Opinion of the Scientific Committee on Plants regarding the genetically modified cotton, tolerant to glyphosate herbicide notified by the Monsanto Company (notification C/ES/97/01) (Opinion expressed by the SCP on 14 July 1998)

1. Title

Application for consent to place on the market herbicide protected cotton expressing the gene **cp4 epsps**, conferring tolerance to glyphosate herbicide (Notification C/ES/97/01).

2. Terms of reference

The Scientific Committee on Plants (SCP The Working Group Plant GMOs comprises members from the following Scientific Committees: Plants, Animal Nutrition, Food, and Toxicity, Ecotoxicity and the Environment) is asked to consider whether there is any reason to believe that production and marketing of varieties of Roundup Ready® Cotton line RRC 1445 and any progeny derived from crosses between Cotton line RRC 1445 and other cotton varieties and import of commodity cotton grain that contains Roundup Ready Cotton® grain mixed with other genetically modified and non-modified cotton grain, is likely to cause any adverse effects on human health and on the environment.

3. Background

Directive 90/220/EEC (Council Directive 90/220/EEC of 23 April 1990 on the deliberate release into the environment of genetically modified organisms, O.J. no. L 117, 08/05/1990, p. 15-27) requires 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. For genetically modified plants, the assessment must be based on information outlined in Annex II B of Directive 90/220/EEC and take into account the proposed uses of this product.

Following the entry into force of the regulation on Novel Foods and Novel Food Ingredients (EC N° 258/97) (Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients, O.J. no. L 043, 14/02/1997, p. 1-7) on 15 May 1997, in order for this cotton and its derived products to be placed on the market for food purposes, the requirements of the regulation will have to be satisfied. Such 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 were already set in the Council Directive 93/58/EEC.

4. Proposed uses

Seeds shall be imported, planted, grown, harvested, and processed to non-viable products. Cottonseed from glyphosate-tolerant cotton will be utilised in the same manner as other cottonseed products from cotton varieties produced or imported into the European Union (EU).

5. Description of the product

The product consists of cotton (**Gossypium hirsutum**) cultivar Coker 312 , which has been transformed using plasmid PV-GHGT07. The transgenic line produced, called RRC line 1445, expresses the 5-enolpyruvylshikimate-3-phosphate synthase protein (CP4 EPSPS). This protein is encoded by the **cp4 epsps** gene (origin: **Agrobacterium** strain CP4).

6. Opinions of the committee

6.1. Molecular / Genetic Aspects

6.1.1. Transformation technique: According to the information provided, the construct was introduced into cells of cotton hypocotyl sections by **Agrobacterium tumefaciens** - mediated transformation. Plantlets were regenerated after selection on kanamycin, and were assayed for glyphosate tolerance.

6.1.2. Vector construct: Line RRC 1445 was produced with vector PV-GHGT07, which is a single border vector that has only a right border containing the following elements: the 0.4 kb **ori** V fragment from the RK2 plasmid fused to the 3.0 kb segment of pBR322 allowing maintenance in **E.coli** and in **Agrobacterium tumefaciens.** This was fused to the 90 bp DNA fragment from pTiT37 plasmid which contains the 25 bp nopaline-type T-DNA right border. The remaining **nptII** portion consists of three genes engineered for plant expression, **cp4, nptII, gox**, and a bacterial selectable marker (**aad**). The **cp**4 synthetic gene is fused at the 5Â' end to the region that codes for the chloroplast transit peptide from **Arabidopsis thaliana** EPSPS and the CMoVb promoter. The 3Â' region is derived from the 3Â' non-translated region of the **rubisco E9** gene from **Pisum sativum**. This is fused to the **aad** gene (allowing bacterial selection on spectinomycin or streptomycin) isolated from Tn7 transposon under the control of its own promoter and terminator. The gene for selection on kanamycin which consists of the cauliflower mosaic virus 35S promoter, the neomycin phosphotransferase (**nptII**) gene and the non-translated region of the 3Â' region of the nopaline synthase gene (**nos 3Â'**) is located downstream of the **aad** gene. The **gox** gene is fused at the 5Â' end to the sequence coding for the chloroplast transit peptide from **Arabidopsis thaliana** EPSPS and the CMoVb promoter. The 3Â' region is derived from the 3Â' non-translated region of the nopaline synthase gene (**nos**).

The vector construct has been characterised and analysed in good detail.

6.1.3. Transgenic construct in the GM plant: The **ori**322 region and the **gox** gene, present in PV-GHGT07, was not transferred in the genome of RRC line 1445 as shown by Southern analysis.

Approximately 6.1 kb of the left hand border of plasmid PV-GHGT07 is integrated into the genome of RRC line 1445 and includes the CMoVb promoter, the **aad**, **nptII**, and the **cp4 epsps** genes and a portion (200 bp) of the **ori-V** origin. The **aad** gene, under the control of a

bacterial promoter is not expressed in the RRC line 1445 (lack of detection of the protein confirmed by an ELISA for the AAD protein).

Southern and genetic analyses demonstrate that one single copy has been transferred at one single locus. The stability of the insert has been demonstrated over three generations of backcrossed derivatives of RRC line 1445.

6.2. Safety aspects

6.2.1. Potential for gene transfer: Although the final construct contains two antibiotic resistance markers, **nptII** conferring resistance to neomycin/kanamycin and **aad** conferring resistance to streptomycin/spectinomycin, it is unlikely that either gene survives processing in a functioning form. The defatted seed meal remaining after oil extraction is used only as animal feed, the bulk of which is fed to ruminants able to tolerate the presence of the terpinoid gossypol and the cycloprenoid fatty acids which are toxic to other livestock species. Removal of these components allows a limited amount of cottonseed meal to be used in the diets of pigs, poultry and fish. The physical and heat treatment used to obtain maximum oil recovery is adequate to coagulate protein and to damage substantially the DNA present.

It is theoretically possible that DNA containing an antibiotic resistance marker gene or **cp4 epsps** could survive processing, that this DNA could transform an intestinal bacterium and, in the case of **nptII** or **cp4 epsps,** recombination could bring the gene under the control of a bacterial promoter. Even if this extremely unlikely chain of events occurred, the potential to compromise chemotherapy in humans is non-existent. Both kanamycin and streptomycin resistant bacteria are relatively common in nature and introduction of either resistance gene would not increase the existing risks to any significant extent.

In the equally remote possibility that **cp4 epsps** was transformed and expressed, 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 the gene products /metabolites:

Safety of gene products: The **nptII** gene product, neomycin phosphotransferase II, is present in whole seeds at concentrations of approximately 7 m g/g fresh weight while CP4 EPSPS occurs at ten-fold higher concentrations (approximately 70 m g/g fresh weight). These values would be proportionally higher in the extracted seed meal but without biological activity because of the heat treatment applied. Even if some activity remained, the shikimate pathway is absent from mammals and the presence of the **cp4epsps** gene product would not create a direct hazard. Elevation of the concentration of CP4 EPSPS in the GM cotton plant also seems free from downstream metabolic consequences and thus from indirect effects for the consuming animal. Production in leaves of anthocyanins, tannins and from flavonoids was no greater in RRC line 1445 compared to the parental control line (Coker 312). No toxic effects have been observed in acute and short-term toxicity studies made with the isolated gene products and no homologies have been found between CP4 EPSPS or NPTII and any known allergens.

Residue assessment: The metabolism of glyphosate has been investigated in several varieties of plants, the metabolic pathway in tolerant crops being the same as in non-tolerant crops. In tolerant plants containing the enzyme CP4 EPSPS, like cotton line RRC 1445, glyphosate is

only slowly metabolised to AMPA (aminomethylphosphonic acid) as in non-tolerant crops. No detectable residues will occur in cottonseed oil.

Studies with livestock animals also show that glyphosate and AMPA are not metabolised or only insignificantly so that residues will not be present in meat, milk and eggs of animals fed with tolerant or non-tolerant crops after treatment with the herbicide glyphosate.

6.2.3. **Substantial equivalence:** Compositional analysis of the intact seeds and extracted seed oil were compared from samples of the transformed line RRC 1445, glyphosate-treated line RRC 1445 and its parent line Coker 312 taken from six sites during two growing seasons. Some minor differences in proximate analysis between the control, the modified line and the treated modified line ($p<0.05$) were detected, but all values fell within the normal range for cotton. However, such differences, which were not consistent between years, would be expected from material grown at multiple sites. Detailed analysis of amino acid content showed no significant differences between the control and the line RRC 1445. The absence of difference between the aromatic amino acid content is particularly important since it provides added evidence that CP4 EPSPS did not affect the metabolism of the shikimate pathway. Application of glyphosate did not affect amino acid composition. Small differences were also seen in the fatty acid composition of seed oil and in total lipids, with the transformed plant either with or without glyphosate application, showing higher total lipids. However differences were minor and remained within the expected range. The gossypol content was higher in the transformed line compared to the control, but still within the range found for field-grown cotton; no difference was seen in the content of cyclopropenoid fatty acids. Overall the Committee was satisfied that the transgenic cotton line RRC 1445 is substantially equivalent to non-transgenic cotton except for the transferred traits and that this conclusion is not altered when glyphosate is applied.

6.3. Environmental Aspects

6.3.1. **Potential for gene transfer/escape**: Cotton (**Gossypium hirsutum**), a member of the **Malvacea** family, is a perennial plant which is planted and harvested annually. It is mainly self-pollinating, but pollen is also transferred by insects (in particular various species of bees and bumblebees).

Outcrossing rates of up to 28% to other cotton cultivars have been observed under field conditions in adjacent plots, declining rapidly with distance. Given proximity and the availability of insects as pollen vectors, Roundup Ready® Cotton line (RRC) 1445 is likely to hybridise with other cotton varieties.

Other species of the Gossypiaea tribe are not native to the EU but are cultivated as ornamental plants or vegetables (e.g. Hibiscus, Okra or LadyÂ's fingers) in Member States which also grow cotton. Hybridisation experiments with several species either failed or resulted in cottonseeds. Taking into account also the need of close proximity, synchronous flowering and the availability of insect pollinators, the probability of fertile hybrids can be considered to be very unlikely. The potential transfer of genetic material to microorganisms in the soil is considered to be very low against a background of the natural occurrence of kanamycin and streptomycin resistance in soil microbes.

6.3.2. **Treatment of volunteers**: There are no specific problems with cotton as a weed. Cottonseed may remain in the field after harvesting and germinate under favourable

conditions. Seeds may also survive mild and dry winters. However, no wild populations of cotton are known in the E.U. Germination, vegetative vigour and reproduction of cotton line RRC 1445 are equivalent to non-modified varieties.

Suitable treatments for any volunteers in the next crop include cultivation and the use of herbicides other than glyphosate.

6.3.3. **Safety to non-target organisms**: Exposure of non-target species to seeds can be considered very low, due to the morphology of the boll. Feeding studies with birds (seeds) and mammals (both proteins) indicate very low toxicity of the proteins which also occur ubiquitously in the environment in plants and microorganisms. Field studies on agronomic performance showed equivalent susceptibility of line RRC 1445 and non-modified varieties to diseases and insect pests.

6.3.4. **Resistance and tolerance issues**: Any selective advantage of cotton line RRC 1445 is restricted to cases where no herbicide other than glyphosate is used on early stages of cotton. Under normal application rates, the introduced glyphosate-tolerance is effective up to the 4-leaf stage only. Other herbicide, cultivation of rotational crops or winter conditions will kill both modified and non-modified plants. Volunteers should be dealt with by standard agricultural practice except that glyphosate should not be used.

7. Overall assessment and conclusion

The Commission requested the Scientific Committee on Plants to consider whether the placing on the market of glyphosate resistant cotton line RRC 1445 (expressing the CP4 EPSPS enzyme) is likely to cause any adverse effects on human health or the environment.

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 placing on the market of line RRC 1445 (expressing the CP4 EPSPS enzyme) with the purpose to be used as any other cotton is likely to cause adverse effects on human health and the environment.

The present opinion relates to the assessment provided for under Directive 90/220/EEC, all applications relating to the placing on the market of this cotton and its derived products intended for food use purposes must also comply with the provisions and procedures of EC Regulation No 258/97 on Novel Foods and Food Ingredients of 15 May 1997 including, as appropriate, consultation of the Scientific Committee on Food.