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Scientific Steering Committee

OPINION ON

GENETICALLY MODIFIED COTTON AND MEDICAL DEVICES

ADOPTED BY THE SCIENTIFIC STEERING COMMITTEE
AT ITS MEETING OF

28-29 JUNE 2001

GENETICALLY MODIFIED COTTON AND MEDICAL DEVICES

OPINION

Two genetically modified cotton lines have been assessed previously[1]. One modified line (C/ES/97/01) is tolerant to the herbicide glyphosate and the other (C/ES/96/02) is resistant to insect damage. The Scientific Steering Committee has been asked by the Commission for advice on two issues related to the use of genetically modified cotton, in relation to their potential use in feminine hygiene products, baby or adult incontinence products and other garments. Specifically the following questions were submitted:

1. Are there features or characteristics of fibres derived from the genetic modification process of cotton plants which significantly influence the risk assessment of products from those fibres?

2. Is there a significant difference in the safety of feminine hygiene products (e.g. tampons, sanitary pads etc), baby or adult incontinence products (e.g. nappies, etc), "medical cotton" product (cotton balls, make-up pads, gauze, etc) and cotton fabrics and garments derived from genetically modified as opposed from currently available cotton fibres?

The opinion hereafter is based on a report (attached) prepared by experts of the Scientific Steering Committee, the Scientific Committee on Plants and the Scientific Committee on Medicinal Products and Medical Devices.

Molecular inserts

The first question was to consider whether the molecular inserts were likely to have any impact on the structural integrity of the fibres from modified cotton plants. The C4 epsps gene which is inserted into the herbicide tolerant cotton encodes an enzyme which is an integral part of the shikimic acid pathway in plants and some metabolites derived from this pathway can be used to synthesis components of cotton fibre. However, biochemical analysis has provided evidence that the pathway has not been significantly modified in rate by the transgene and therefore it is extremely unlikely that the composition of fibre from GM cotton varies from its non GM counterpart. In GM cotton lines expressing genes such as cry1A(b) or cry1A(c) which encode for B.t.k toxins, the active insecticidal (toxic) protein interacts with the midgut epithelium of susceptible insects to elicit a change in osmotic balance which results in cell lysis. For several Bt proteins specific, high–affinity binding sites have been shown to exist on the midgut epithelium of susceptible insects. The Cry1A(b) protein encoded by the *Btk* gene is specific to lepidopterans. The Cry1A(b) and Cry1A(c) proteins have no known enzyme functionality that could cause modifications in the metabolic pathways responsible for cotton fibre formation.

[1] http://europa.eu.int/comm/food/fs/sc/scp/out17_en.html
http://europa.eu.int/comm/food/fs/sc/scp/out18_en.html

Raw cotton is processed extensively through harsh chemical and heat treatments to prepare cotton lint for textiles. Analysis of raw and processed fibres from these 2 GM cotton lines suggests that any protein, either endogenseous or introduced into cotton plants by genetic modification, should be denatured or removed by processing.

Medical cotton products

The second question was to consider the consequences for human safety. Although there is no real data on the potential for these 2 genetically modified cottons to have a significant adverse impact on the safety of feminine hygiene, incontinence and similar products, the SSC sees no reasons for any additional risk of such products interacting in intimate contact with the skin, vaginal mucosa, endometrium, or other tissues as compared to products from non-genetically modified cotton.

Recommendation

The Committee recommends that, if future genetically modified plant products are considered for use in medical and hygiene cotton products, risk assessments should be carried out on a case-by-case basis to include: definition of the molecular inserts and their effect on metabolism and fibre structure, analysis of raw and processed fibres for protein content, evidence of the substantial equivalence[2] of the physico-chemical characteristics of medical and hygiene products derived from either non-GM or GM plant material.

[2] Substantial equivalence refers here to the same concept as also used in the context of the evaluation of genetically modified organisms.

REPORT OF THE WORKING GROUP

I. MANDATE

The Scientific Steering Committee has been asked by the Commission for advice on two issues related to the use of genetically modified cotton, in relation to their potential use in feminine hygiene products, baby or adult incontinence products and other garments. Specifically the following questions were submitted:

1. Are there features or characteristics of fibres derived from the genetic modification process of cotton plants which significantly influence the risk assessment of products from those fibres?
2. Is there a significant difference in the safety of feminine hygiene products (e.g. tampons, sanitary pads etc), baby or adult incontinence products (e.g. nappies, etc), "medical cotton" product (cotton balls, make-up pads, gauze, etc) and cotton fabrics and garments derived from genetically modified as opposed from currently available cotton fibres?

The Scientific Steering Committee asked experts on the Scientific Committee on Plants and the Scientific Committee on Medicinal Products and Medical Devices to prepare a report on these genetically modified cotton lines and their use in hygiene and incontinence products. This report follows hereafter.

II. BACKGROUND: GENETICALLY MODIFIED COTTON

Two genetically modified cotton lines have been considered previously by the Scientific Committee on Plants for full marketing consent under DIR 90/220 EEC and positive opinions were published on 14 July 1998. [3] [4]

A genetically modified cotton line which is tolerant to the herbicide glyphosate was notified by the Monsanto company (notification C/ES/97/01). 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).

A second genetically modified cotton line which is tolerant to insect attack was notified by the Monsanto company (notification C/ES/96/02). 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.

III. RISK ASSESSMENT OF THE MOLECULAR INSERT

[3] http://europa.eu.int/comm/food/fs/sc/scp/out17_en.html
[4] http://europa.eu.int/comm/food/fs/sc/scp/out18_en.html

III.1. A genetically modified cotton line, tolerant to the herbicide glyphosate notified by the Monsanto company (notification C/ES/97/01)

Molecular inserts in the GM Cotton

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* (glyphosate resistance) genes and a portion (200 bp) of the *ori-V* origin. Southern and genetic analyses demonstrate that one single copy has been transferred at one single locus.

Potential effects of expressing the *C4 epsps* gene on fibre composition

Cellulose biosynthesis

Cotton fibres are one of the classical objects for studies on cellulose microfibrils and their orientated deposition in growing and thickening plant cell walls. The cell walls of cotton have a high cellulose content (ca 80% in the thickened secondary cell wall) but also contain other neutral, as well as acidic, polysaccharides. The primary cell wall of cotton fibres is similar to the primary cell walls of other dicotyledons (see Ryser 1985, for details on chemical composition of fibres). Glucose is the primary starting substrate for cellulose synthesis and is metabolised to UDPglucose prior to addition to growing glucan chains. Other polysaccharides of the cell wall are composed of more than one type of sugar residue or linkage. These are assembled by specific synthases from nucleoside-activated sugar pre-cursors then transported from the Golgi and extruded to the cell wall. Here the synthesis of heteropolymers requires the concerted action of a number of different nucleotide sugar transferases/synthases (see McDougall *et al.* 1993 and references therein). The *epsps* enzyme catalyses a specific reaction in the shikimic acid pathway which is not involved in cellulose biosynthesis. There is no reason to believe that expression of this gene will modify cellulose content or composition.

Suberin synthesis

The *epsps* gene encodes for the 5-enolpyruvylshikimate-3-phosphate synthase enzyme, an enzyme on the shikimic acid pathway. The shikimic acid pathway does give rise to metabolites used in the production of suberin which is a component of raw cotton fibres.

The safety of the introduced protein from *Agrobacterium* strain CP4 has been assessed. This class of proteins has a long history of safe use due to the presence of related EPSPS proteins in all plants, microbes, yeast and fungi. There is no evidence that elevated concentration of the enzyme in GM plant significantly alters the production of aromatic amino acids which might have been expected if this enzyme catalysed a rate-limiting enzyme. As a result, production of other C6-C3 compounds which derive from phenylalanine also would not be expected to be changed. The dossier showed that production in leaves of anthocyanins, tannins and flavonoids was no greater in the RRC line 1445 compared to the parental control line (Coker 312). 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

upset the rates of reactions within the shikimate pathway. Similarly, application of glyphosate did not affect amino acid composition.

There is therefore no reason to believe that the composition of cotton fibres will be modified by the expression of the *cp4 epsps* gene as there is no indication that metabolic fluxes have been sufficiently modified to cause significant changes in the levels of compounds used in fibre synthesis.

Raw cotton fibre is processed extensively before its final use in products (Sims *et al.* 1996). Combed lint is produced by a mechanical-air cleaning step applied to raw lint which removes plant material from ginning. Combed lint fibres are then subjected to various degrees of bleaching, washing and other chemical processes depending on the intended use of the fabric (Perkins *et al.* 1984). Linters, the short fibres associated with the seed, are composed primarily of cellulose and are highly processed for both chemical and non-chemical uses (NCPA 1989). Linters are removed during the mechanical delinting step of processing cottonseed and are converted to brown stock with an alkaline wask (>0.75 M sodium hydroxide) and temperatures >100^{o}C (AOCS 1991). The processing steps which are used to prepare cotton lint for textiles (cleaning, bleaching and dying) and cellulose for chemical uses (alkaline wash, heat, bleaching) should denature or remove any protein, either endogenous or introduced into the cotton plant by genetic modification.

The CP4 EPSPS protein was detected at low levels by western blot in combed lint (<0.5 μg/g) but not in processed linter brown stock (Sims *et al.* 1996). Inactivation of this protein in the first processing step for linters indicates that the protein will not be present in cotton linter products.

III.2. A genetically modified cotton, insect-protected by expressing a gene for *B.t.k.* endotoxin (notification C/ES/96/02)

The product consists of cotton (*Gossypium hirsutum*) cultivar Coker 312, which has been transformed to expresses the *cry1A(c)* gene or rather a 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.

Molecular inserts in the Cotton

The *aad* gene, under the control of a bacterial promoter is present in the genome of IPC 531 line but ELISA confirmed the lack of detectable expression of the AAD 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 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.

Potential effects of expressing the *cry1A(c)*, *aad* and *npt*II genes on cotton composition

No AAD protein is detectable by ELISA. The *B.t.k.* toxin is present at a concentration of less than 1 μ g/g fresh weight in whole seeds and the *npt*II gene

product, neomycin phosphotransferase II, at approximately 2.5 μ g/g fresh weight. Values for AAD and NPTII proteins in the processed cotton fibres are not available but fibre processing would be expected to substantially reduce or destroy any biological activity. The CRYA(c) protein was detected by western blot and insect bioassay in raw cotton linters (0.17μ g/g) but was not detected in either raw or combed lint (Sims *et al.* 1996). Inactivation of the protein in the first processing step for linters indicates that the protein will not be present in cotton linter products. There is no evidence from the genetic construct used that specific protein targeting to the developing fibres (cell walls) of cotton bols would occur which makes is very unlikely that any of the gene products would be accumulated to any extent in freshly harvested bols. Even if this were the case no toxic effects have been observed in acute and short-term toxicity (feeding) studies made with *B.t.k.* protein produced in *E. coli*.. No homologies have been found between the *B.t.k.* toxin or NPTII protein and any known allergens.

The c*ry* genes which encode B.t.k toxin are derived from the naturally occurring soil organism *Bacillus thuringiensis* . The active ingredient produced in the GM plant is a truncated form of the δ-endotoxin protein. The active insecticidal protein interacts with the midgut epithelium of susceptible insects to elicit a changes osmotic balance and cell lysis. For several Bt proteins specific, high – affinity binding sites have been shown to exist on the midgut epithelium of susceptible insects. The Cry1A(b) protein encoded by the *Btk* gene is specific to lepidopterans. The Cry1A(b) and Cry1A(c) proteins have no known enzyme functionality that could cause modifications in the metabolic pathways responsible for cotton fibre formation.

III.3. Conclusion for Question 1

The C4 epsps gene inserted into the herbicide tolerant cotton encodes an enzyme which is an integral part of the shikimic acid pathway in plants and some metabolites derived from this pathway can be used to synthesis components of cotton fibre. However, biochemical analysis has provided evidence that the pathway has not been significantly modified in rate by the transgene which makes it extremely unlikely that the composition of fibre from GM cotton varies from its non GM counterpart. In GM cotton lines expressing genes such as cry1A(b) or cry1A(c) which encode for B.t.k toxins, the active insecticidal (toxic) protein interacts with the midgut epithelium of susceptible insects to elicit a change in osmotic balance which results in cell lysis. For several Bt proteins specific, high – affinity binding sites have been shown to exist on the midgut epithelium of susceptible insects. The Cry1A(b) protein encoded by the *Btk* gene is specific to lepidopterans. The Cry1A(b) and Cry1A(c) proteins have no known enzyme functionality that could cause modifications in the metabolic pathways responsible for cotton fibre formation.

IV. GENETICALLY MODIFIED COTTON IN HYGIENE, COSMETIC AND MEDICAL PRODUCTS

IV.1. Assessment

In coming to a view as to whether genetically modified cotton could be associated with a significant difference in safety of hygiene and associated products, it is necessary to identify the possible chemical, structural and performance differences between unmodified and modified cotton products and to relate any such differences to the mechanisms by which these hygiene products could induce adverse effects in users of the products. In dealing with the latter point, it is considered that the user of tampons is most at risk since these products come into contact with the haemorrhaging endometrium. In practice, tampons are only rarely associated with any adverse effects. Four possibilities do exist and have to be addressed, direct toxicity, allergies, modified absorbency and toxic shock.

In relation to toxicity, tampons are made from materials, primarily cotton and rayon, which are intrinsically non-toxic. In recent years there have been some concerns over the possibilities of adverse effects from additives or residues in tampons, specifically asbestos and dioxins, but investigations have shown that these concerns have been without foundation. It is not considered possible for genetically modified cotton to have any impact on the non-toxic status of the material.

Allergy to the cotton fibre itself being made of cellulose, does not occur. However, cosnideration needs to be given to protein residues that may be present in a finished product and which may have potential to cause immediate hypersensitivity reactions. An analogy to this is the protein present in medical and hygiene devices made from natural rubber latex (e.g., surgical gloves, condoms). Such immediate hypersensitivity reactions from cotton products is not a problem and it is considered, therefore, that there is no significant risk of increased allergies associated with the use of these two specified GM cottons in relation to feminine hygiene and incontinence products. However, a risk analysis should be performed should any other GM cotton lines be considered for this use, concentrating on the presence of residual protein structures and their potential to initiate an allergic response.

With respect to modified absorbency, the greatest risks for adverse effects from tampons are vaginal dryness and ulceration when tampons are used that are more absorbent than is required. Since tampons are already available with different degrees of absorbency, and since this factor should be specified on product labels, there is no inherent risk associated with using materials with higher or lower absorbency rates. It is not known whether genetically modified cotton products do exhibit significantly different performance with respect to absorbance. However, there is no reason to believe that they do and, even if they did, this would not be of any consequence provided the manufacturer determined and specified the absorbance quality, and this came within the normal range of these products.

Finally, with respect to toxic shock syndrome (TSS), it is known that this rare and potentially fatal disease is caused by bacterial toxins, most often streptococci or staphylococci. In the past there has been a connection between tampons and TSS, although without a clearly understood causal relationship. Some high absorbency products appear to have been associated with a higher risk. The incidence of tampon related TSS is now very low and not considered

to be a serious risk. The deliberate modification of a cotton by a transformation that expresses a gene for an endotoxin suggests that serious consideration has to be given to the potential relationship between this material and a fatal syndrome that is known to occur, however rarely, in users of these cotton products, through the involvement of bacterial toxins. It has been demonstrated with the GM cotton line 2 mentioned above that the *B.t.k* toxin is present at a concentration of less than 1μg/g in whole seeds, and that fibre processing would be expected to reduce or destroy any biological activity. In a situation in which TSS is extremely rare, it is not considered that this particular modification poses any significantly increased risk, but the lack of a clear causal relationship between tampon material and the onset of TSS cannot rule this out unequivocally.

IV.2 Conclusion for Question 2.

In considering the potential for genetically modified cotton to have a significant adverse impact on the safety of feminine hygiene, incontinence and similar products, there is no evidence that the altered cotton can have any effect on the principal ways in which such products interact with the skin, endometrium or other tissues of the users. The lack of such evidence in general, and the outcome of a specific risk analysis performed with these two GM lines, however, cannot unequivocally rule out increased risks. Risk analyses should take account of the intended introduction of, for example, bacterial toxins into the cotton fibres and the relationships between proteins and allergic responses, and between bacterial toxins and TSS.

V. REFERENCES

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VI. ACKNOWLEDGEMENTS

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