Opinion of the Scientific Committee on Plants regarding submission for placing on the market of fodder beet tolerant to glyphosate notified by DLF-Trifolium, monsanto and danisco seed (notification C/DK/97/01) (Opinion expressed by SCP on 23 June 1998)

1. Title

Application for consent to place on the market of fodder beet tolerant to the herbicide glyphosate (Notification C/DK/97/01).

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 fodder beet tolerant to glyphosate with the purpose to be used as any other fodder beet is likely to cause any adverse effects on human health and the environment.

3. Background

Directive 90/220/EEC 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, in order for this fodder beet 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 (aminomethylphosphonic acid) is in progress under Directive 91/414/EEC. Maximum residue levels (MRLs) for residues of glyphosate in products of animal origin were already set in the Council Directive 93/57/EEC. 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.

4. Proposed uses

The product that is the subject of this application is seeds and beet of glyphosate tolerant Roundup Ready® (RR) beet varieties (**Beta vulgaris**) and seeds and beet of any progeny derived from line A5/15 by conventional breeding. The application includes the production of Roundup Ready® (RR) fodder beet in the European Union as well as processing, feed use, and any other uses of the derived products.

5. Description of the product

The product consists of fodder beet (**Beta vulgaris** L **.** sp. **vulgaris**) transformed using the **Agrobacterium tumefaciens** vector system based on plasmid pMON17204 to introduce the **cp**4 **epsps** gene (derived from **Agrobacterium** sp. strain CP4) into fodder beet. Transformed line A5/15 tolerant to glyphosate expresses only one new protein CP4 EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) which is tolerant to glyphosate and thereby confers tolerance to Roundup Ready® herbicide on the fodder beet.

6. Opinions of the committee

6.1. Molecular/Genetic Aspects

6.1.1. Transformation technique: Based on the information provided, a disarmed **Agrobacterium tumefaciens** plant transformation system was used to produce A5/15 from a proprietary line DP15 which is a yellow diploid multigerm fodder beet pollinator. Detached cotyledons were cocultivated with a disarmed **Agrobacterium tumefaciens** and placed on selection medium containing glyphosate. After subcultivation and rooting, transformed plantlets were transferred to the greenhouse.

6.1.2 Vector constructs: The plant transformation vector pMON17204 used is a disarmed **Agrobacterium tumefaciens** binary vector containing four genes between the left and right borders. The vector also contains a bacterial selectable marker gene (**aad; Tn**7 AAD3" adenyltransferase; Sp^R and Str^R) located outside the borders. pMON17204 has been characterised at the nucleotide sequence level and comprises 15755 bp.

The genetic elements present between the right and left borders are well characterised and include:

(i) The **cp**4 **epsps** gene cassette consisting of the figwort mosaic virus promoter, a chloroplast targeting sequence from **A. thaliana**, the **cp**4 **epsps** coding region from **Agrobacterium** sp. strain CP4 and a 3Â’ untranslated region from pea;

(ii) The **gus** gene cassette containing the 35S promoter from cauliflower mosaic virus, the **uid**A coding region for the ß-D-glucuronidase from **E. coli** and a 3Â’ untranslated region from pea;

(iii) The **gox** gene cassette containing the figwort mosaic virus promoter, a chloroplast targeting sequence from **A. thaliana**, the **gox** coding region (glyphosate oxidoreductase) from **Ochrobactrum anthropi** and a 3Â’ untranslated region of the nopaline synthase gene;

(iv) The **npt**II gene cassette containing the 35S promoter from cauliflower mosaic virus, the **npt**II coding region for the neomycin phosphotransferase protein, and the 3Â’ untranslated region of the nopaline synthase gene.

Information included in the dossier on genetic transfer capabilities of the vector and the frequency of mobilisation of the vector is deduced from the fact that the plasmid has no inherent capability to transfer DNA to plants. Based on available information on the properties of the vector, the conclusions reached appear to be appropriate.

6.1.3. Transgenic construct in the genetically modified plant: Vector pMON17204 was designed to transfer DNA located between the right and left borders. In the A5/15 construct it was determined that only part of the DNA between the borders was transferred. Molecular analysis based on the Southern blot technique showed that the insert contains only the **cp**4 **epsps** gene. The **uid**A, **gox** and **npt**II genes located between the borders in the vector were not incorporated into line A5/15. By PCR experiments it was stated that the plasmid origins of replication (oriV and oriColE1) were not incorporated into line A5/15. The T-DNA was truncated in the E9 3Â' element before the 35S promoter and the **uid**A gene resulting in a fully functional **cp**4 **epsps** gene and no other elements of pMON17204 are inserted into line A5/15. Southern blot analysis showed that there is one copy of T-DNA inserted into line A5/15.

The **cp**4 **epsps** gene confers tolerance to glyphosate and was used for selection of the transgenic shoots during the transformation experiments.

Stability of the insert was determined in two ways:

(a) Testing of multiple generations of RR hybrids for tolerance suggested that the levels of tolerance are consistent between generations.

(b) Physical stability testing by Southern blot and PCR walking experiments were performed on the original transformation event (T_0) and on 5-6 plants from each of the subsequent 3 generations (T_1 to T_3). It is indicated that no differences in the banding pattern were observed among the generations.

The fact that no meaningful differences between the ranges and mean levels of CP4 EPSPS in A5/15 were observed over 2 years in samples from field trials is consistent with stable insertion and expression of the RR gene over generations.

6.2. Safety Aspects

6.2.1. Potential for gene transfer: Beet line A5/15 contains only the functional **cp4-epsps** gene (under the control of a plant virus promoter and an **Arabidopsis** chloroplast-targeting sequence) conferring the glyphosate-tolerant phenotype. In the unlikely event of intestinal bacteria being transformed by this gene, its expression could not occur unless some recombinational event placed the gene under the control of a bacterial promoter. Even if this extremely remote possibility did occur, the resulting protein would share common sequence and catalytic properties with the corresponding plant enzymes consumed in far larger amounts as a normal part of human and livestock diets.

6.2.2. Safety of gene products and metabolites:

Safety of gene products: Since the shikimate pathway is absent in mammals, the presence of the **cp4 epsps** gene product does not present a direct hazard for ruminant animals, the target species for the whole product. In addition, the 5-enolpyruvylshikimate-3-phosphate synthase protein would be expected to be substantially degraded by the rumen microflora. Indirect effects of the gene product in the target species were not observed. Elevated concentration of the enzyme in the GM plant did not significantly alter the production of aromatic amino acids which might have been expected if this were a rate-limiting enzyme. As a result, production of other C_6-C_3 compounds which derive from phenylalanine also would not be expected to

be changed. Products (sucrose) extracted for human consumption would be essentially free from protein (including the gene product which is found in greatest concentration in leaves) and DNA.

Residue assessment: The metabolism of glyphosate has been investigated in several varieties of plants; the metabolic pathway in tolerant crops is the same as in non-tolerant. In tolerant plants containing the enzyme CP4 EPSPS like fodder beet line A5/15, glyphosate is only slowly metabolised to AMPA (aminomethylphosphonic acid) like in non-tolerant crops. Studies with livestock animals show that glyphosate and AMPA are either not metabolised or are insignificantly metabolised and that residues will not be present in meat, milk and eggs of animals that consume feed prepared from tolerant or non-tolerant crops after treatment of glyphosate according to Good Agricultural Practice.

The WHO has recommended the following acceptable daily intake (ADI) for the sum of glyphosate and aminomethylphosphonic acid: 0.3 mg/kg b.w. (1997 Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group).

6.2.3. Substantial equivalence: Pooled material from 30 plants from each of 15 locations collected over three growing seasons was analysed. Tops (leaf tissue) and roots (brei) were separately treated. Some minor differences in proximate analysis between control and modified lines within years ($p<0.05$) were detected, but all values fell within the normal range for beet. Significant differences were not present when the data for all three growing seasons were collectively analysed. Detailed analysis of amino acid content also showed minor, but significant, within-year differences between the control and the A5/15 line. However, such differences, which were not consistent between years, would be expected from material grown at multiple sites. Seen in the context of the limited contribution made by beet protein to the total protein in the ration of a dairy cow, these differences are inconsequential. The saponin concentration found in roots or tops of the modified line did not differ significantly from the control. Substantial equivalence with respect to the fractions of nutritional value and the presence of saponins has been demonstrated.

6.3. Environmental Aspects

6.3.1. Potential for gene transfer/escape: Fodder beet is a cultivated biennial form of the beet, **Beta vulgaris**, which is an outbreeding, wind pollinated species which is self-sterile. Large amounts of pollen are produced which can travel long distances. Assuming proximity, synchrony of flowering and suitable conditions, **B. vulgaris** may freely hybridise with other varieties, **B. maritima** (sea-shore beet) and the wild beets **B. macrocarpa** and **B. atriplicifolia**. Hybridisation within the section **Beta** may give fertile offspring but is unlikely with other members of the Chenopodiaceae family. Annual weed beet is found in wild populations. The normally biennial cultivated beet may become vernalised by cold weather which induces bolting and the reproductive phase within season.

The best cultural practice to prevent outcrossing is to prevent flowering of the herbicide tolerant fodder beet in the same way that unmodified cultivars are grown. Fodder beet is harvested before the natural onset of the reproductive phase. For seed production there are clear seed certification rules prescribing minimum distances to foreign pollen sources of the genus **Beta**.

6.3.2. Treatment of volunteers: Volunteer plants in the crop may arise from the presence of wild beet, the bolting of fodder beet plants, the development of groundkeepers which arise initially from vegetative growth of beet crowns or tops left after harvest or the germination of seed (which may be dormant in the soil for up to 10 years). Beet is sensitive to tillage and most broad-leaved herbicides commonly used in rotational crops. Volunteer plants should be controlled by standard agricultural practice (other than the use of glyphosate). Bolting plants should also be removed by standard agricultural practice before pollen release.

6.3.3. Safety to non-target organisms: Roundup Ready® beet is as susceptible as non-modified beet to predation by insects, nematodes and mammals. It is equally susceptible to viruses and fungi and shows the same behaviour to fungicides, insecticides and herbicides (other than glyphosate). Safety to mammals has been established by a toxicity study. The environmental impact of glyphosate-tolerant fodder beet is not expected to be any different from that of any other beet variety used for the same purpose.

6.3.4. Resistance and tolerance issues: Beet is biennial, highly sensitive to frost and poorly competitive. The sensitivity of this transformed beet to non-glyphosate herbicides is the same as the sensitivity of non-modified beet. In non-cropped habitats, any modified plants will have no selective advantage in the absence of glyphosate. In the case of field volunteer plants they should be dealt with by standard agricultural practice. The notifiers should establish a detailed code of practice and work closely with growers to ensure Good Agricultural Practice which should minimise the establishment of herbicide tolerance outside the crop.

7. Overall assessment

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 fodder beet tolerant to glyphosate notified by Monsanto and Danisco Seed with the purpose to be used as any other fodder beet is likely to cause any adverse effects on human health and the environment".

In the assessment of the dossier against the criteria set out in Directive 90/220/EEC, the Committee has reached the following conclusions:

1. 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 indicating that the use of the fodder beet tolerant to glyphosate with the purpose to be used as any other fodder beet is likely to cause any adverse effects on human health and the environment.

2. The Committee was also of the opinion that the notifiers should establish a detailed code of practice and work closely with growers to ensure Good Agricultural Practice which should minimise the spread of herbicide tolerance. The Scientific Committee wishes to be kept informed of progress in this area.