SUMMARY NOTIFICATION INFORMATION FORMAT (SNIF) FOR PRODUCTS CONTAINING GENETICALLY MODIFIED HIGHER PLANTS (GMHPs)

INSECT-PROTECTED COTTON LINE DERIVED FROM EVENT 531

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LIST OF ABREVIATION

aad	DNA sequence that encodes for the aminoglycoside adenylyltransferase
AAD	Aminoglycoside adenylyltransferase
B.t.	*Bacillus thuringiensis*
B.t.k.	*Bacillus thuringiensis kumamotoensis*
bp	Base pairs
CaMV	Cauliflower mosaic virus
CBW	Cotton bollworm
Cry	Crystal protein, a diverse group of insecticidal proteins produced by *B.t.*
Cry1Ac	Cry protein with insecticidal properties directed against certain Lepidoptera
cry1Ac	DNA sequence that encodes for the protein, Cry1Ab
DNA	Deoxyribonucleic acid
E. coli	*Escherichia coli*
E.U.	European Union
ELISA	Enzyme-linked immunosorbent assay
fw	Fresh weight
GMHP	Genetically modified higher plant
GMO	Genetically modified organism
ha	Hectare
IPC	Insect-protected cotton
Kb	Kilobases
µg	Microgram
nptII	DNA sequence that encodes for the enzyme neomycin phosphotransferase II
NPTII	Neomycine phosphotransferase II
NTO	Non-target organism
OECD	Organisation for Economic Cooperation and Development
ORF	Open reading frame
PBW	Pink bollworm
PCR	Polymerase chain reaction
PV-GHBK04	Plasmid vector containing the genes of interest
spp	Species
T	tonne
T-DNA	Transferred DNA
tRNA	Transfer RNA
U.S.	United States
USDA	United States Department of Agriculture

A. GENERAL INFORMATION

1. Details of notification

(a) Member State of notification: Spain

(b) Notification number: C/ES/96/02

(c) Name of the product (commercial and other names):

The name of the product is insect-protected cotton line derived from event 531[1]. Varieties of cotton derived from this genetically modified line will be sold under the Bollgard®[2] trade name.

This application under Directive 2001/18/EC is for the cultivation and marketing into the European Union of IPC 531 and any progeny derived from crosses, for the purposes of production, importation, storage and processing to non-viable products.

(d) Date of acknowledgement of notification: December 02, 1996

2. Notifier

(a) Name of notifier: Monsanto Company represented by Monsanto Europe S.A.

(b) Address of notifier:

Monsanto Europe S.A.
270-272 Avenue de Tervuren
B-1150 Brussels
BELGIUM

Monsanto
800 N. Lindbergh Boulevard
St. Louis, Missouri 63167
U.S.

(c) Is the notifier: domestic manufacturer: [ ]
importer: [X]

(d) In case of an import the name and address of the manufacturer shall be given

IPC 531 will be manufactured in countries outside of the European Union where the appropriate approvals are obtained. The manufacturers are cotton seed companies with commercial licenses from of Monsanto Company to sell cotton varieties containing the *cry1Ac* gene.

3. General description of the product

(a) Name of the recipient or parental plant and the intended function of the genetic modification

To produce IPC 531, a DNA sequence encoding i) the Cry1Ac protein which confers protection to certain lepidopteran insects, ii) the AAD protein (aminoglycoside adenylyltransferase) which provides resistance towards spectinomycin or streptomycin for bacterial selection purposes and iii) the NPTII protein (neomycin phosphotransferase II) which provides resistance towards kanamycin for cotton plant cell selection purposes, was inserted into cotton cells using *Agrobacterium tumefaciens* mediated transformation utilizing the plant expression vector, PV-GHBK04.

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[1] Thereafter referred as IPC 531
[2] Bollgard is a registered trademark of Monsanto LLC Technology

(b) Any specific form in which the product must not be placed on the market (seeds, cut-flowers, vegetative parts, etc.) as a proposed condition of the authorisation applied for

IPC 531 has been demonstrated to be equivalent to other cotton, apart from its protection against certain lepidopteran insect pests and therefore cottonseed and processed products derived from IPC 531 will be used in the same manner as any other cotton products.

(c) Intended use of the product and types of users

There are no specific differences when IPC 531 is compared to traditional cotton except for its protection against certain Lepidoptera. IPC 531 has been shown to be substantially equivalent, with exception of the introduced trait, to cotton currently in commerce and therefore, the proposed uses and the types of users for IPC 531 are identical to those for traditional cotton.

(d) Any specific instructions and/or recommendations for use, storage and handling, including mandatory restrictions proposed as a condition of the authorisation applied for

IPC 531 has been demonstrated to be substantially equivalent to other cotton, apart from its protection against certain lepidopteran insect pests. No specific instructions or recommendations for storage of seeds, plants, or products derived from IPC 531 are envisaged.

(e) If applicable, geographical areas within the E.U. to which the product is intended to be confined under the terms of the authorisation applied for

Cottonseed production in the E.U. is focused in Spain and Greece. Cottonseed products are utilized and processed in all member states of the European Union.

(f) Any type of environment to which the product is unsuited

Cottonseed derived from IPC 531 will be used where traditional cottonseed is currently produced.

(g) Any proposed packaging requirements

Insect-protected cotton has been shown to be substantially equivalent to other cotton varieties. Therefore, insect-protected cotton in the form of cottonseed and processed products will be used in the same manner as with other cotton and no specific packaging is foreseen.

(h) Any proposed labelling requirements in addition to those required by law

In accordance with the requirements of Directive 2001/18/EC, repealing Directive 90/220 on 17 October 2002, Monsanto will:

a) inform European and International traders of the approval for production into the European Union of IPC 531,

b) provide all traders with the commercial name of the product and any agreed European and/or international unique identifier,

c) advise all traders, and other operators using the products, that IPC 531 are subject to the traceability and labelling requirements of Directive 2001/18/EC.

d) in accordance with the requirements of Annex IV of Directive 2001/18, the product will be labelled with the following words "This product contains genetically modified organisms". Packages and bags containing the seeds will be identified as Bollgard to allow farmers to know they are purchasing an insect-protected cotton variety derived from event 531. As for any other variety, all the usual pieces of information including variety name, seed quality, seed treatment, manufacturer's name and full address, will be given on the seed package.

(i) Estimated potential demand

(i) in the Community

In 2000, Spain and Greece produced respectively 3.14 T and 3.05 T per hectare and planted more than 90.6 and 413.6 thousand hectares, respectively. The corresponding cotton seed production is of 284,300 and 1,259,628 MT, respectively.

(ii) in export markets for EC supplies

Not relevant

(j) Unique identification code(s) of the GMO(s)

The unique identifier for IPC 531, MON-00531-6, has been attributed based on the guidance for the designation of a unique identifier for transgenic plants developed by OECD's Working Group on Harmonisation of Regulatory Oversight in Biotechnology.

4. ***Has the GMHP referred to in this product been notified under part B of Directive 2001/18/EC and/or Directive 90/220/EEC ?***

Yes [X] No [ ]

If no, refer to risk analysis data on the basis of the elements of Part B of Directive 2001/18/EC

Not relevant.

5. ***Is the product being simultaneously notified to another Member State ?***

Yes [ ] No [X]

If no, refer to risk analysis data on the basis of the elements of Part B of Directive 2001/18/EC

See following sections.

OR

Has the product being notified in a third country either previously or simultaneously?

Yes [X] No [ ]

If yes, please specify

Argentina, Australia, China, Columbia, India, Indonesia, Israel, Japan, Mexico, South Africa, Thailand, Turkey and the US.

6. ***Has the same GMHP been previously notified for marketing in the Community?***

Yes [ ] No [X]

I f yes, give notification number and Member State

7. ***Measures suggested by the notifier to take in case of unintended release or misuse as well as measures for disposal and treatment***

Insect-protected cotton varieties will be marketed as cotton varieties in Europe or imported in mixture with other commodity cottonseeds. Insect-protected cotton varieties can only be legally marketed and used in the E.U. if they are certified to meet the criteria of uniformity, stability, and homogeneity for a variety according to the Organization of Economic Co-operation and Development (OECD) standards and registered in the European Common Catalogue. Insect-protected cotton varieties will also be the subject of a request for registration on the European Common Catalogue. No particular measures will be taken beyond those taken for other cotton varieties.

Cottonseed from insect-protected cotton will be utilised in the same manner as other cottonseed products from cotton varieties produced or imported into E.U. These mixtures of bulk commodity cottonseed will be employed for food, feed, and industrial uses in the same manner as currently.

The measures for waste disposal and treatment for IPC 531 products are the same as those for other cotton products.

B. NATURE OF THE GMHP CONTAINED IN THE PRODUCT

INFORMATION RELATING TO THE RECIPIENT OR (WHERE APPROPRIATE) PARENTAL PLANTS

8. ***Complete name***

(a) Family name: Malvaceae

(b) Genus: *Gossypium*

(c) Species: *hirsutum*

(d) Subspecies: Not applicable

(e) Cultivar/breeding line: Coker 312

(f) Common name: cotton

9. **(a) Information concerning reproduction**

(i) Mode(s) of reproduction

Cotton is a perennial plant that is planted and harvested annually. Although natural crossing can occur, cotton is normally considered to be a self-pollinating crop. The pollen is heavy and sticky and transfer by wind is unlikely. It is transferred instead by insects, in particular, by various wild bees, bumble bees (*Bombus* spp.), and honeybees (*Apis mellifera*).

(ii) Specific factors affecting reproduction, if any

The range over which natural crossing occurs appears to be limited. McGregor (1976) traced movement of pollen by means of fluorescent particles and found that, even among flowers located only 50 to 60 meters from a cotton field which was surrounded by a large number of bee colonies to ensure ample opportunity for transfer of pollen, fluorescent particles were detected on only 1.6% of the flowers. For comparison, the isolation distances for foundation, registered and certified cottonseed in the U.S. are approximately 450 meters, 450 meters and 220 meters, respectively.

(iii) Generation time

The cultural cycle for cotton ranges from 120 to 200 growing days from seedling emergence to maturity. Rainfall, temperature, sunshine, and spring warming all impact optimal growth.

(b) Sexual compatibility with other cultivated or wild plant species

i) gene transfer to cultivated genotypes

In as much as similar cotton genotypes are fully compatible, any pollen that is transferred has the potential to produce a hybrid seed. The degree of out-crossing in a production field is strongly dependent upon the geographic location of the field (which means upon the crop ecology) and the kinds and numbers of insect pollen vectors. Bumble bees (*Bombus* spp.) and honey bees (*Apis mellifera*) are the most significant with the former being the most efficient pollinator.

Considerable work has been done on the degree of out-crossing between adjacent plants, rows and plots of cultivated cotton. Molecular techniques have been used to determine out-crossing from transgenic cotton plots buffered by cotton. It has been shown that no more than 6% out-crossing occurred to border rows and the percentage dropped rapidly in rows successively distant from the plot.

ii) gene transfer to wild plant species

The criterion of sexual compatibility greatly limits the potential of gene flow from cultivated *Gossypium* in the geopolitical boundaries of Spain, Greece or other countries of the European Union. No genera in the Gossypieae tribe occur naturally in these countries.

10. Survivability

(a) Ability to form structures for survival or dormancy

Cotton is a perennial plant that is harvested and planted annually and is not considered to have weedy characteristics. Seeds are the only survival structures. Cotton is not considered to have seed which can persist in the environment for long periods of time.

(b) Specific factors affecting survivability, if any

Cultivated cotton does not possess any of the attributes associated with long term survivability such as seed dormancy, long soil persistence, germination

under diverse environmental conditions, rapid vegetative growth, a short life cycle, high seed output, high seed dispersal or long distance dispersal of seeds. In most cotton growing areas of Europe some of the seed remaining in the field following harvest and cultivation may germinate in autumn if conditions are favourable. The seeds not germinating are likely to rot and die. In cotton growing regions with mild and dry winters, such as in Spain and Greece, cottonseed may over-winter and germinate the following spring. These cotton volunteers can be easily controlled by current agronomic practices including cultivation and the use of appropriate herbicides such as atrazine, bromoxynil, glufosinate and paraquat.

11. Dissemination

(a) Ways and extent of dissemination

Cotton is a perennial plant that is harvested and planted annually. Dissemination occurs only by means of seeds. Genetic material can be disseminated by pollen movement.

(b) Specific factors affecting dissemination, if any

Seed dissemination is impacted by mechanical harvesting and transport as well as wind damage, which may cause some mature bolls to fall to the ground. Pollen dispersal is influenced by insect vectors, particularly, bumble bees (*Bombus* spp.) and honey bees (*Apis mellifera*), with the former being the most efficient pollinator.

12. Geographical distribution of the plant

Plants of the tribe Gossypiae originated in the tropics and subtropics. Except as a cultivated crop, they are essentially excluded from temperate climates. They also tend to be plants of the southern hemisphere.

13. In the case of plant species not normally grown in the Member State(s), description of the natural habitat of the plant, including information on natural predators, parasites, competitors and symbionts

In the E.U., cotton is commercially grown in Spain and Greece.

14. Potentially significant interactions of the plant with other organisms in the ecosystem where it is usually grown, including information on toxic effects on humans, animals and other organisms

Cotton is known to interact with other organisms in the environment including a range of beneficial and pestiferous arthropods, fungal diseases and surrounding weed species. Cotton is cultivated in Spain and Greece and has a history of safe use in those countries. Cotton is not considered harmful nor pathogenic to humans, however the plant does produce gossypol and cyclopropenoid fatty acids, which are natural toxicants.

15. Phenotypic and genetic traits

The same as the recipient cotton cultivar Coker 312.

INFORMATION RELATING TO THE GENETIC MODIFICATION

16. Description of the methods used for the genetic modification

IPC 531 was modified by incorporation of a DNA fragment derived from plasmid vector PV-GHBK04 into the maize genome using an *Agrobacterium tumefaciens* mediated transformation. The use of *Agrobacteria* in transformation ensures that only T-DNA is integrated in the plant genome and the border sequence is not.

17. Nature and source of the vector used

The plasmid vector, PV-GHBK04, is an 11.4 Kb single border binary transformation vector. It contains well-characterised DNA segments required for selection and replication of the plasmid in bacteria as well as a right border for initiating the region of DNA (T-DNA) integrated into the plant genomic DNA.

18. Size, source [name of donor organism(s)] and intended function of each constituent fragment of the region intended for insertion

Summary of the genetic elements intended for insertion in IPC 531

Sequence	Size (Kb)	Source	Function
7S 3'	0.44	Soybean	Ends transcription and directs polyadenylation
cry1Ac	3.54	*Bacillus thuringiensis* subsp. *kurstaki*	ORF encoding resistance to Lepidoptera
P-e35S	0.62	Cauliflower Mosaic Virus (CaMV)	Promoter
aad	0.79	Transposon Tn7	ORF allowing bacterial selection
NOS 3'	0.24	*Agrobacterium tumefaciens*	Ends transcription and directs polyadenylation
nptII	0.97	Transposon Tn5	ORF allowing plant cell selection
P-35S	0.32	Cauliflower Mosaic Virus (CaMV)	Promoter
Ori-V	0.39	Plasmid RK2	Origin of replication

INFORMATION RELATING THE GMHP

19. Description of the trait(s) and characteristics which have been introduced or modified

Insect-protected cotton plants provide effective control of cotton bollworm (CBW, *Helicoverpa armigera*) and the pink bollworm (PBW, *Pectinophora gossypiella*) in cotton. The tissues of IPC 531 contain a *cry1Ac* gene, derived from the common soil bacterium *Bacillus thuringiensis* subsp. *kurstaki* (*B.t.k.*), which encodes a *B.t.* Cry1Ac protein for cotton bollworm and pink bollworm control. IPC 531 also contains two antibiotic resistance marker genes: *nptII* and *aad*.

The CBW and PBW feed on the leaves, squares and bolls of cotton plants inflicting serious damage or destroying these structures. Such plant damage can result in a significant reduction in yield. Repeated applications of chemical insecticides are currently used for the control of the CBW. Chemical insecticides for control of PBW are of limited value, as applications must be made prior to the time the insect bores into the cotton boll to ensure contact of the insecticide with the insect.

The use of insect-protected cotton would enable the farmer to effectively control the CBW and PBW providing protection of potential cotton yield and a reduction of the use of chemical insecticides. Insect-protected cotton would provide benefits to growers, the general public, and the environment, including: (1) a more reliable, economical, and less labor intensive means to control the CBW and PBW, (2) insect control without harming non-target species, (3) a means for growers to significantly reduce the amount of chemical insecticides now applied to the crop thereby achieving CBW and PBW control in a more environmentally compatible manner than is currently available, (4) a reduction in the manufacturing, shipment, and storage of chemical insecticides used in cotton, (5) a reduction in the exposure to workers to the pesticide and pesticide spray solution, (6) a reduction in the number of empty pesticide containers and amount of spray solution that must be disposed of according to applicable environmental regulations, (7) a fit with integrated pest management (IPM) and sustainable agricultural systems, and (8) both large and small growers will benefit from the planting of Insect-Protected cotton as no additional labor, planning, or machinery is required.

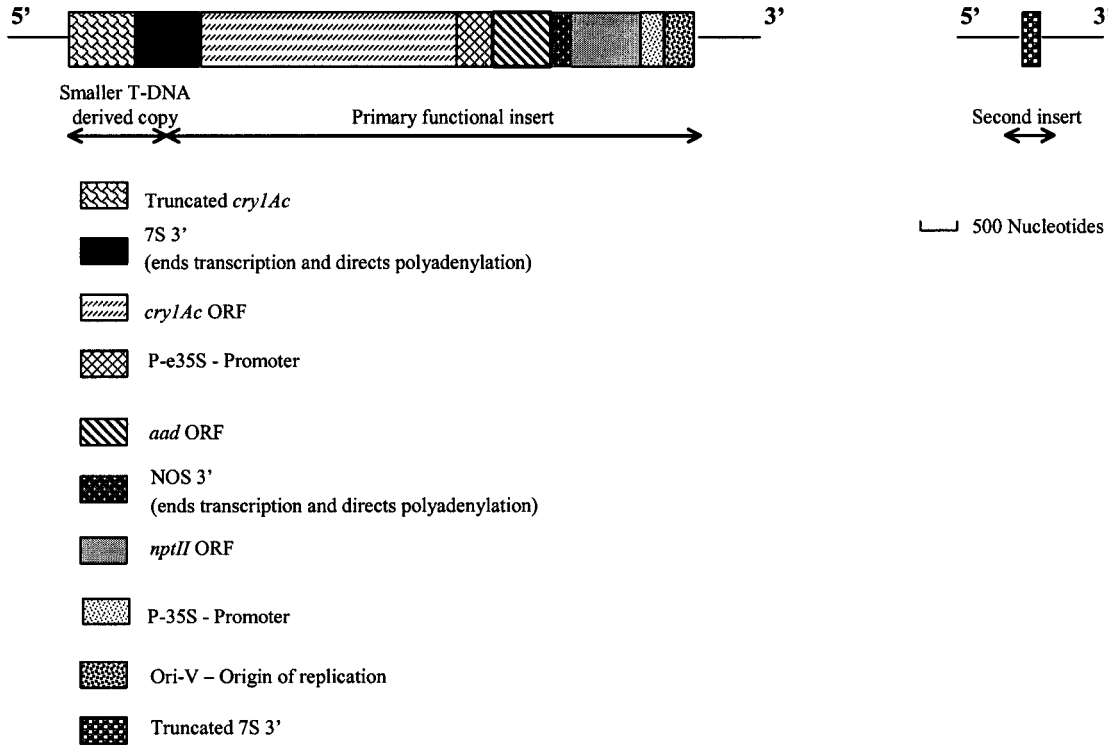
20. Information on the sequences actually inserted/deleted/modified

(a) Size and structure of the insert and methods used for its characterisation, including information on any parts of the vector introduced in the GMHP or any carrier or foreign DNA remaining in the GMHP

Southern blot analyses, PCR, DNA sequencing and genome walking were conducted to characterise the inserted T-DNA. IPC 531 contains two T-DNA inserts. The primary functional insert contains single copies of the full-length *cry1Ac* gene, the *nptII* gene and the *aad* antibiotic resistance gene and is no larger than 8.2 Kb. This T-DNA insert also contains a smaller T-DNA derived copy, an 892 bp portion of the 3' end of the *cry1Ac* gene fused to the 7S 3' transcriptional termination sequence. This segment of DNA is at the 5' end of the insert, is contiguous and in the reverse orientation with the full-length *cry1Ac* gene cassette and does not contain a promoter. The second T-DNA

insert contains 242 bp of a portion of the 7S 3' polyadenylation sequence from the terminus of the *cry1Ac* gene and is not functionally active in the plant genome.

Schematic representation of the insert DNA in IPC 531



(b) In case of deletion, size and function of the deleted region(s)

Not applicable

(c) Location of the insert in the plant cells (integrated in the chromosome, chloroplasts, mitochondria, or maintained in a non-integrated form), and methods for its determination

Southern blot analysis was conducted to confirm the location of the inserted DNA in the nuclear genome of IPC 531.

(d) Copy number and genetic stability of the insert

Analysis demonstrated that the larger insert containing an intact fragment containing the *cry1Ac* and *nptII* genes has been integrated into the cotton nuclear genome. This T-DNA copy is no larger than 8.2 Kb, and so maximally contains the *cry1Ac*, *nptII* and *aad* genes, as well as part of the oriV site.

Further analysis indicates that this insert also contains a second, but smaller T-DNA derived copy (0.44 Kb of 7S 3' termination sequence, and 0.89 Kb of the 3' end of the *cry1Ac* gene). This portion of the *cry1Ac* gene is not insecticidally active and so has no impact on the action of the introduced trait.

The larger insert contains two T-DNA copies are contiguous to each other, and in a tail-to-tail arrangement.

The second insert is also in the nuclear genome and contains only a portion of the transcriptional termination sequence from the *cry1Ac* gene.

Stability of the T-DNA insertions in IPC 531 was determined by analysing the R5 and R6 generations, as well as two commercial cotton lines containing cotton event 531 by Southern blot analysis. The results from these experiments indicated that the functional insert was present in all the generations of cotton event 531 that were analysed.

The 242 bp T-DNA segment containing a portion of the 7S 3' genetic element was detected in the R5 and R6 inbred generations, but was not detected by Southern blot analysis in the two commercial lines containing cotton event 531 that were tested. A likely explanation for the absence of the 242 bp insertion in the commercial lines is that it segregates independently of the functional insertion since the commercial lines were derived from the original cotton event 531 through traditional breeding methods.

(e) In case of modifications other than insertion or deletion, describe function of the modified genetic material before and after the modification as well as direct changes in expression of genes as a result of the modification

Not applicable

21. Information on the expression of the insert

(a) Information on the expression of the insert and methods used for its characterisation

Levels of Cry1Ac, NPTII and potentially AAD proteins were evaluated in young leaf (3-6 week plantlets) and seed tissues collected from six field locations during the 1992 U.S. growing season using ELISA and western blot methods. In addition, at one field site young leaf tissue was collected at 3 time points throughout the season after the initial sampling and whole, mature cotton plants were collected just prior to defoliation and harvest to establish the consistency of expression throughout the season.

(b) Parts of the plant where the insert is expressed (*e.g.* roots, stem, pollen, etc.)

The levels of expression of the Cry1Ac protein for the tissues RRC 1445 comprised between 0.0012-0.0019 and 0.0004-0.0013 mg/g FW, in leaves and seeds, respectively. The level of Cry1Ac protein detected in pollen was 11.5ng/ g FW of pollen and in nectar below limit of detection (1.6 ng/ g FW of nectar).

The levels of expression of the NPTII protein for the tissues of RRC 1445 are comprised between 0.0025-0.0038 and 0.002-0.003mg/g FW, in leaves and seeds, respectively.

In both tissues, the AAD protein was below detection level (detection levels were below 0.008×10^{-3} mg/g FW).

22. *Information on how the GMHP differs from the recipient plant in*

(a) Mode(s) and/or rate of reproduction

Data and information collected from field trials conducted in the United States demonstrated no significant morphological, growth or developmental differences between IPC 531 and control cotton. The following characteristics were observed: seed germination; plant morphology; time to flowering, fruiting, boll formation; boll development and yield.

Based on those observations, no differences are anticipated in the reproductive capability of IPC 531 when compared to the control cotton and therefore IPC 531 should behave similarly to conventional cotton.

(b) Dissemination

i. pollen transfer to wild species

For gene flow to occur via normal sexual transmission certain conditions must exist: the two parents must be sexually compatible, their periods of fecundity must coincide, a suitable pollen vector must be present and capable of transferring pollen between the two parents and resulting progeny must be fertile and ecologically fit for the environment in which they find themselves.

Based upon these criteria, out-crossing to wild species is not considered possible in Spain, Greece and the other countries comprising the European Union as no other genera in the Gossypieae tribe are endemic to this geographic area.

ii. pollen transfer to cultivated genotypes

In as much as similar cotton genotypes are fully compatible, any pollen that is transferred has the potential to produce a hybrid seed. The degree of out-crossing in a production field is strongly dependent upon the geographic location of the field, which depends upon the crop ecology. The most important factors are the kinds and numbers of insect pollen vectors. Bumble bees (*Bombus* spp.) and honey bees (*Apis mellifera*) are the most significant with the former being the most efficient pollinator. Typical out-crossing percentages for a number of locations in the U.S. cottonbelt range from 0 to 28%. Consequently, the transgenic material can be expected to be transferred to other cultivated genotypes over time.

While limited out-crossing to cultivated cotton (*Gossypium hirsutum*) can be expected, such out-crossing would not be expected to cause any adverse effects because cotton plants receiving the trait will behave in the same manner as insect protected cotton. Apart from insect resistance, the introduced trait does not provide any selective advantage.

iii. transfer of genetic information to species to which it cannot interbreed

We are not aware of any other species within the countries of the European Union with which *Gossypium hirsutum* is able to successfully exchange pollen and produce viable hybrid plants. There is no evidence that plants can exchange genes with any other organisms in nature.

(c) Survivability

Studies in laboratory and in the field have been performed to determine whether IPC 531 improved survival and/or over-wintering characteristics which could increase the possibility of IPC 531 to become a weed.

The results obtained in laboratory suggested that under certain conditions (*i.e.* cold temperatures) IPC 531 may be more vigorous than Coker 312. However, those results were not confirmed in the field where no meaningful differences exist in the germination or survival rates of IPC 531 and Coker 312. In addition, under field conditions, IPC 531 did not possess an increased potential for over-wintering.

(d) Other differences

No other differences observed.

23. Potential for transfer of genetic material from the GMHP to other organisms

Based on the fact that pollen production and pollen viability as measured by yield and germination of progeny are unchanged by the genetic modification, the out-crossing frequency to other cotton varieties or to wild relatives (which are not present in the E.U.) is unlikely to be different for IPC 531 when compared to other varieties. Also, none of the genetic elements introduced in the plant carries genetic transfer function.

24. Information on any harmful effects on human health and the environment, arising from the genetic modification

An assessment of the human safety of the Cry1Ac and NPTII proteins was conducted based upon the extensive characterization of those proteins.

The human safety of the Cry1Ac and NPTII proteins has been established based upon the following considerations: (1) no amino acid sequence similarity to known toxins, other than *B.t.* proteins in the case of Cry1Ac, and no immunologically relevant sequence similarity with known allergens, (2) rapid degradation under conditions which simulate mammalian digestive systems, (3) no indications of acute toxicity in mice administered Cry1Ac or NPTII protein by oral gavage, (4) very low dietary exposure, and (5) a history of safe use.

Finally, the nutritional equivalence of cottonseed from IPC 531 has been established by compositional analysis.

25. Information on the safety of the GMHP to animal health, where the GMHP is intended to be used in animal feedstuffs, if different from that of the recipient/parental organism(s)

There is no difference between IPC 531 and the recipient organism in terms of safety to animals.

An assessment of the safety of the Cry1Ac and NPTII proteins was conducted based upon the extensive characterization of those proteins.

The safety of the Cry1Ac and NPTII proteins has been established based upon the following considerations: (1) no amino acid sequence similarity to known toxins, other than *B.t.* proteins in the case of Cry1Ac, and no immunologically relevant sequence similarity with known allergens, (2) rapid degradation under conditions which simulate mammalian digestive systems, (3) no indications of acute toxicity in

mice administered Cry1Ac or NPTII protein by oral gavage, (4) very low dietary exposure, and (5) a history of safe use.

The Cry1Ac protein is present in commercially available insecticides, which are considered environmentally acceptable, because they are specific for the target insect pest and are typically harmless to plants and other non-target organisms. Cry1Ac cotton has been grown commercially on 14.145 million hectares since 1996.

26. Mechanism of interaction between the GMHP and target organisms (if applicable), if different from that of the recipient/parental organism(s)

Insect-protected cotton plants provide effective control of the tobacco budworm (*Heliothis virescens*), cotton bollworm (*Helicoverpa armigera*) and the pink bollworm (*Pectinophora gossypiella*) in the United States. In Spain and Greece the cotton bollworm (CBW) and pink bollworm (PBW) are principal pests of cotton (the tobacco budworm is not found in European cotton growing regions). CBW damage to cotton plants includes feeding on the leaves and fruiting structures such as the squares and bolls. The PBW feeds on cotton bolls. The Cry1Ac protein, produced by IPC 531, must be ingested by these insects for the protein to bind to the midgut epithelial cells, which results in cell disruption, gut paralysis and eventual mortality. As the insects must feed on the plant to ingest the protein, no change in the way they interact with the plant is expected.

27. Potentially significant interactions with non-target organisms, if different from the recipient or parental organism(s)

Since the Cry1Ac insect control protein is very specific in its range of control, beneficial insects and other non-target organisms will be unaffected by the protein expressed in IPC 531. In addition there have been no changes in the levels of natural toxicants present.

28. Description of detection and identification techniques for the GMHP, to distinguish it from the recipient or parental organism(s)

Southern blot or PCR techniques may be employed for the detection and identification of the inserted nucleotide sequences and ELISA for detection of the expressed Cry1Ac and NPTII proteins.

Additionally, an insect bioassay employing sensitive lepidopteran insect species such as tobacco hornworm (*Manduca sexta*), cabbage looper (*Trichoplusia ni*), tobacco budworm (*Heliothis virescens*), cotton bollworm or pink bollworm, may be utilised to identify plants expressing the Cry1Ac protein.

INFORMATION ON THE POTENTIAL ENVIRONMENTAL IMPACT FROM THE RELEASE OF THE GMHP

29. Potential environmental impact from the release or the placing on the market of GMOs (Annex II, D2 of Directive 2001/18/EC), if different from a similar release or placing on the market of the recipient or parental organism(s)

The behaviour and characteristics of insect-protected cotton plants have been studied in a range of field environments since 1991 and no significant differences, compared

to other cotton varieties, have been observed, apart from protection from certain lepidopteran insects.

30. Potential environmental impact of the interaction between the GMHP and target organisms (if applicable), if different from that of the recipient or parental organism(s)

As the target organisms must feed on the plant to ingest the protein produced by IPC 531, no change in the way they interact with the plant is expected.

31. Possible environmental impact resulting from potential interactions with non-target organisms, if different from that of the recipient or parental organism(s)

Since the Cry1Ac protein is very specific in its range of control, non-target organisms will not be affected by the Cry1Ac protein expressed in IPC 531.

(a) Effects on biodiversity in the area of cultivation

IPC 531 cotton has been grown commercially in the U.S., Australia, Mexico, South Africa, China, Argentina, Indonesia and India since initial commercial introduction in the US in 1996. No unusual plant pest characteristics or unintended environmental effects have been observed that are attributed to the inserted DNA and expressed proteins in IPC 531 cotton, as confirmed by the extensive studies developed prior to, and subsequent to, regulatory approvals and market introduction.

There is a history of safe use of the Cry1Ac protein in microbial *Bt*-based products. The Cry1Ac protein is highly specific against Lepidoptera pest species, such as pink bollworm (*Pectinophora gossypiella*), corn earworm (*Helicoverpa zea*) and tobacco budworm (*Heliothis virescens*), and do not have any deleterious effects to non-target organisms such as beneficial insects (other than closely-related Lepidopterans), birds, fish, and mammals, including humans. The safety of the Cry1Ac protein expressed in IPC 531 cotton to non-target organisms was confirmed on several representative organisms. Therefore, based on the demonstrated low hazard of the Cry1Ac protein to non-target organisms, no adverse effects from IPC 531 cotton are predicted or have been observed for non-target insects, mammals, birds, non-insect aquatic animals, and non-insect soil organisms. Therefore IPC 531 cotton poses comparable or fewer risks to biodiversity as traditional cotton treated with commercially approved insecticides. Furthermore, insect pests in cotton have been traditionally controlled with numerous applications of broadspectrum chemical insecticides which have been reduced with the use of IPC 531 cotton containing Cry1Ac protein. Therefore, with the use of IPC 531 cotton with in plant *Bt*-technology, non-target species are less likely to be harmed as they are with many broad spectrum insecticidal sprays, thereby preserving biodiversity in the area of cultivation. In fact, several studies have shown that non-target organisms can be more active in IPC 531 cotton.

Based on the available data and experience collected to date, IPC 531 cotton poses comparable or fewer risks to the environment as traditional cotton treated with commercially approved insecticides. Rather, the reduction of insecticide

applications as a result of using IPC 531 affords significant environmental and biodiversity benefits.

(b) Effects on biodiversity in other habitats

Cotton is predominantly a self-pollinating crop, but can be cross-pollinated by certain insects. However, outcrossing of the *cry1Ac* coding sequence from IPC 531 cotton to other *Gossypium* species or to others of the malvacea family is extremely unlikely for the following reasons:

- Cultivated cotton is an allotetraploid and is incompatible with cultivated or wild diploid cotton species; therefore, it cannot cross and produce fertile offspring.
- Although outcrossing to wild or feral allotetraploid *Gossypium* species can occur, commercial cotton production generally does not occur in the same geographical locations as the wild relatives.
- There are no identified non-cotton plants that are sexually compatible with cultivated cotton.

Crossing of the insect protection trait into other cultivated cotton genotypes is possible should the plants be in close proximity; however, studies have shown that this occurs at a very low frequency and is not considered to be a concern as it is unlikely to cause any adverse impact to the environment.

IPC 531 cotton does not exhibit different agronomic or morphological traits compared to controls that would confer a competitive advantage over other species in the ecosystem in which it is grown. Also, there is little probability that any *Gossypium* species crossing with IPC 531 cotton could become weedier. All wild and feral relatives of cotton are tropical, woody, perennial shrubs other than a few herbaceous shrubs. In most instances, the distribution of these species is determined by soil and climatic conditions. As perennials, the plants are not particularly programmed to produce seed each year. Based on these mechanistic arguments, it is unlikely that transfer of the insect-protection trait to wild or feral cotton would have any significant impact on biodiversity of the wild or feral cotton habitat.

(c) Effects on pollinators

Cotton is normally considered to be a self-pollinating crop. The pollen is heavy and sticky and transfer by wind is unlikely. Pollen is transferred instead by various insects, in particular by various wild bees, including bumble bees *Bombus* spp., Melissodes bees and honey bees (*Apis mellifera*). The Cry1Ac protein was not detected in nectar collected from IPC 531 cotton using an assay with a limit of detection of 1.6 ng/g fresh weight of the nectar. The Cry1Ac protein is present in pollen collected from IPC 531 cotton at very low levels just above the limit of detection of the assay used to evaluate the Cry1Ac protein concentrations: 11.5 ng/g fresh weight of the pollen. Therefore, the pollinating insects of cotton are exposed to very low levels of the Cry1Ac protein expressed in IPC 531 cotton. Furthermore, the literature has established that the Cry1Ac protein is selective for lepidopteran insects, binds specifically to receptors on the mid-gut of lepidopteran insects and has no deleterious effect on beneficial/non-target insects. The safety of the Cry1Ac protein expressed in IPC 531 cotton to non-target organisms was confirmed on several representative organisms, including bees. Honey bee larva and adults were

exposed to concentrations of Cry1Ac protein in the insect diet that were more than 1600 times the maximum Cry1Ac protein expression level in either pollen or nectar. Results of these studies showed that the Cry1Ac protein produced in IPC 531 cotton tissues has no measurable deleterious effects on honey bee larvae or adults. Therefore, with the use of IPC 531 cotton with in plant *Bt*-technology, non-target, beneficial insects including pollinators are not harmed as they are with many broad spectrum insecticidal sprays. Therefore, since use of IPC 531 cotton has resulted in a reduction in conventional synthetic insecticide applications increased populations of beneficial insects in IPC 531 cotton fields are expected. In fact, several studies have shown that non-target organisms can be more active in IPC 531 cotton as biological control agents for secondary pests.

(d) Effects on endangered species

The potential risk to endangered species of IPC 531 cotton plants containing the Cry1Ac protein was first assessed by analyzing the specificity and potential hazard of the Cry1Ac protein to different groups of endangered non-target species based on our knowledge of mechanisms of action and spectrum of insecticidal activity for *Bacillus thuringiensis* crystalline (Cry) proteins. Secondly the possible exposure of susceptible species to IPC 531 cotton producing the Cry1Ac protein was evaluated.

IPC 531 cotton has been grown commercially in the U.S., Australia, Mexico, South Africa, China, Argentina, Indonesia and India since initial commercial introduction in the US in 1996. No unusual plant pest characteristic or unintended environmental effects have been observed that are attributed to the inserted DNA and expressed proteins in IPC 531 cotton, as confirmed by the extensive studies developed prior to, and subsequent to, regulatory approvals and market introduction.

There is a history of safe use of the Cry1Ac protein in microbial *Bt*-based products.

The next step in the risk assessment is to determine whether endangered Lepidoptera species may potentially be exposed to hazardous levels of the Cry1Ac protein in IPC 531 cotton. Lepidoptera larvae are principally herbivorous and the adults feed mainly on nectar. Adults and larvae of endangered Lepidoptera could possibly be exposed to the Cry1Ac protein from IPC 531 cotton by feeding directly on cotton plants, on nectar (adults), or on wind-drifted pollen. No endangered Lepidoptera are known to be pests of cotton and hence do not feed extensively on cotton. Therefore, the exposure assessment focused on exposure via feeding on nectar or pollen drift. The Cry1Ac protein was not detected in nectar collected from IPC 531 cotton using an assay with a limit of detection of 1.6 ng/g fresh weight of the nectar. The Cry1Ac protein is present in pollen collected from IPC 531 cotton at very low levels just above the limit of detection of the assay used to evaluate the Cry1Ac protein concentrations: 11.5 ng/g fresh weight of the pollen. Therefore, minimal exposure of endangered Lepidoptera to the Cry1Ac protein via nectar is expected. Regarding exposure via pollen, cotton pollen grains are sticky and relatively large in diameter, which makes their movement by wind unlikely. Therefore, off-site exposure of endangered Lepidoptera to wind-drifted IPC 531 cotton pollen is not likely. Pollination of cotton occurs mainly through insect

pollinators, principally bees, which have been shown not to be susceptible to the Cry proteins as discussed earlier.

These data indicate that non-pest Lepidoptera are unlikely to be exposed to the Cry1Ac protein in IPC 531 cotton.

Based on these observations, no significant impact or exposure of endangered Lepidoptera to the Cry1Ac protein is predicted from the use of IPC 531 cotton.

Furthermore, insect pests in cotton have been traditionally controlled with numerous applications of broadspectrum chemical insecticides which have been reduced with the use of IPC 531 cotton containing Cry1Ac protein. Therefore, with the use of IPC 531 cotton with in plant *Bt*-technology, endangered insect species are less likely to be harmed as they are with many broad spectrum insecticidal sprays. In fact, several studies have shown that non-target organisms can be more active in IPC 531 cotton as biological control agents for secondary pests.

As a result of the use of IPC 531 cotton, insecticide use has been reduced and therefore associated exposure of non-endangered, as well as endangered species to chemical insecticides used in cotton has also been reduced.

C. INFORMATION RELATED TO PREVIOUS RELEASES

32. History of previous releases notified under Part B of the Directive 2001/18/EC and under Part B of Directive 90/220/EEC by the same notifier

(a) Notification number

Releases of IPC 531 have been notified under Part B of the Directive 90/220/EEC in Greece (B/GR/97/01; B/GR/97/06; B/GR/98/06) and Spain (B/ES/96/05; B/ES/97/12; B/ES/98/16; B/ES/00/01; B/ES/01/01).

(b) Conclusions of post-release monitoring

The conclusions of the field trials with IPC 531, relate to the assessment of agronomic performance, efficacy and selectivity, yield potential, residues determination, compositional analysis and breeding. No significant evidence that IPC 531 are likely to cause any adverse effects to human or animal health or to the environment.

(c) Results of the release in respect to any risk to human health and the environment (submitted to the Competent Authority according to Article 10 of Directive 2001/18/EC)

Post-release general surveillance from environments provided no significant evidence that IPC 531 is likely to pose any risk of adverse effects to human or animal health or to the environment.

33. ***History of previous releases carried out inside or outside the Community by the same notifier***

(a) Release country

First year of experiment and commercialisation

Country	First year of experiment	First year of commercialisation
Argentina	1994	1998
Australia	1992	1996
China	1995	1998
India	1996	2002
Indonesia	1998	2001
Mexico	1995	1996
South Africa	1995	1997
U.S.	1991	1996
Japan	Import only	Import approval 1997
Thailand	1997	-
Turkey	1998	-
Columbia	1997	2002 semi-commercial
Israel	1997	-
Spain	1996	-
Greece	1998	-

(b) Authority overseeing the release

Argentina: Secretary of Agriculture (SAGPyA) - CONABIA

Australia: GMAC and now OGTR (Office of Gene Technology Regulator)

China: Ministry of Agriculture

Colombia: Colombian Institute of Agriculture (ICA)

Greece: Ministry of Environment

India: Genetic Engineering Approval Committee under Ministry of Environment and Forests

Indonesia: National Biosafety and Food safety Committee for Genetically Modified Agriculture Products, and Variety Release Committee

Israel: Ministry of Agriculture and Rural Development

Japan: Ministry of Agriculture, Forestry and Fisheries (MAFF)

Mexico: Agriculture and Health Ministries

South Africa: National Department of Agricutlure

Spain: Comisión Nacional de Bioseguridad (1996-2002), and Consejería de Agricultura de la Junta de Andalucía (2000-2002)

Thailand: Ministry of Agriculture

Turkey: Ministry of Agriculture and Rural Affairs (MARA)

U.S.: United States Department of Agriculture and Environmental Protection Agency

(c) Release site

Argentina: cotton area, Northern part of Argentina

Australia: Cotton Growing regions of Queensland and New South Wales

China: Yellow River and Yangzte River regions

Colombia: Humid Caribbean zone at Cordoba department (Province)

Greece: two sites: county of Larissa, Messorachi and county of Fthiotis, Avlaki

India: entire country

Indonesia: Selected regencies in South Sulawesi Province

Israel: entire cotton growing favourable area

Japan: isolated field regulated by MAFF's guideline

Mexico: cotton growing area

South Africa: entire cotton production region

Spain: Andalucía (Sevilla, Córdoba, Jaén, Cádiz)

Thailand: Nakornsawan, Nakornrajsrima, Petchboon and Leoi Provinces

Turkey: South, Southeast and Southwestern part

U.S.: multiple field locations in the following states : California, Arizona, Texas, Louisiana, Mississippi, Arkansas, Georgia, Alabama, North Carolina

(d) Aim of the release

Argentina: Compare different hybrid phenotypes and yielding performance

Australia: Assess the performances: efficacy, yield, breeding

China: Assess the performance: efficacy, yield

Colombia: Assess the performances: efficacy, yield. Pollen flow and risk of cross-pollination. Affect of the technology on target and non-target arthropods and annelids.

Greece: check the adaptability of GM cotton varieties under Greek conditions

India: assess performances

Indonesia: commercial cultivation

Israel: assess the performances: efficacy, yield and use for animal feed

Japan: conduct the environmental safety assessment for import approval

Mexico: test biological efficacy and obtain cost/benefit data

South Africa: conditional general release – full commercial

Spain: determine the protection, benefits and compatibility with recommended Integrated Pest Management practices

Thailand: biosafety study and requirement for production approval

Turkey: for field trial purpose

U.S.: assess agronomic performances: efficacy of the trait, yield, lint quality

(e) Duration of the release

Argentina: annual field trial permit

Australia: ongoing

China: 3-4 years for field trials; commercialised

Colombia: not determined yet

Greece: one year

India: three years (2002-2005) for the commercial release; one year for experimental purpose

Indonesia: extendable one yearly release

Israel: one year

Japan: not applicable

Mexico: one or two years

South Africa: conditional until IPR demonstrates no resistance development

Spain: one year

Thailand: one year

Turkey: 1999-2000

U.S.: before commercial approval of IPC 531, experimental field trials were conducted from 1991 to 1995 (planted from February to June; harvest completed between September and November)

(f) Aim of post-releases monitoring

Argentina: check for volunteer plants. No cotton is allowed to be grown in the same plot for three years

Australia: compliance with regulatory requirements and in particular to monitor for any changes in pest resistance

China: Environmental safety assessment : resistance appearance; impact on NTOs

Colombia: monitoring behaviour of *Anthonomus grandis*, principal cotton pest under IPC 531 cotton crops. Determine susceptibility base line to the *Bt* protein for target species. Build up database of social economical, health and environmental impacts

Greece: no post monitoring

India: evolve IRM strategy and monitor benefits to cultivars

Indonesia: mandatory monitoring by regulatory agency to secure extension permit

Israel: effectiveness of technology

Japan: not applicable

Mexico: to check in target insects their response to Bt protein and that growers put refugee areas in place

South Africa: determination if resistance development can be contained

Spain: susceptibility and insect resistance development (only after commercial release); overwintering capability

Thailand: to confirm safety and performance of product

Turkey: no monitoring

U.S.: before commercial approval, all field trials were monitored for volunteers for 1 year after final harvest, as part of the weediness assessment comparing IPC 531 and conventional cotton. Any volunteers were controlled by herbicides or cultivation practices. After commercial approval, IPC 531 production is monitored annually for compliance with Insect Resistance Management (IRM) requirements: grower compliance with IRM (refuge size, distance between refuge and IPC 531 fields); insect susceptibility monitoring

(g) Duration of post-releases monitoring

Argentina: three years

Australia: ongoing

China: ongoing monitoring on commercialized varieties

Colombia: on going. Not determined yet

Greece: no post monitoring

India: three years

Indonesia: three to five years

Israel: permanent under supervision of Plant Protection and Inspection Service and the Israeli Cotton Board

Japan: not applicable

Mexico: permanent and consist in analysis of target pests for their susceptibility to Cry1Ac

South Africa: on going until success can be defined

Spain: one year

Thailand: six months

Turkey: not determined yet

U.S.: 12 months

(h) Conclusions of post-release monitoring

Argentina: technology very effective to control target pests (*i.e. Alabama argillacea*, *Heliothis virescens*, *Helicoverpa gelotopoeon*, and *Pectinophora gossypiella*)

Australia: no changes in resistance to date

China: no risk to environmental safety

Colombia: risk of cross pollination is very low and at 1 meter apart with other *Gossypium hirsutum*. Technology is effective to control target pests, no effect on beneficial fauna and other non-target arthropods and annelids

Greece: no post monitoring

India: no risk to the environment. A refuge of 20 % is required.

Indonesia: no hazard identified

Israel: technology has proved very effective in controlling the target pests

Japan: not applicable

Mexico: positive impact (insecticide use reduction)

South Africa: so far no resistance development has been seen from the study of 5 populations of *H. armigera*

Spain: overwintering and dissemination risks are equivalent to those foe conventional cotton varieties

Thailand: it is safe to environment and has real benefit to farmers

Turkey: still under process

U.S.: Pre-commercial: There was no difference in volunteers between IPC 531 and conventional cotton varieties. The IPC 531 trait does not change the volunteer potential of cotton. Post-commercial: Typically grower compliance with IPC 531 cotton IRM requirements is high (92%), and no significant change in susceptibility of target insect pests to the Cry1Ac protein.

(i) Results of the release in respect to any risk to human health and the environment

Argentina: no evidence of any risk but a significant reduction of pesticides applied to cotton has been evidenced

Australia: no adverse effect

China: no risk to human health and environment

Colombia: no evidence of any risk

Greece: no such a kind of risk has been defined

India: no evidence of any risk

Indonesia: no risk was identified to human health and the environment

Israel: no negative results

Japan: not applicable

Mexico: no evidence of any risk

South Africa: only beneficial – insecticide reductions – reduced admittance to clinics at small grower level due to reduce pesticide exposure

Spain: no evidence of any risk

Thailand: has not approved any results of the release. If they approve, results will be that transgenic is as safe to human health and the environment as non-transgenic

Turkey: no risk detected

U.S.: No unusual plant pest characteristics have been reported for IPC 531 cotton, and no adverse effects on human health reported that are associated with production of IPC 531 cotton, and human consumption of cotton food products.

D. INFORMATION RELATING TO THE MONITORING PLAN – IDENTIFIED TRAITS, CHARACTERISTICS AND UNCERTAINTIES RELATED TO THE GMO OR ITS INTERACTION WITH THE ENVIRONMENT THAT SHOULD BE ADDRESSED IN THE POST COMMERCIALISATION MONITORING PLAN

1. Confirmation that any assumptions regarding the occurrence and impact of potential adverse effects of the GMO or its use in the E.R.A. are correct.

The results of the environmental risk assessment (E.R.A.) of IPC 531 (Annex II) show effectively zero overall risk arising from the placing on the market of this cotton relating to:

- Persistence or invasiveness
- Selective advantage
- Potential for gene transfer
- Impact on target organisms
- Impact on non-target organisms
- Effects on biogeochemical processes due to direct or indirect interactions with target and non-target organisms
- Changes in agricultural practice

Moreover, the risk assessment has demonstrated that IPC 531 presents effectively zero risk to human and animal health relating to:

- Persons in proximity or contact with the release
- The consumption of products derived from IPC 531

These conclusions having been reached on the basis of scientific data and analysis, rather than on the basis of assumptions, case-specific monitoring of IPC 531 is not relevant.

2. Identification of the occurrence of adverse effects of the GMO or its use on human health or the environment which were not anticipated in the E.R.A.

The environmental and human health safety assessment for IPC 531 did not identify any specific risks related to its placing on the market during production, storage, processing and other uses. Therefore the monitoring plan for IPC 531 is focused on general surveillance for unanticipated, adverse effects.