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SCIENTIFIC COMMITTEE ON PLANTS

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OPINION OF THE SCIENTIFIC COMMITTEE ON PLANTS REGARDING "SUBMISSION FOR PLACING ON THE MARKET OF GLUFOSINATE TOLERANT MAIZE (*Zea mays*) TRANSFORMATION EVENT T25" BY THE AGREVO COMPANY (NOW AVENTIS CROPSCIENCE)

(NOTIFICATION C/F/95/12/07)

(Amended opinion adopted by the Scientific Committee on Plant, on 20 July 2001,
Replaces document SCP/GMO/006-Final adopted on 10 February 1998)

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1. TITLE

Opinion of the Scientific Committee on Plants regarding the submission for placing on the market of glufosinate tolerant maize (*Zea mays*) transformation event T25 by the AgrEvo Company, now Aventis CropScience (Notification C/F/95/12/07)
(Opinion adopted by the Scientific Committed on Plants on 20 July 2001)

2. TERMS OF REFERENCE

The Scientific Committee on Plants was asked to consider whether there is any reason to believe that the placing on the market of the T25 genetically modified maize with the purpose to be used as any other maize, is likely to cause any adverse effects on human health and the environment.

3. BACKGROUND

Directive 90/220/EEC foresees that an assessment has 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.

Following the entry into force of the Regulation on Novel Foods and Novel Food Ingredients (EC No 258/97) on 15 May 1997, 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.

Glufosinate ammonium has not so far been authorized for direct application onto maize plants. This issue comes under the scope of other Community legislation, such as Directive 91/414/EEC.

4. PROPOSED USES

The genetically modified maize is proposed to be used as any other maize.

5. DESCRIPTION OF THE PRODUCT

The dossier concerns maize line HE/89 transformation event T25, transformed with modified pUC18 plasmid and containing:

(i) one functional copy of a synthetic version of the *pat* gene from *Streptomyces viridochromogenes* (encoding phosphinothricin acetyltransferase - PAT), under the regulation of cauliflower mosaic virus (CaMV) 35S promoter and

terminator; the enzyme inactivates glufosinate ammonium, thereby conferring an increased tolerance to this herbicide;

(ii) a truncated, non-functional fragment of the bacterial *AmpR* gene (when complete, encodes β-lactamase conferring resistance to ampicillin);

(iii) one copy of Col E1 origin of replication of pUC18.

The product is seed derived from the transformation event and any progeny derived from crosses of event T25 (yellow dent maize) with traditional maize varieties. The product contains a gene conferring an increased tolerance to glufosinate ammonium thereby allowing the use of this herbicide for post-emergence weed control.

6. OPINION OF THE COMMITTEE

6.1. Molecular/Genetic Aspects

6.1.1. ***Transformation Technique:*** The glufosinate tolerant maize T25 was transformed with a plasmid containing the *pat* gene. The plasmid DNA was introduced into maize protoplasts by a direct uptake technique in the presence of polyethylene glycol. The selection was based on L-PPT (L-isomer of phosphinothricin). The approach seems appropriate.

6.1.2. ***Vector Construct:*** The inserted sequence contains a synthetic *pat* gene encoding phosphoinothricin acetyl transferase, a CaMV 35S promoter and terminator, part of *ampR* gene, and the ColE1 origin of replication of the pUC plasmid. The molecular analyses seem appropriate. The stability of the transformation event has been shown. The vector was totally sequenced. The complete sequence of the final recombinant plasmid is given as well as the sequences of native and synthetic *pat* genes. The vector construct is thus well characterized.

6.1.3. ***Transgenic Construct in the Genetically Modified Plant:*** PCR and Southern analyses have been performed on transformation event T25. They indicate quite clearly that approximately 25% of the *ampR* gene is missing and that the expression of ampicillin resistance protein would not occur.

6.2. Safety Aspects

6.2.1. ***Potential for Gene Transfer:*** *Antibiotic (ampicillin) resistance gene* — A truncated bacterial ampicillin resistance gene *ampR* is present in the plant together with a replication origin of an *E. coli* plasmid pUC18. Theoretically, this construct could be transformed into intestinal *Enterobacteriaceae*. However, as approximately 25% of the gene is missing, the expression of ampicillin resistance would not occur. Lack of β-lactamase activity is shown, confirming no risk of transfer of ampillicin resistance from the insert.

pat gene — The gene is under the control of a plant promoter which is not functional in bacteria. Consequently, in the unlikely event of gene transfer from the transgenic maize to intestinal bacteria, expression of the *pat* gene would not occur. Even if it is assumed that, due to genetic recombination events, the gene would be expressed in intestinal micro-organisms or in human or animal cells (the probability of which is remote), no negative effects are expected since the only known substrate of phosphinothricin acetyltransferase (PAT) is the herbicide glufosinate ammonium.

6.2.2. ***Safety of Gene Products/Metabolites (Food and Feed Aspects)****:*

Safety of gene products: The protein product of the phosphinothricin acetyltransferase gene is not present in humans, animals, intestinal micro-organisms or in traditional food and feed plants. The gene encoding PAT has been isolated from the gram-positive soil actinomycete *Streptomyces viridochromogenes*. The bacterium is not pathogenic for humans or animals. The nucleotide sequence has been modified to provide codons preferred by plants without changing the amino acid sequence of the protein. Sequence comparisons show that the PAT protein does not have homology to known allergens. Acute toxicity tests on PAT protein in rats showed no negative effects. The safety of PAT protein has been further assessed in broilers fed with either non transgenic or T25 maize. There was no effect of the GM maize on growth performance and body composition.

The weight of evidence provided by the company and available elsewhere concerning safety leads the Committee to conclude that there is no significant risk to humans or livestock following ingestion of the gene product. However, the applied *in vitro* methodology to study the survival of the PAT can be improved. Similarly, use of the isolated protein in toxicity studies does not adequately model degradation of the same protein when fed as an integral component of the diet.

Residue assessment: The principal residue identified in transgenic maize plants after post - emergence use of glufosinate ammonium was N-acetyl-glufosinate with lesser quantities of glufosinate and 3-methylphosphinico-propionic acid (MPP) which is also found in non-transgenic plants.

In maize grain, which exhibits much lower residues than the other plant parts, the principal residue identified was MPP with lesser amounts of N-acetyl-glufosinate.

In maize grain, approximately 5% of 300 samples analysed in US-trials exhibited residues >= 0.05 mg/kg. About 80 field trials were conducted with different application rates in Europe and in the harvested grain the residue of each metabolite was < 0.05 mg/kg.

The glufosinate-derived residues do not concentrate in any maize processed fraction which are relevant to food or feed items such as flour, starch, grits or oil. Residues are not detectable in crude and refined oil.

In green maize, forage and fodder, higher residues of around 2 - 5 mg/kg are possible.

In ruminant and poultry feeding studies no detectable residues were found in meat, milk or eggs at the dose calculated to represent the highest residues in livestock feed under Good Agricultural Practice and taking into account the potential use of glufosinate herbicide in several tolerant crops.

It can be concluded on the basis of the available data that residues of glufosinate ammonium and its metabolites, N-acetyl-glufosinate and 3-methylphosphinico-propionic acid expressed as glufosinate free acid equivalents, will be below 0.2 mg/kg in imported field maize grain as well as in maize grain from plants grown under European conditions. Levels will be below 4 mg/kg and 6 mg/kg in forage and fodder derived from glufosinate-tolerant maize, respectively. These are the time-limited tolerances set by the US EPA (Federal Register Vol. 62, No. 24, p. 5333, 1997). No residues above the limit of detection are to be expected in food of animal origin derived from livestock fed with GM maize treated with glufosinate herbicide.

6.2.3. ***Substantial Equivalence:*** Data are provided on compositional analysis, morphological studies and agronomic performance from field trials in the USA and Europe. The compositional analyses included fatty acid profiles, protein, amino acid, crude fiber, ash, phytate and moisture contents determined from grain and silage of GM and non-GM plants. The integration of the *pat* gene into the maize genome and the expression of PAT protein in glufosinate tolerant maize do not seem to cause any negative pleiotropic effects on the characteristics of the plant relevant to its safety for human and animal consumption, i.e., the nutritional composition and the content of the natural anti-nutritional factors are within the normal range. The genetically modified maize plant is thus substantially equivalent to its non-GM counterpart except for the introduced trait.

6.3. Environmental Aspects

6.3.1. ***Potential of Genetic Transfer:*** The risk of genetic escape from modified crop plants of this anemophilous species, i.e. largely pollinated by wind and gravity will be limited by poor dispersal and the absence of sexually-compatible plants either of the same or different species. *Zea mays* is not an invasive crop but is a weak competitor with limited powers of seed dispersal. Since pollen production and viability are unchanged by genetic modification, dispersal and outcrossing frequency should be no different from other maize varieties. There are no plant species closely-related to maize in the wild in Europe and the risk of genetic transfer to other species appears remote.

6.3.2. ***Treatment of Volunteers:*** The risks of volunteer maize plants surviving are considered to be remote. In growing areas free from winter frost which will kill any residual plants, any subsequent volunteers may be controlled by agronomic practices including cultivation and the use of alternative non-selective herbicides.

6.3.3. ***Safety to Non-Target Organisms:*** The available data indicate no qualitative differences in the susceptibility of GM and non-GM maize to insects and disease. Although risks to birds and other non-target species that frequent maize fields are considered to be low there is no direct data available from field experimentation. Risks to soil organisms and soil function through degradation of modified plant material and contamination of ground water are considered to be extremely low.

6.3.4. ***Resistance and Tolerance Issues:*** In view of the remote possibility of transfer of genes from GM maize to other plant species, the transfer of tolerance to glufosinate ammonium is not considered to be a problem.

7. OVERALL ASSESSMENT

The Commission requested the Scientific Committee on Plants to consider whether there is any reason to believe that the placing on the market of the T25 genetically modified maize with the purpose to be used as any other maize, is likely to cause any adverse effects on human health and the environment. In the assessment of the dossier provided by the notifier against the criteria set out in Directive 90/220/EEC, the Committee has reached the following conclusions:

The Committee after examining and considering the existing information and data provided in the dossier, against the background of available knowledge in the areas concerned, considers that there is no evidence to indicate that the use of the genetically modified maize as any other maize, is likely to cause adverse effects on human or animal health and the environment.