groups of 40 male or female animals were fed an experimental diet from day 1 to 38 (males) and 40 (females). The 7 diets included either GA21 maize, the parental maize line from 2 locations, or 4 commercial maize lines. No relevant differences were found between GA21 and its parental lines with respect to weight increase, feed conversion, and fat pad weight at study termination. No other studies have been performed with target animal species.

Residue assessment:

The residues of glyphosate and the main metabolite aminomethylphosphonic acid (AMPA) in the grain of resistant maize plants were reported to be < 0.05 - 0.34 mg/kg (range) and < 0.05 - 1.4 mg/kg (range) respectively; the residues in fodder were reported to be 1.8 - 41 mg/kg (range) and < 0.05 - 4.7 mg/kg (range) respectively at a pre-harvest interval of 6-8 days. The residues of glyphosate and AMPA in forage (green) from resistant maize were reported to be < 0.05 - 0.52 mg/kg (range) and 0.06 - 1.1 mg/kg (range) respectively at 50 days pre-harvest interval according to the reported registered use outside of the EU. These levels do not raise toxicological concerns given the WHO[8] recommended ADI[9] of 0.3 mg/kg body weight for humans.

Feeding studies with livestock animals (dairy cattle, pigs and poultry) have been reported by the JMPR[10] (1986). Animals were fed mixtures of glyphosate and AMPA (3+1) at 10, 30 and 100 ppm in the diet for 30 days. Taking the reported results into account and the glyphosate and AMPA residue levels in maize grain and forage, no detectable residues of glyphosate and AMPA are expected in meat, fat, liver, milk and eggs; residues of glyphosate in the kidneys of ruminants and pigs can occur but they are covered by the MRLs fixed in Directive 98/82/EC; AMPA residues may be expected in kidney of ruminants at or about the limit of determination. There were no adverse reactions in the animals in the reported feeding studies.

It can be concluded that the metabolism of glyphosate in genetically modified plants is similar to that in unmodified plants, and that residue levels of glyphosate and AMPA on genetically modified plants are of no toxicological concern.

Expression levels of the novel genes:

The content of endogenous and modified EPSPS (mEPSPS) in transgenic GA21 maize ranged from 46.6-210.4 μg/g fresh weight (average 118.7 μg/g) in forage and from 1.4-4.9 μg/g (average 3.2 μg/g) in grains (validated ELISA assay). The content of mEPSPS has not been determined separately due to the near-identity of mEPSPS to wild-type EPSPS. The endogenous EPSPS could not be demonstrated in grains from the negative control line (limit of determination 0.8 μg/g). In addition, EPSPS was detectable in forage from four out of five locations, but amounts were too low for quantification (limit of determination 4 μg/g). The antibodies directed against petunia EPSPS (anti-pEPSPS), which were used for the ELISA, have been raised in goats. The rationale for employing pEPSPS as antigen is that insufficient mEPSPS is available for antibody production. Anti-pEPSPS cross reacts with mEPSPS in an Ouchterlony assay.

Northern blots (RNA) indicate that the truncated mEPSPS gene and two additional open reading frames are not expressed.

---

8 World Health Organisation.
9 Acceptable daily intake.
10 Joint Meeting on Pesticide Residues.

Composition:

Maize line GA21 and control lines, which are negative progeny of the same crossing event and do not contain mEPSPS gene, have been grown at five different locations in 1996. The plants were self-fertilised and forage and grains collected. Grains samples were obtained from 9 to 16 ears for each maize line and location. The forage samples were composed of 2 to 4 plants at the soft dough stage, without roots, for each line and location. Forage was analysed for proximate composition: ash, calcium, carbohydrates, fibre (acid detergent fibre [ADF] and neutral detergent fibre [NDF]) moisture, and phosphorus, protein, and fat. Grains were analysed for protein, fat, ash, carbohydrate (calculated), fibre (ADF and NDF), amino acid and fatty acid composition, calcium and phosphorus. A short description of the analysis methods is also presented. In 1997, grain and forage samples from 7 US locations and 4 European locations were assayed for the same parameters as in 1996. Plants used for compositional analysis in 1996 were not treated, while plants in 1997 were treated with the herbicide glyphosate (Roundup). No statistically significant differences were found.

Substantial equivalence:

Data on the chemical analysis of the grain have been particularly well documented on the basis of analysis performed on samples collected on 7 sites in the USA and on 4 sites in the South of Europe. Average values concerning the proximate analysis fall in the range of values indicated on the tables. Data on amino acids and fatty acids are also acceptable as well as complementary data concerning trace elements, trypsin inhibitors, phytic acid and Vitamin E. Data on proximate analysis of the forage have also been satisfactorily supplied and analysed, including data on NDF and ADF contents.

Using animal feeding studies, the data assessed, very clearly demonstrated the nutritional equivalence of grain to isogenic material, on the basis of growth performance and body composition of broilers receiving GM maize compared to isogenic grain for 40 days.

These data satisfactorily demonstrate the substantial equivalence of the GA21 maize to its conventional counterparts.

4.5 Environmental Aspects

4.5.1 *Potential of gene transfer in the environment*

The risk of gene transfer from the modified crop will be limited by the absence in Europe of sexually compatible plants of different species. There are indeed no wild plant species that are closely related to maize present in Europe and therefore the risk of genetic transfer to other species is remote. *Zea mays* is not an invasive crop but is a weak competitor with limited powers of seed dispersal. The mEPSPS protein is expressed in all tissues of the modified plant (roots, stem, leaves and pollen). Except for glyphosate tolerance of the maize line GA21, there appear to be no detected phenotypic differences between modified and non-modified maize in this wind-pollinated crop. Dispersal and outcrossing frequency should be no different from other maize varieties.

The probability of horizontal gene flow from plants to micro-organisms is considered to be extremely small, as noted in section 4.4.1. EPSPS genes are naturally present in soil microflora.

4.5.2 *Treatment of volunteers*

The risk of volunteer maize plants surviving to become established is considered to be remote. In growing areas that are free from winter frost, which will normally kill residual plants, any subsequent volunteers in the next crop may be controlled by agronomic practices including cultivation and the use of alternative non-selective herbicides.

4.5.3 *Safety to non-target organisms*

The available information indicates no qualitative differences in the susceptibility of GM and non-GM maize to insects and diseases. Although risks to birds and other non-target species that frequent corn fields are considered to be low, there is no direct data available from field experimentation. Risks to soil organisms and related functions through degradation of modified plant material and contamination of ground water are considered to be extremely low.

4.5.4 *Resistance and tolerance issues*

In view of the remote possibility of transfer of genes from GM maize to any different plant species, the development of tolerance to glyphosate is not considered to be a problem. The notifier should however establish a monitoring plan to identify unexpected and unusual events and analyse grower experiences, in order to develop and implement any necessary changes in crop management practices in response to the results of monitoring.

4.6 Conclusion

The Commission requested the Scientific Committee on Plants to consider whether there is any scientific reason to believe that the placing on the market of genetically modified maize (*Zea mays*) line GA21 tolerant to glyphosate herbicide with the purpose to be used as any other maize is likely to cause any adverse effects on human health and the environment within the scope of Directive 90/220/EEC. The Committee, after examining the information and data provided in the dossier and using available background knowledge underpinning the areas concerned, considers that there is no evidence to indicate that the placing on the market of the modified maize line (*Zea mays* GA21) with tolerance to glyphosate herbicide is likely to cause any adverse effects on human health and the environment.

5. DOCUMENTATION MADE AVAILABLE TO THE COMMITTEE

1. Questions to Monsanto regarding its submission for placing on the market of genetically modified maize (*Zea mays*) line GA21 with tolerance to glyphosate herbicide (Doc. SCP/GMO/239-rev.1).

2. Response to questions from the SCP (SCP/GMO/239-Rev. 1) submitted by Monsanto (Doc. SCP/GMO/246).

3. Response to question 2 (SCP/GMO/239-Rev. 1) submitted by Monsanto, (Doc. SCP/GMO/265).

4. Response to question 1 (SCP/GMO/239-Rev. 1) submitted by Monsanto (Doc. SCP/GMO/268).

5. A dossier comprising:
   - The objection of the Member States authorities;
   - A table summarising these objections;
   - The statement of the Spanish competent authorities;
   - The complete dossier submitted by Monsanto;
   - Additional information submitted by Monsanto in response to Member States comments and objections.

6. ACKNOWLEDGEMENTS

The Committee wishes to acknowledge the contributions of the working group that prepared the initial draft opinion:

GMO WG: F. O'Gara (Chair) and Committee Members: H. Davies, M-P. Delcour-Firquet, R. Hans, A. Hardy, S. Kärenlampi, H. Kuiper, H. Koepp, A. Silva Fernandes, G. Speijers, and invited experts: L.-A. Aumaitre, A. Chesson, B. Moseley, P. Puigdomenech Rossell, M. Vighi and A. von Wright.