

Opinion of the Scientific Committee on Plants on the genetically modified cotton line, insect-tolerant notified by the Monsanto company (notification C/ES/96/02) (Opinion expressed by SCP on 14 July 1998)

1. Title

Application for consent to place on the market insect-protected cotton expressing a gene for **B.t.k.** endotoxin (notification C/ES/96/02)

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 the production and marketing of varieties of seed from IPC 531 cotton line, tolerant to feeding by certain lepidopteran insects and any progeny derived from crosses between Cotton line IPC 531 and other cotton varieties and the import of commodity cotton grain that contains Cotton line IPC 531 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.

4. Proposed Uses

Seeds shall be imported, planted, grown, harvested, and processed to non-viable products. Cottonseed from insect-protected 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-GHBK04. The transgenic line produced is called IPC 531, and expresses the **cry1A(c)** gene (origin: *Bacillus thuringiensis* subsp. **kurstaki**) which encodes a modified **CRY1A(c) B.t.k.** protein.

6. Opinions of the committee

6.1. Molecular/Genetic Aspects

6.1.1. Transformation technique: According to 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 insect resistance.

6.1.2. Vector construct: Line IPC 531 was produced with vector PV-GHBK04 containing the following elements.

The 0.4 kb **oriV** fragment from the RK2 plasmid fused to the 3.4 kb segment of pBR322 allowing maintenance in *E. coli* and in *Agrobacterium tumefaciens*. This was fused to the 0.36 kb DNA fragment from pTiT37 plasmid which contains the nopaline right border. The remaining portion consists of two chimeric genes that encode the **B.t.k.** HD 73 and NPTII protein and a bacterial selectable marker (**aad**). The chimeric gene consists of the 35S promoter, the modified **cry1A(c)** gene [part of the 5' end of the **cry1A(b)** gene with a portion of the **cry1A(c)** gene] which encodes the **B.t.k.** protein and the mRNA polyadenylation signals (7S 3' terminator sequence) of the soybean alpha subunit of the beta-conglycin gene. This is fused to the **aad** gene isolated from Tn7 transposon (allowing bacterial selection on spectinomycin or streptomycin). The chimeric 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 3' region of the nopaline synthase gene (**nos**) is located downstream of the **aad** gene.

6.1.3. Transgenic construct in the GM plant: The **ori322** region, present in PV-GHBK04, was not transferred in the genome of IPC 531 line as shown by Southern analyses. The **aad** gene, under the control of a bacterial promoter is present in the genome of IPC 531 line, but for the AAD protein, an ELISA confirmed the lack of detectable expression of the protein. Southern and genetic analyses demonstrate that two copies are inserted in a head-to-tail arrangement into the genome of IPC 531 line. One T-DNA insert contains a full-length **cry1A(c)** gene and a **nptII** gene and the second insert contains an inactive 3' portion of the **cry1A(c)** gene. The two inserts are linked and behave genetically as a single locus. The stability of the insert has been demonstrated over four generations of backcrossed derivatives of IPC 531 lines in several elite cultivars.

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 as animal

feed, the bulk of which is fed to ruminants able to tolerate the presence of the terpinoid gossypol and the cyclopropenoid 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 a resistance marker gene could survive processing, that this DNA could transform an intestinal bacterium and, in the case of **nptII**, 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 the introduction of either resistance gene would not increase the existing risks to any significant extent.

6.2.2. Safety of the gene products/metabolites: The **B.t.k.** toxin is present at a concentration of less than 1 mg/g fresh weight in whole seeds and the **nptII** gene product, neomycin phosphotransferase II, at approximately 2.5 mg/g fresh weight. The **aad** gene product could not be detected using ELISA. These values would be proportionally higher in the extracted seed meal but without biological activity. No toxic effects have been observed in acute and short-term toxicity studies made with **B.t.k.** protein produced in **E. coli**. Widespread use of **B.t.k.** as an insecticide spray has not produced evidence of any allergenic response. Similarly no homologies have been found between the **B.t.k.** toxin or NPTII and any known allergens.

The weight of evidence provided by the Company and available elsewhere leads the Committee to conclude that there is no significant risk to humans or livestock following ingestion of these gene products.

6.2.3. Substantial equivalence:

Compositional analyses of the intact seeds and extracted seed oil samples of the transformed line 531 were compared with those of its parent line Coker 312, taken from six sites during one growing season and a further four sites the following season. The yield and fraction of the seed dry matter represented by the major components did not differ significantly and fell within the published range for other cotton varieties. Small significant differences were observed in the composition of the extracted oil for three of the eleven measured fatty acids. All values however, fell within the normal published range and were not a consequence of the introduced traits. The concentration of potential toxicants (gossypol and the cyclopropenoid fatty acids) did not differ significantly. On the basis of this evidence, the Committee is of the opinion that the transgenic cotton line 531 is substantially equivalent to non-transgenic cotton except for the transferred traits.

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, Insect Protected Cotton line 531 is likely to hybridise with other cotton varieties.

Other species of the *Gossypia* tribe are not native to the EU but are cultivated as ornamental plants or vegetables (e.g. Hibiscus, Okra or Lady'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 micro-organisms 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. Germination, vegetative vigour and reproduction of IPC line 531 are equivalent to non-modified varieties.

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

6.3.3. Safety to non-target organisms: The target pests in Europe are the cotton bollworm (***Helicoverpa armigera***) and the pink bollworm (***Pectinophora gossypiella***). The **cryIa(c)** protein is specifically toxic to certain Lepidopteran larval pests on ingestion and appears non-toxic to other arthropod species. Exposure of non-target species to seeds can be considered as especially low, due to the morphology of the boll. Feeding studies with birds and mammals indicate very low toxicity of the **cryIa(c)** protein. Field studies on agronomic performance showed equivalent susceptibility of IPC line 531 and non-modified varieties to diseases and arthropod pests other than Lepidoptera (to which **cryIa(c)** is toxic). The toxicity of the **cryIa(c)** protein was further tested in laboratory and field experiments in a wide range of arthropod species from different orders, including honey bees, lacewing larvae, ladybird beetles and parasitic Hymenoptera. Laboratory studies showed very low toxicity for all species except Lepidopteran larvae. In the field, populations of non-target arthropods were frequently higher in IPC line 531 plots than in those of non-modified cotton, attributed to less use of insecticides in the IPC line 531 fields.

6.3.4. Resistance and tolerance issues: The development of resistance in target pests will be delayed by the rigorous adoption of a comprehensive resistance management strategy. To be effective, this should require the active involvement of the notifier to monitor for control failure, to provide technical support and to educate growers to implement the strategy.

The speed with which resistance to **cryIa(c)** protein develops will depend on the rigour and efficiency of any insect resistance management strategy. Such a programme designed to delay resistance development requires:

- - knowledge of pest biology and ecology
- - gene deployment strategy (full-season, constitutive, optimal dose **cryIa(c)** expression to control insects heterozygous for resistance alleles)
- - refuges to support the development of **B.t.k.** - susceptible insects
- - monitoring and reporting of incidents of resistance development

- - employment of integrated pest management practices to encourage ecosystem diversity and provide multiple tactics for insect control
- - communication and education plan
- - development and deployment of products with alternative modes of action.

The notifier intends to recommend resistance monitoring and management strategies, addressing the elements listed above. The success of such resistance management strategy will depend on the ability of any monitoring programmes to detect resistance as soon as possible, and the extent and quality of advice given to farmers. The proposed plan if rigorously carried out with the active involvement of the company should provide an adequate framework to delay the onset of resistance in the target pests.

The Scientific Committee on Plants should be kept informed of the results of the proposed surveillance of resistance in the target pests in Member States.

7. Overall assessment and conclusion

The Commission requested the Scientific Committee on Plants to consider whether the placing on the market of insect-resistant cotton line IPC 531 (expressing the modified **cry1A(c)** gene) is likely to cause any adverse effects on human health or on the environment.

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 to indicate that the placing on the market of line IPC 531 (expressing a **B.t.k.** toxin) with the purpose to be used as any other cotton is likely to cause adverse effects on human health and on the environment.

2. The Committee was also of the opinion that the proposed plan for risk management with regard to **B.t.k.** endotoxin resistance provides an adequate framework to delay the onset of such resistance in the target pests. The Scientific Committee on Plants should be kept informed of monitored progress in the field.

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.