Opinion of the Scientific Committee on Plants regarding Chicory derived from genetically modified male sterile, Glufosinate tolerant parental lines (RM3-3, RM3-4 and RM3-6) notified by Bejo Zaden (notification C/NL/94/25-A) (SCP/GMO/087-Final) - (Opinion adopted by the Scientific Committee on Plants, 18 December 1998)

1. TITLE

Outcome of Discussion on Application for the Placing on the Market of Bejo Zaden genetically modified male sterile, glufosinate-tolerant parental lines of chicory and hybrids derived from them.

2. TERMS OF REFERENCE

The Scientific Committee on Plants is asked to consider whether there is any reason to believe that the placing on the market of the genetically modified chicory described below with the purpose to be used as any other chicory 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 chicory 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.

A previous Commission Decision on Chicory (96/424/EC pursuant to Council Directive 90/220/EEC) concerns the same **Chicorium intybus L.** cv. Radicchio Rosso lines RM3-3, RM3-4, RM3-6 (Notification n° C/NL/94/25-A). The decision covers the use of the product for breeding activities.

4. PROPOSED USES

Crop production and trading for the use of the product as any other chicory, including food and feed, under the following conditions: that it shall be indicated on the label of each package of seeds to be used for crop production that 50 % of the seeds may be tolerant to the herbicide glufosinate-ammonium and that therefore volunteer plants cannot be controlled by herbicides with glufosinate ammonium as active ingredient.

5. DESCRIPTION OF THE PRODUCT

The product consists of chicory (**Chicorium intybus** L.cv. Radicchio Rosso) lines RM3-3, RM3-4 and RM3-6 and all the hybrids obtained from these lines with non-transgenic chicory.

Lines RM3-3, RM3-4 and RM3-6 were derived by transformation via **Agrobacterium tumefaciens** and contain a **barnase** gene (origin **Bacillus amyloliquefaciens**) coding for a ribonuclease expressed only in the tapetum cells that leads to lack of viable pollen and male sterility; a **bar** gene (origin **Streptomyces hygroscopicus**) coding for phosphinothricin acetyl transferase conferring tolerance to herbicides based on glufosinate ammonium and the **npt**II gene (origin **Escherichia coli**) coding for neomycin phosphotransferase II used as selection marker during transformation.

6. OPINIONS OF THE COMMITTEE

6.1. Molecular/Genetic Aspects

6.1.1. Transformation Technique: Based on the information provided, the DNA was introduced in lines RM3-3, RM3-4 and RM3-6 by **Agrobacterium tumefaciens**-mediated transformation, a standard technology. The transformants were successfully tested for the absence of the **Agrobacterium** strain used for transformation.

6.1.2. Vector Constructs : Lines RM3-3, RM3-4 and RM3-6 were produced with plasmid pTTM8RE by cointegration with plasmid pGV3000. Plasmid pTTM8RE contained between the right and left borders: the anther-specific promoter PTA29 from **Nicotiana tabacum**; the coding and 3´noncoding region of **barnase** gene from **Bacillus amyloliquefaciens**; part of the 3´untranslated region of **nos** gene of **Agrobacterium tumefaciens**; the pSsuAra green tissue-specific promoter and a transit peptide sequence from **Arabidopsis thaliana**; the coding region of the **bar** gene of **Streptomyces hygroscopicus**; 3´untranslated sequences of the T_L gene 7 of **Agrobacterium tumefaciens;** the promoter p **nos** from **Agrobacterium tumefaciens**; the coding region of the **npt**II gene of **Escherichia coli**-Tn **5**; the 3´untranslated region of **ocs** gene of **Agrobacterium tumefaciens**. As shown in the dossier, residual sequences near the borders with no coding capacity are not transcribed into the RNA. Sequences outside the borders on pTTM8RE contained: an **npt**II gene to encode kanomycin resistance, derived from Tn **903**; origin of replication of pBR322 (**Escherichia coli**); a **barstar** gene with regulatory sequences for expression in **Escherichia coli**; a marker streptomycin/spectinomycin (Sm/Sp) resistance gene for selection in **Escherichia coli** and **Agrobacterium tumefaciens.**

Information included in the dossier on genetic transfer capabilities of the vector and the frequency of mobilisation of the vector is deduced from the already published properties of the vector and not based on a direct experimental evaluation. 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: In both RM3-3 and RM3-4 the integrated DNA consists of a single copy and behaves as a single genetic locus. It is shown that the insertions are stable and follow standard Mendelian inheritance. In parental RM3-6 the inserted DNA was present in two copies in different loci and through selection in the offspring stable single copy plants have been identified.

The absence in the plants of sequences corresponding to regions outside the borders of pTTM8RE is shown by the results of Southern blot analyses for all three parental lines. PCR analyses have been carried out of the sequences surrounding the insertion site in the plant genome for regions flanking the T-DNA left border. No imperfect integration seems to have occurred and no plant sequences were amplified.

Transgene expression and cryptic expression are addressed with appropriate techniques (Northern blot). The **bar** and **npt**II genes are expressed in leaves, heads and roots. The **barnase** gene expression cannot be detected in these tissues. Expression of residual sequences between the borders was not detected. Evidence for absence of cryptic expression (sense probes) is provided.

The NPTII protein (the product of the **npt**II gene) and the PAT protein (the product of the **bar** gene) levels were assessed in heads and leaves of the parental lines with appropriate techniques.

6.2. Safety Aspects

6.2.1. Potential for Gene Transfer:

The **npt**II is under the control of nopaline synthase promoter. Even if transformed into intestinal bacteria the probability of it being expressed is remote. The **bar** gene is under the control of a plant promoter which is not functional in bacteria. Consequently, in the unlikely event of transformation, it would not be expressed. Even if, due to genetic recombination, the gene would be expressed in intestinal micro-organisms or human or animal cells, the probability of which is remote, no negative effects are expected because the only known substrate of phosphinothricin acetyltransferase (PAT) is the herbicide glufosinate ammonium. The **barnase** gene is under the control of a plant promoter and consequently not expressed in bacterial cells. Even if, due to genetic recombination, the gene would be expressed in intestinal micro-organisms, the probability of which is remote, no negative effects are expected because ribonucleases and inhibitors are ubiquitous among bacteria, including those present in the digestive tract.

6.2.2 Safety of the gene products/metabolites:

The modified chicory heads and leaves contain detectable amounts of the PAT and NPTII proteins. The biosafety of the PAT and NPTII proteins has been extensively assessed with regard to the allergenicity, toxicity and degradation in the digestive tract. No toxic or allergenic effects should be expected.

Residue assessment: only 50% of the plants of the crops derived from the transgenic chicory will be tolerant to glufosinate ammonium. This herbicide is used only for selection purposes in breeding and is not authorised for use in chicory crop production and, accordingly, the assessment of residues is not applicable to this notification.

6.2.3 Substantial equivalence:

Compositional analyses of the chicory leaves were performed on samples of untransformed and transformed lines during two growing seasons. There were no differences in the content of major macro and trace elements. Only minor differences in some components (i.e. dry

matter, potassium and manganese) were detected in proximate analyses between control and modified lines, but variation was within the normal range for chicory leaves. Additional results of a taste study conducted in humans failed to demonstrate any difference in texture, taste, after taste (bitterness) and overall palatability between the genetically modified product and the conventional variety of chicory. The data add to the evidence that no change in the composition occurred as a result of the genetic modification.

Overall the Committee was satisfied that the transgenic chicory is substantially equivalent to non-transgenic chicory except for the introduced traits.

6.3 Environmental Aspects

6.3.1 Potential for gene transfer/gene escape:

When cultivated chicory is grown as a vegetable crop it is harvested before it would flower naturally. If any plants become vernalised inducing premature flowering, no pollen will be produced since the GM plants are male sterile. The potential transfer of genetic material to micro-organisms in the soil is considered to be very low against the background of natural occurrence of kanamycin resistance in soil microbes.

6.3.2 Treatment of volunteers:

Any volunteer in subsequent crops should be controlled by cultivation or appropriate other methods (except for glufosinate ammonium).

6.3.3 Safety to non-target organisms:

There are no specific data on non-target organisms although there are no reports of differences in the behaviour of pollinating insects observed during seed production. Published literature on the toxicity of these transgenes suggest that the modified chicory should not present a risk to other non-target organisms.

6.3.4 Resistance and tolerance issues:

Appropriate agronomic practice should control any volunteers in subsequent crops. In the unlikely event of transfer to wild chicory, any progeny will have no selective advantage in the absence of glufosinate ammonium.

7. OVERALL ASSESSMENT

The notification examined by the SCP seeks the placing on the market of transgenic chicory (**Chicorium intybus L.** cv. Radicchio Rosso) lines with male sterility (lack of viable pollen) and tolerance to glufosinate ammonium (GA)-based herbicides and to kanamycin (as selectable marker). The transgenic lines will be used as an alternative technology to produce hybrid seed by crossing with conventional, non-transgenic chicory lines. The notification also seeks the cultivation of the hybrid chicory and its use as any other chicory variety. Since only 50% of plants of the hybrid crop will contain two new, linked characters (tolerance to glufosinate ammonium-based herbicides and male sterility), GA-based herbicides will not be used in this crop. The linkage of the herbicide resistance gene to male sterility reduces even more the low probability of gene flow to wild chicory. The SCP after examining the existing

information and data presented in the dossier considers that, against the background of available knowledge, there is no evidence to indicate that the placing on the market of transgenic chicory lines RM3-3, RM3-4, RM3-6 and their hybrids will cause adverse effects on human health and the environment.