Application for authorization of MON 15985 and MON 15985 × MON 1445 cotton in the European Union, according to Regulation (EC) No 1829/2003 on genetically modified food and feed

Part II
Summary

A. GENERAL INFORMATION

1. Details of application

a) Member State of application

United Kingdom

b) Application number

Not available at the time of application

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

The Monsanto development codes for both genetically modified cotton products are: MON 15985 and MON 15985 × MON 1445. In countries where MON 15985 and MON 15985 × MON 1445 are being cultivated, packages of these cottonseeds are marketed under the name of the varieties, in association with the trademarks Bollgard II®[1] or Bollgard II® with Roundup Ready®[1], indicating clearly to growers that the cotton is protected from specific lepidopteran insect pests or protected from specific lepidopteran insect pests and tolerant to Roundup®[1] herbicide, containing the active ingredient glyphosate.

d) Date of acknowledgement of valid application

Not available at the time of application

2. Applicant

a) Name of applicant

Monsanto Company, represented by Monsanto Europe S.A.

b) Address of applicant

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

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

[1] Bollgard II®, Roundup Ready® and Roundup® are registered trademarks of Monsanto Technology LLC.

c) Name and address of the person established in the Community who is responsible for the placing on the market, whether it be the manufacturer, the importer or the distributor, if different from the applicant (Commission Decision 2004/204/EC Art 3(a)(ii))

MON 15985 and MON 15985 × MON 1445 will be traded and used in the E.U. in the same manner as the equivalent products from current commercial cotton and by the same operators currently involved in the trade and use of traditional cotton.

3. Scope of the application

( ) GM plants for food use
( ) Food containing or consisting of GM plants
(X) Food produced from GM plants or containing ingredients produced from GM plants
( ) GM plants for feed use
( ) Feed containing or consisting of GM plants
(X) Feed produced from GM plants
( ) Import and processing (Part C of Directive 2001/18/EC)
( ) Seeds and plant propagating material for cultivation in Europe (Part C of Directive 2001/18/EC)

4. Is the product being simultaneously notified within the framework of another regulation (*e.g.* seed legislation)?

Yes ( )	**No (x)**
If *ye*s, specify	

5. Has the GM plant been notified under Part B of Directive 2001/18/EC and/or Directive 90/220/EEC?

Yes ( )	**No (x)**
If *n*o, refer to risk analysis data on the basis of the elements of Part B of Directive 2001/18/EC *See* following sections	

6. Has the GM plant or derived products been previously notified for marketing in the Community under Part C of Directive 2001/18/EC or Regulation (EC) 258/97?

Yes ( )	No (x)
If *yes*, specify	

7. Has the product been notified in a third country either previously or simultaneously?

Yes (x)	No ( )
If *yes*, specify Outside the E.U., such as in U.S. and Australia, MON 15985 and MON 15985 x MON 1445 are authorized for all uses, corresponding to the full range of used of traditional cotton. The scope of the approvals already granted for these genetically modified cotton products and the status of pending regulatory reviews, which are currently in progress in numerous countries around the world, depend on the country and its local regulatory framework. Final approvals wherein countries require specific approvals are posted by these regulatory agencies on their official websites.	

8. General description of the product

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

MON 15985 was developed to produce two *Bacillus thuringiensis* proteins conferring protection against lepidopteran pests. This product is the result of the transformation of MON 531 which contains the genetic material necessary to express the Cry1Ac insect protection protein and the NPTII selectable marker protein. Genetic modification was used in the development of MON 531. The transformation of MON 531 introduced a second genetic modification, resulting in the production of the Cry2Ab2 and GUS proteins; this second genetic modification is referred to as MON 15947. The combination of the genetic material responsible for the Cry1Ac and the Cry2Ab2 production from MON 531 and MON 15947 respectively is known as MON 15985. Therefore, MON 15985 produces both Cry1Ac and Cry2Ab2 insect protection proteins for the effective control of major lepidopteran insect pests of cotton, including the cotton bollworm, tobacco budworm, and the pink bollworm.

MON 1445 contains the genetic material necessary to express the CP4 EPSPS which imparts tolerance to glyphosate and the NPTII selectable marker protein.

MON 15985 × MON 1445 has been produced by the traditional breeding of MON 15985 and MON 1445. Although genetic modification was used in the development of MON 15985 and MON 1445, no additional genetic modifications were involved for the production of MON 15985 × MON 1445.

b) Types of products planned to be placed on the market according to the authorisation applied for

The scope of this application is for uses of MON 15985 and MON 15985 × MON 1445 for food and feed, specifically cottonseed oil and its constituents. The range of uses of both cotton for food and feed will be identical to the full range of equivalent uses of traditional cotton. It does not include environmental release.

c) Intended use of the product and types of users

MON 15985 and MON 15985 × MON 1445 food and feed products will be traded and used in the E.U. in the same manner as current commercial cotton and by the same operators currently involved in the trade and use of traditional cotton.

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

MON 15985 and MON 15985 × MON 1445 are substantially equivalent to other cotton varieties except for the introduced traits, namely protection from target lepidopteran pests or protection from target lepidopteran pests with tolerance to glyphosate, which are traits of agronomic interest. Both were shown to be as safe and as nutritious as traditional cotton. Therefore MON 15985 and MON 15985 × MON 1445 food and feed products will be stored, packaged, transported, handled and used in the same manner as other commercial cotton products. No specific conditions are warranted or required for the food and feed use of MON 15985 and MON 15985 × MON 1445.

e) Any proposed packaging requirements

MON 15985 and MON 15985 × MON 1445 are substantially equivalent to traditional cotton varieties (except for the protection from targeted lepidopteran insect pests or the protection from targeted lepidopteran insect pests with tolerance to glyphosate). Therefore, MON 15985 and MON 15985 × MON 1445 food and feed products will be used in the same manner as other cotton and no specific packaging is foreseen. (For labelling, *See* question 8.(f)).

f) A proposal for labelling in accordance with Articles 13 and Articles 25 of Regulation (EC) 1829/2003. In the case of GMOs, food and/or feed containing, consisting of GMOs, a proposal for labelling has to be included complying with the requirements of Article 4, B(6) of Regulation (EC) 1830/2003 and Annex IV of Directive 2001/18/EC.

The scope of this application covers foods and feeds produced from MON 15985 and MON 15985 × MON 1445. According to Regulation (EC) N° 1829/2003, Articles 13 and 25, the operators placing on the market food and feed products produced from MON 15985 and MON 15985 × MON 1445 shall ensure that those products are labeled with the words "*produced from genetically modified cotton*". In the case of products for which no list of ingredients exists, operators shall ensure that an indication that the food or feed product is produced from this GM plant is transmitted in writing to the operator receiving the product.

g) Unique identifier for the GM plant (Regulation (EC) 65/2004; does not apply to applications concerning only food and feed produced from GM plants, or containing ingredients produced from GM plants)

Not applicable as the scope of this application is foods and feeds produced from MON 15985 and MON 15985 x MON 1445.

h) If applicable, geographical areas within the EU to which the product is intended to be confined under the terms of the authorisation applied for. Any type of environment to which the product is unsuited

MON 15985 and MON 15985 × MON 1445 are suitable for food and feed use throughout the E.U.

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

Misuse of MON 15985 and MON 15985 × MON 1445 is unlikely, as the proposed food and feed uses for both cotton products include the current food and feed uses of traditional cotton. MON 15985 and MON 15985 × MON 1445 are substantially equivalent to other cotton except for the introduced traits, which are traits of agronomic interest. Both MON 15985 and MON 15985 x MON 1445 have been shown to be as safe and as nutritious as traditional cotton. Therefore, any measures for waste disposal and treatment of MON 15985 and MON 15985 × MON 1445 products are the same as those for traditional cotton. No specific conditions are warranted or required for the placing on the market of MON 15985 and MON 15985 × MON 1445 for food and feed.

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

1. Complete name

a) Family name

Malvaceae

b) Genus

Gossypium

c) Species

hirsutum (4n = 52)

d) Subspecies

N/A

e) Cultivar/breeding line or strain

MON 15985 and MON 15985 × MON 1445

f) Common name

Cotton

2. a) Information concerning reproduction

(i) Mode(s) of reproduction

Cotton production is generally carried out with seeds. Cotton is a perennial plant that is harvested and planted annually. Cross-pollination can occur, but cotton is normally considered to be a self-pollinating crop.

(ii) Specific factors affecting reproduction

Although natural crossing can occur, cotton is considered to be a self-pollinating crop. The pollen is heavy and sticky and transfer by wind is unlikely. Regardless, there are no morphological barriers to cross-pollination based on flower structure. Pollen is transferred instead by insects, in particular by various wild bees, bumble bees (*Bombus* sp.), and honeybees (*Apis mellifera*).

(iii) Generation time

The cultural cycle for cotton ranges from 120 to 200 growing days from seedling emergence to maturity.

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

The scope of the current application does not include the environmental release of MON 15985 and MON 15985 × MON 1445.

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.

Cross-pollination decreased from five to less than one percent from one to seven meters, respectively, away from the source plot.

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 the E.U. No genera in the Gossypieae tribe occur naturally in this region.

3. 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.

b) Specific factors affecting survivability

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 the E.U. some of the seed remaining in the field following harvest and cultivation may germinate in the 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. However, it should be noted that cultivation or import of cottonseed of MON 15985 and MON 15985 × MON 1445 is not in the scope of this application.

4. 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

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.

5. Geographical distribution and cultivation of the plant, including the distribution in Europe of the compatible species

There are five prominent types of cotton being grown commercially around the world including Egyptian, Sea Island, American Pima, Asiatic and Upland. Cotton is grown worldwide between latitudes of 45° north and 30° south, in areas that have at least 160 frost free days. Cotton is a 'heat loving' plant, however more than 50% of the world crop is grown in temperate zones above 30° N latitude. Additionally, cotton is grown under similar climatic and soil constraints. The majority of cotton is grown in areas that receive between 50 and 150 cm of rainfall per year.

The major cotton producing countries in the world include the United States, Peoples Republic of China, India, Pakistan and the Republic of Uzbekistan. Brazil, Australia, Egypt, Argentina, Turkey, Greece, Syria and others produces significant, but lesser amounts.

There are no close wild relatives of cotton in the E.U.

6. 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, however cotton cultivation or the import of cottonseed of MON 15985 and MON 15985 × MON 1445 in the E.U. is not within the scope of this application.

7. Other potential interactions, relevant to the GM plant, of the plant with organisms in the ecosystem where it is usually grown, or used elsewhere, 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. Both gossypol and cyclopropenoid fatty acids contents are reduced via processing of the cottonseed into oil or meal.

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Information on MON 531 and MON 1445 has been previously described in the notifications pursuant to Regulation (EC) No 258/97.

1. Description of the methods used for the genetic modification

MON 15985
MON 15985 is produced by the transformation of MON 531, which was previously genetically modified via *Agrobacterium tumefaciens* mediated transformation. MON 15985 was generated using the particle acceleration transformation system.

MON 15985 x MON 1445
Not applicable since MON 15985 × MON 1445 is produced by the traditional cotton breeding cross of MON 15985 with MON 1445. Genetic modification was used in the development of MON 15985 and MON 1445.

2. Nature and source of the vector used

MON 15985
MON 15985 is produced by the transformation of MON 531. The plasmid vector used to generate MON 15985, PV-GHBK11, is an 8.7 Kb high copy number, pUC-based plasmid. It contains well-characterized DNA elements for selection (*nptII*) and replication (ori-pUC) of the plasmid in bacteria.

MON 15985 x MON 1445
Not applicable since MON 15985 x MON 1445 results from traditional breeding.

3. Source of donor DNA, size and intended function of each constituent fragment of the region intended for insertion

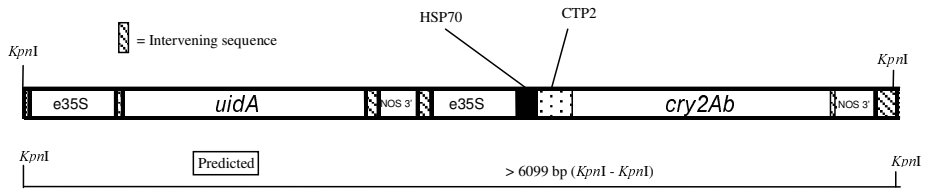
MON 15985

The linearized segment of the vector used in the transformation, PV-GHBK11L**,** contained the two genes to be introduced in MON 531 cotton plant cells, *i.e.*, the chimearic *cry2Ab2* gene (encoding the agronomic trait) and the *uidA* gene (selectable marker). The expression cassettes (Table 1 and Figure 1) corresponding to these two genes consist of respectively: a *cry2Ab2* coding sequence regulated by the e35S plant promoter, heat shock protein leader (HSP70), ctp2 and the NOS 3' polyadenylation sequence; and the *uidA* coding sequence regulated by the e35S plant promoter and the NOS 3' polyadenylation sequence.

Table 1. Elements of the transformation fragment PV-GHBK11.

Genetic Element	Approximate Size (Kb)	Description/source
uidA cassette		
e35S	0.6	Cauliflower mosaic virus (CaMV) promoter with the duplicated enhancer region used to drive expression of the *uidA* coding sequence.
uidA	1.8	DNA sequence coding for the β-D-glucuronidase (GUS) protein from *E. coli.*
NOS 3'	0.26	3' nontranslated region of the nopaline synthase (*nos*) gene from *Agrobacterium tumifaciens* which terminates transcription and directs polyadenylation.
cry2Ab2 cassette		
e35S	0.6	Cauliflower mosaic virus (CaMV) promoter with the duplicated enhancer region used to drive expression of the *cry2Ab2* gene.
HSP70	0.1	Petunia heat shock protein 70 5' untranslated leader sequence.
ctp2	0.23	DNA sequence coding for the N-terminal chloroplast transit peptide from *Arabidopsis* thaliana *epsps* gene.
cry2Ab2	1.9	DNA sequence coding for a synthetic Cry2Ab2 protein of *Bacillus thuringiensis.*
NOS 3'	0.26	3' nontranslated region of the nopaline synthase (*nos*) gene from *Agrobacterium tumefaciens* which terminates transcription and directs polyadenylation.

Figure 1. Transformation vector: DNA segment PV-GHBK11L



The *Kpn*I DNA segment, PV-GHBK11L was used as transformation vector to generate MON 15985 by particle acceleration technology.

MON 15985 x MON 1445

MON 15985 × MON 1445 has been produced by the traditional breeding of MON 15985 and MON 1445. The inserted DNA fragment from both parental lines are inherited in MON 15985 x MON 1445.

The individual components and the size, source and function of these inherited DNA sequences are given in Tables 2 and 3. while schematic representations of those inserts are shown in Figures 2 and 3.

Table 2. Summary of genetic elements of the inserts in MON 15985.

Genetic Element	Approximate Size (Kb)[1]	Description/source
Genetic elements associated to the functional *cry1Ac* insert (MON 531)		
cry1Ac cassette		
7S 3'	0.44	3' nontranslated region from soybean 7S seed storage protein gene which terminates transcription and directs polyadenylation of the *cry1Ac* mRNA
cry1Ac	3.54	DNA sequence coding for a synthetic variant of the Cry1Ac protein of *Bacillus thuringiensis*
e35S	0.6	Cauliflower mosaic virus (CaMV) promoter with the duplicated enhancer region used to drive expression of the *cry1Ac* coding sequence.
aad gene		
aad	0.79	Bacterial gene comprising its own regulatory elements and coding for an aminoglycoside-modifying enzyme, 3'(9)-O-nucleotidyltransferase from the transposon Tn7
nptII cassette		
NOS 3'	0.24	3' nontranslated region of the nopaline synthase (*nos*) gene from *Agrobacterium tumefaciens* which terminates transcription and directs polyadenylation
nptII	0.97	DNA sequence isolated from the bacterial transposon Tn5 coding for neomycin phosphotransferase type II. Expression of this sequence in plant cells confers resistance to kanamycin and serves as a selectable marker for transformation. The *nptII* cassette also contains a 153 bp portion of the 378 bp (*ble*) gene encoding the bleomycin binding protein.
35S	0.32	Cauliflower mosaic virus (CaMV) promoter
ori-V	0.39	Origin of replication for *Agrobacterium* derived from the broad host range plasmid RK2.

[1] Sizes of the same genetic element may differ slightly between the *cry1Ac* and *cry2Ab2* coding regions due to revisions in the annotation of the Monsanto proprietary sequence database.

Table 2. Summary of genetic elements of the inserts in MON 15985 – continued.

Genetic Element	Approximate Size (Kb)[1]	Description/source
Genetic elements associated to the *cry2Ab2* insert (MON 15947)		
uidA cassette		
e35S	0.3	Cauliflower mosaic virus (CaMV) promoter with a duplicated enhancer region used to drive expression of the *uidA* coding sequence.
uidA	1.8	DNA sequence coding for the β-D-glucuronidase (GUS) protein from *E. coli*
NOS 3'	0.26	3' nontranslated region of the nopaline synthase (*nos*) gene from *Agrobacterium tumefaciens* which terminates transcription and directs polyadenylation
cry2Ab2 cassette		
e35S	0.6	Cauliflower mosaic virus (CaMV) promoter with the duplicated enhancer region used to drive expression of the *cry2Ab2* gene.
HSP70	0.1	Petunia heat shock protein 70 5' untranslated leader sequence.
ctp2	0.23	DNA sequence coding for the N-terminal chloroplast transit peptide from *Arabidopsis thaliana epsps* gene.
cry2Ab2	1.9	DNA sequence coding for a synthetic Cry2Ab2 protein of *Bacillus thuringiensis*.
NOS 3'	0.26	3' nontranslated region of the nopaline synthase (NOS) gene from *Agrobacterium tumefaciens* which terminates transcription and directs polyadenylation.

[1] Sizes of the same genetic element may differ slightly between the *cry1Ac* and *cry2Ab2* coding regions due to revisions in the annotation of the Monsanto proprietary sequence database.

Table 3. Summary of genetic elements of the insert in MON 1445.

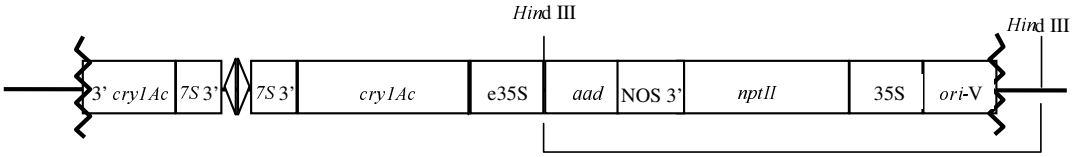
Genetic Element	Approximate Size (Kb)	Description/source (Reference)
Right Border	0.02	DNA sequence derived from *Agrobacterium* containing the right border essential for transfer of the T-DNA.
cp4 epsps cassette		
E9 3'	0.64	3' nontranslated region of the pea ribulose-1,5-bisphosphate carboxylase small subunit (rbcS) E9 gene, terminates transcription and directs polyadenlyation of the mRNA.
cp4 epsps	1.37	DNA sequence coding for the synthetic CP4 EPSPS protein (5-enolpyruvylshikimate-3-phosphate synthase) from *Agrobacterium* sp. strain CP4 (*aroA* gene).
ctp2	0.29	DNA sequence coding for the N-terminal chloroplast transit peptide from *Arabidopsis thaliana* EPSPS gene.
FMV	0.56	35S promoter derived from figwort mosaic virus.
aad gene		
aad	0.83	Bacterial gene comprising its own regulatory elements and coding for an aminoglycoside-modifying enzyme, 3''(9)-O-nucleotidyltransferase from the transposon Tn7

Table 3. Summary of genetic elements of the insert in MON 1445 – continued.

nptII cassette		
NOS 3'	0.25	3' nontranslated region of the nopaline synthase (NOS) gene from *Agrobacterium tumefaciens* which terminates transcription and directs polyadenylation.
nptII	0.79	DNA sequence isolated from the bacterial transposon Tn5 (Beck *et al.*, 1982) coding for neomycin phosphotransferase type II. Expression of this sequence in plant cells confers resistance to kanamycin and serves as a selectable marker for transformation.
35S	0.32	Cauliflower mosaic virus (CaMV) promoter.
ori-V	0.22	Origin of replication for *Agrobacterium* derived from the broad host range plasmid RK2.

Figure 2. Schematic representation of the inserts in MON 15985

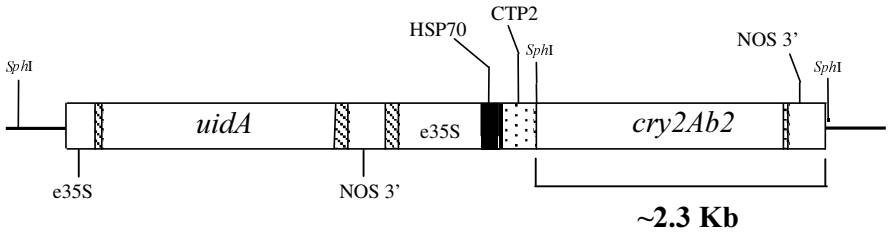
a/ MON 531 insert



~4.0 Kb

Schematic representation of the functional *cry1Ac* insert denoting the expected fingerprint band that should be detected by probing *Hin*d III digested MON 531 DNA with the ^{32}P-labeled *ori*-V element.

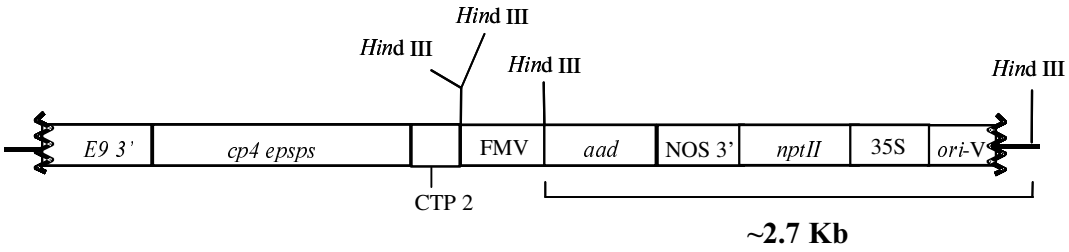
b/ MON 15947 insert



~2.3 Kb

Schematic representation of the *cry2Ab2* insert denoting the expected fingerprint band that should be detected by probing *Sph* I digested MON 15985 DNA with the ^{32}P-labeled *cry2Ab2* element.

Figure 3. Schematic representation of the *cp4 epsps* insert in MON 1445.



Schematic representation of the cp4 espsp insert denoting the expected fingerprint band that should be detected by probing *Hind* III digested MON 1445 DNA with the 32P-labeled *ori*-V element.

D. INFORMATION RELATING TO THE GM PLANT

Information on MON 531 and MON 1445 has been previously described in the notifications pursuant to Regulation (EC) No 258/97.

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

MON 15985

MON 15985 plants provide effective control of cotton bollworm (CBW, *Helicoverpa armigera*), pink bollworm (PBW, *Pectinophora gossypiella*) and tobacco budworm (TBW, *Heliothis virescens*) in cotton. These genetically modified cotton plants produce the Cry1Ac and Cry2Ab2 insect protection proteins derived from the common soil bacterium *Bacillus thuringiensis* subsp. *kurstaki*. Previously, MON 531 was found to have value beyond a replacement for insecticide applications for specific pests. The other direct benefits of MON 531, continued in MON 15985, and supported by data in the current literature, are improved control of agricultural pests, improved yield, reduced production costs, improved grower profitability, reduced occupational risk, improved opportunity to grow cotton, and improved economic outlook for the cotton industry. There also are a number of indirect benefits associated with the reduction in insecticide use, which include improved beneficial insect and wildlife populations, reduced runoff of insecticides, reduced air pollution, and reduction of chemical handling for farm workers.

MON 15985 is also expected to provide an additional tool to delay the development of lepidopteran resistance in cotton, because MON 15985 produces both the Cry1Ac and Cry2Ab2 proteins. MON 15985 provides equivalent or increased control of the major insect pests of cotton (tobacco budworm, pink bollworm, and cotton bollworm) compared to MON 531, with additional control of secondary lepidopteran insect pests such as beet and fall armyworm.

MON 15985 x MON 1445
MON 15985 x MON 1445 has been produced by the traditional breeding of MON 15985 and MON 1445. MON 15985 x MON 1445 expresses the insect-protection trait found in MON 15985, as well as the CP4 EPSPS protein which confers tolerance to glyphosate. The insect protection trait provides effective control of lepidopteran insects which are economically damaging pests in most cotton growing regions (*See* above). The glyphosate tolerance trait provides a novel, highly efficacious weed control option for farmers, and allows the farmer to take advantage of the favorable environmental properties exhibited by Roundup®.

2. Information on the sequences actually inserted or deleted

a) The copy number of all detectable inserts, both complete and partial

MON 15985
MON 15985 genomic DNA was analyzed by Southern blotting to determine the number of insertions and the copy number of the inserted DNA from MON 15947 and MON 531 genetic elements in MON 15985. It has been demonstrated that MON 15947 DNA contains one single insert made of one copy of the genetic elements of the transformation vector PV-GHBK11L.

MON 15985 x MON 1445
To confirm that the DNA inserts in MON 15985 x MON 1445 are the same as those that occur in MON 15985 and MON 1445, a Southern blot analysis was conducted to confirm the presence of the product-specific fingerprints for both MON 15985 and MON 1445 in MON 15985 x MON 1445.

The fingerprint analyses indicate that each of the parental inserts are present in MON 15985 x MON 1445.

Tables 2 and 3 summarize the genetic elements of the DNA inserts in MON 15985 and MON 1445.

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

Not applicable.

c) Chromosomal location(s) of insert(s) (nucleus, chloroplasts, mitochondria, or maintained in a non-integrated form), and methods for its determination

MON 15985

In addition to the MON 531 insert, MON 15985 contains in its nuclear genome an insert with one single copy of the elements present in transformation vector PV-GHBK11L. This insert is defined as MON 15947 insert. The presence of MON 15947 insert in the nuclear genome is best shown by the Chi square analysis of the segregation results. The Chi square analysis of the segregation pattern was consistent with a single site of insertion into the cotton DNA and segregation according to Mendelian genetics. This result is therefore consistent with DNA integration into nuclear DNA.

MON 15985 x MON 1445

The traditionally bred MON 15985 x MON 1445 contains the DNA inserts from both MON 15985 and MON 1445 at separate sites in the nuclear genome, as they were inherited from the MON 15985 and MON 1445 single trait material.

d) The organisation of the inserted genetic material at the insertion site

MON 15985

Genomic DNA from MON 15985 was analyzed by Southern blotting to determine the integrity of the inserted promoters, coding regions, and polyadenylation sequences, and the presence or absence of plasmid backbone sequences associated with the second insert MON 15947. In addition, the 5' and 3' junctions between the insert and the plant DNA were confirmed by PCR.

MON 15985 contains one complete copy of the *cry2Ab2* cassette linked to one copy of the *uidA* cassette, which is missing approximately 260 bp at the 5' end of the enhanced CaMV 35S promoter. MON 15985 does not contain any detectable plasmid backbone sequence.

MON 15985 x MON 1445

MON 15985 x MON 1445 is the result of traditional breeding of MON 15985 and MON 1445. There is no indication that the location of the inserts and the 5' and 3' flanking sequences have been altered during the breeding process; the molecular analysis of MON 15985 x MON 1445 confirms the presence of both inserts.

3. Information on the expression of the insert

a) Information on developmental expression of the insert during the life cycle of the plant

The scope of the current application covers cottonseed oil and its constituents produced from MON 15985 and MON 15985 x MON 1445. In support of notifications for MON 531 and MON 1445 under Regulation (EC) No 258/97, it has been demonstrated that there is no detectable level of protein in refined cottonseed oil produced from cotton modified through biotechnology or traditional cottonseeds. Therefore, the following information related to the expression of the insert can be considered as mainly informative.

MON 15985

A study was conducted to measure the amount of Cry2Ab2, Cry1Ac, GUS, NPTII, and AAD proteins in various tissue types collected from MON 15985 and control cotton grown in U.S. field trials in 1998. There were two types of controls used for this study including: DP50, a traditional variety, and MON 531, which expresses Cry1Ac and NPTII proteins. The background genetics of the test and control cotton were similar.

Levels of Cry2Ab2 and Cry1Ac proteins were analyzed in leaf, seed, whole plant and pollen because these tissues are most relevant to the insect control performance of the plant. The levels of NPTII and GUS proteins were estimated in leaf and seed samples. Tissue samples were collected from test and control plants grown in eight U.S. field trials conducted during the 1998 growing season.

Enzyme-Linked Immunosorbent Assay (ELISA) methods were developed and validated to quantify the Cry2Ab2, Cry1Ac, GUS, NPTII and AAD levels in cotton tissues. All protein values are expressed as micrograms (µg) of the specific protein per gram (g) of tissue on a fresh weight (fw) basis.

Table 4 presents a summary of the mean level of Cry2Ab2, Cry1Ac, GUS, NPTII and AAD protein levels found in cottonseed in MON 15985, MON 531 and the traditional control.

In conclusion, the Cry1Ac and NPTII protein levels are similar in MON 15985 compared to MON 531. Additionally, the Cry1Ac and NPTII proteins levels are below the limit of detection in the traditional cotton. As expected, the AAD protein was not detected in MON 15985, MON 531 or in the traditional control. The results also confirm that MON 15947 did not affect the levels of Cry1Ac and NPTII proteins expressed in MON 15985, as compared to MON 531. The measured Cry2Ab2 and Cry1Ac protein levels are sufficient to confer protection from cotton pest feeding damage.

Table 4. Summary of Cry2Ab2, Cry1Ac, GUS, NPTII and AAD proteins levels (µg/g fw)[1] measured in seed samples collected in 1998 field season
Mean ± Std Dev.[2] - (Range)[3]

	Cry2Ab2	Cry1Ac	GUS	NPTII	AAD
Seed[4]					
MON 15985	43.2 ± 5.7	3.35 ± 0.63	58.8 ± 13.0	10.8 ± 1.2	N.D.[9]
	(31.8-50.7)	(2.21-4.84)	(37.2-82.3)	(8.88-13.2)	
MON 531	<2.31[5]	3.22 ± 0.77	<4.54[6]	9.92 ± 2.19	N.D.[9]
		(1.50-4.46)		(3.81-12.6)	
Traditional control	<2.31[5]	<0.43[7]	<4.42[6]	<1.17[8]	N.D.[9]

1 Protein levels are expressed as microgram of protein per gram fresh weight of tissue and have been corrected for overall assay bias.
2 The mean and standard deviation were calculated from the analyses of plant samples, one from each of eight field sites except for tissues collected from single site.
3 Minimum and maximum values from the analyses of samples across sites.
4 The sample was of tissue from up to 6 plants per plot from each site.
5 The Limit of Detection for the Cry2Ab2 assay is 2.65 µg/g in leaf tissue and 2.31 µg/g in seed tissue.
6 The Limit of Detection for the GUS assay is 0.91 µg/g in leaf tissue and 4.42 µg/g in seed tissue.
7 The Limit of Detection for the Cry1Ac assay is 0.58 µg/g in leaf tissue and 0.43 µg/g in seed tissue.
8 The Limit of Detection for the NPTII assay is 0.30 µg/g in leaf tissue and 1.17 µg/g in seed tissue.
9 Not Detected (N.D.) since the mean blank O.D. was greater than the mean sample O.D.

MON 15985 x MON 1445

A study was conducted to measure the amount of Cry1Ac, Cry2Ab2, CP4 EPSPS, NPTII, and GUS proteins in various tissues collected from test and control cotton varieties grown in U.S. field trials conducted in 2001. The test for this study was MON 15985 x MON 1445, expressing the Cry1Ac, Cry2Ab2, CP4 EPSPS, NPTII, and GUS proteins. There were three types of controls used for this study consisting of 1) MON 15985 expressing Cry1Ac, Cry2Ab2, NPTII, and GUS proteins; 2) MON 1445 expressing CP4 EPSPS and NPTII proteins; and 3) a traditional cotton control. The background genetics of the test and controls were similar.

Enzyme-Linked Immunosorbent Assay (ELISA) methods were developed and validated to quantify the Cry1Ac, Cry2Ab2, CP4 EPSPS, NPTII, and GUS protein levels in seed and leaf tissues. Seed and leaf tissues were analyzed for all five proteins, seeds being the only relevant tissue to food and feed product safety. Tissue samples were collected from five locations in 2001. The sites included in the production year provided a variety of environmental conditions representative of regions where cotton is grown for commercial use.

Table 5 presents a summary of Cry1Ac, Cry2Ab2, CP4 EPSPS, NPTII and GUS proteins on a fresh weight basis in cottonseed samples collected from the 2001 field season.

The mean levels of the Cry1Ac, Cry2Ab2, CP4 EPSPS, NPTII and GUS proteins were 1.5, 45, 160, 17 and 45 µg/g respectively in MON 15985 x MON 1445. The Cry1Ac levels in MON 15985 was similar to those found in MON 15985 x MON 1445. Additionally, the Cry2Ab2 and GUS protein levels in MON 15985 were similar to those found in MON 15985 x MON 1445. CP4 EPSPS protein levels were slightly higher in MON 15985 x MON 1445 compared to MON 1445. The levels of Cry1Ac, Cry2Ab2, CP4 EPSPS, NPTII and GUS proteins were below the limits of detection in the traditional cotton control.

In conclusion, Cry1Ac, Cry2Ab2, CP4 EPSPS, NPTII and GUS protein levels between the test and cotton controls were generally very similar for a given protein. Where a slight expression difference occurred, the differences are not considered to be meaningful from a safety perspective considering the low levels of protein expression, the protein safety characteristics and the absence of detectable level of protein in refined oil.

Table 5. Summary of Cry1Ac, Cry2Ab2, CP4 EPSPS, NPTII, and GUS protein levels (µg/g fw)[1] measured in cottonseed samples collected in the 2001 field season Mean ± Std Dev. (n = 5)[2] - (Range)[3].

	Cry1Ac	Cry2Ab2	CP4 EPSPS	NPTII[4]	GUS
Traditional control	N.D.[5]	N.D.[5]	N.D.[5]	N.D.[5]	N.D.[5]
MON 15985	1.6 ± 0.23 (1.3-1.9)	44 ± 10 (34-60)	N.D.[5]	5.5 ± 0.59 (4.8-6.2)	46 ± 13 (27-59)
MON 1445	N.D.[5]	N.D.[5]	110 ± 6.8 (100-120)	16 ±2.0 (13-17)	N.D.[5]
MON 15985 x MON 1445	1.5 ± 0.095 (1.3-1.6)	45 ± 5.7 (39-53)	160 ± 28 (130-200)	17 ± 2.6 (14-20)	45 ± 16 (29-67)

[1] Protein levels are expressed as µg/g fw of tissue. Cry2Ab2, CP4 EPSPS, NPTII, and GUS protein levels were corrected for assay bias. Cry1Ac protein levels were corrected for trypsinization using the trypsinization factor.

[2] The mean and SD were calculated across sites from the analyses of plant samples from four plots (i.e. replicates) at each of five field sites.

[3] Minimum and maximum values from the analyses of samples across sites.

[4] The LOQ for the NPTII ELISA in seed tissue is 4.1 µg/g fw.

[5] Not detectable. The LODs for the Cry1Ac, Cry2Ab2, CP4 EPSPS, NPTII, and GUS ELISAs in seed tissue are 0.10, 3.4, 6.8, 1.3, and 6.5 µg/g fw, respectively.

b) Parts of the plant where the insert is expressed

The scope of the current application covers cottonseed oil and its constituents produced from MON 15985 and MON 15985 x MON 1445. Both the food and feed are produced from cottonseed, and therefore, the proteins expressed in the cottonseed should be considered the most important in regard to where the proteins are expressed in the plant. However, in support of notifications for MON 531 and MON 1445 under Regulation (EC) No 258/97, it has been demonstrated that there is no detectable level of protein in refined cottonseed oil produced from cotton modified through biotechnology or traditional cottonseeds.

MON 15985 and MON 15985 x MON 1445 express the insect protection proteins Cry1Ac and Cry2Ab2 or the insect protection proteins Cry1Ac and Cry2Ab2 and the CP4 EPSPS protein that provides tolerance to glyphosate. The expression of these proteins in seed was measured by ELISA analysis and was previously reported in this document (*See* Section 3.a))

4. Information on how the GM plant differs from the recipient plant in

a) Reproduction

Comparative assessments of the phenotypic and agronomic characteristics of MON 15985 and MON 1445 and traditional cotton have been conducted at multiple sites in the U.S. since development of these products began. Further, MON 15985 and MON 1445 are currently registered and grown commercially in the U.S., Australia and elsewhere. The extensive experience from commercial use of these products has demonstrated that, except for the insect protection and glyphosate tolerance traits, there are no biologically significant differences in the reproductive capability, dissemination or survivability of MON 15985 and MON 1445 compared to traditional cotton.

b) Dissemination

The introduced traits have no influence on cotton reproductive morphology or dissemination.

c) Survivability

Cotton is known to be a weak competitor in the wild, which cannot survive outside cultivation without the aid of human intervention. Field observations have demonstrated that MON 15985 and MON 15985 × MON 1445 have not been altered in its survivability when compared to traditional cotton.

d) Other differences

Comparative assessments in the field did not reveal any biologically significant differences between MON 15985 and MON 15985 × MON 1445 and traditional cotton, except for the introduced traits that are of agronomic interest.

5. Genetic stability of the insert and phenotypic stability of the GM plant

MON 15985
A Southern blot analysis was conducted to demonstrate the stability of the inserted elements from MON 531 that are responsible for the expression of the Cry1Ac protein in MON 15985 across four generations. The results indicate that the primary, functional insert of MON 531 is stably maintained across the four generations of MON 15985.

Additionally, the genetic stability of MON 15947 insert in MON 15985 has been demonstrated, by Southern blot analysis across five plant breeding generations.

To determine the phenotypic stability of MON 15985 across generations, a series of progeny tests were conducted based on a qualitative Cry2Ab2 enzyme-linked immunosorbent assay (ELISA) of four generations. The data confirm that the MON 15985 contains a DNA insert at a single locus that segregates according to Mendelian genetics and therefore remains stably integrated in the plant genome over selfed generations and over successive backcross generations.

MON 15985 x MON 1445
The presence of the parental inserts in MON 15985 x MON 1445 was demonstrated using DNA material extracted at the 8th generation (BC_2F_6) of the plant expressing the combined traits. The fact that the two inserts are still present after this high number of generations indicate that, as expected, each of them is stable even when combined over multiple generations.

6. Any change to the ability of the GM plant to transfer genetic material to other organisms

a) Plant to bacteria gene transfer

None of the genetic elements introduced in MON 15985 and MON 15985 x MON 1445 carries a genetic transfer function. Therefore, no changes are expected in the ability of this cotton to transfer genetic material to bacteria.

b) Plant to plant gene transfer

Not applicable. Neither the import of whole seed or cultivation is within the scope of this MON 15985 and MON 15985 x MON 1445 application, and therefore plant to plant gene transfer would have no opportunity to occur. However, 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 outcrossing frequency to other cotton varieties or to wild relatives (which are not present in the E.U.) is unlikely to be different for MON 15985, MON 1445 or MON 15985 x MON 1445 when compared to other cotton.

7. Information on any toxic, allergenic or other harmful effects on human or animal health arising from the GM food/feed

7.1 Comparative assessment

Choice of the comparator

MON 15985 and MON 15985 x MON 1445 were compared with a traditional cotton control and other commercially available cotton.

7.2 Production of material for comparative assessment

a) number of locations, growing seasons, geographical spreading and replicates

MON 15985

Materials for the compositional analysis were produced from a total of 14 U.S. field sites over two years (1998 and 1999). The test, MON 15985, and the traditional cotton control, had similar background genetics and were planted in eight sites in 1998 and in six sites in 1999. In 1998, the test and control cotton were planted in a single block with two 4.5 m row plots at Winnsboro, LA; Florence, SC; Starkville, MS; and Corpus Christi, TX; in a single block with one 9 m row plot at Starkville, MS; and in four replicate blocks at Leland, MS; Loxley, AL; Bossier City, LA; and Maricopa, AZ[2]. In 1999, all locations included four replicate blocks. Eight commercial reference varieties were included for the seed composition comparisons in 1998, four commercial cotton varieties were planted as reference lines in 1999. Additionally, compositional analysis of the cottonseed oil and cottonseed meal from the test variety compared to the control and three reference varieties were reported.

[2] AL: Alabama; AZ: Arizona; LA: Louisiana; MS: Mississippi; SC: South Carolina; TX: Texas

MON 15985 x MON 1445

A study was conducted on the compositional analysis of cottonseed from the test, MON 15985 x MON 1445, and a control cotton. The control cotton was a traditional cotton variety. Additionally, eleven different traditional cotton varieties were included as reference varieties to provide data for the development of a 99% tolerance interval for each component analyzed. The study was conducted at five sites across the U.S. during the 2001 field season. All sites were replicated using a randomized complete block design, with each site having four blocks or replicates of the control, test and reference substance.

b) the baseline used for consideration of natural variations

MON 15985

The study compared MON 15985 to the control. Reference varieties were grown in the same field locations and under the same conditions as the test and control. Where statistical differences occurred, the measured analyte was compared to a confidence interval developed from the reference varieties. Differences were also compared to historical ranges and ranges reported in literature.

MON 15985 x MON 1445

The test MON 15985 x MON 1445 was compared to a traditional, non-transgenic control. Eleven different non-transgenic commercial varieties were included as reference lines to provide data for the development of a 99% tolerance interval for each component analysed. Where statistical differences occurred, the measured analyte was compared to a confidence interval developed from the reference varieties. Differences were also compared to historical ranges and ranges reported in literature.

7.3 Selection of material and compounds for analysis

The compounds that were selected for analysis in the compositional studies were chosen on the basis of internationally accepted guidance, and animal feed manufacturers specifications.

The results of the compositional analyses conducted for MON 15985 and MON 15985 x MON 1445 in comparison to control cotton demonstrate equivalence and do not indicate a need for further analysis of selected compounds in these cotton products.

7.4 *Agronomic traits*

The results from field trials and the experience from commercial planting in North America has provided a weight of evidence that when compared with traditional cotton varieties, MON 15985 and MON 15985 x MON 1445 have:

- equivalent growth, developmental and morphological characteristics;
- equivalent plant health, vigour and pest susceptibility (except for predation by specific leptidopteran insect pests);
- equivalent agronomic performance, including yield potential.

These results also infer that MON 15985 and MON 15985 x MON 1445 have equivalent biological fitness, dissemination and survival characteristics (*i.e.* similar lack of persistence in the field and lack of invasiveness into natural environments) as any other cotton.

7.5 *Product specification*

MON 15985

MON 15985 comprises all traditionally bred progeny that express the MON 15985 traits. MON 15985 contains MON 15947 insert, and produces the Cry2Ab2 protein. Therefore, MON 15985 is detectable using the product-specific PCR method for detecting the introduced DNA present from MON 15947.

MON 15985 x MON 1445

MON 15985 x MON 1445 comprises all traditionally bred cotton produced by the combinations of MON 15985 and MON 1445. As MON 15985 x MON 1445 is the result of a traditional cross of MON 15985 and MON 1445, it contains all the respective DNA inserts from both single trait cotton products. Therefore, MON 15985 x MON 1445 is detectable using either the product-specific PCR method for detecting the introduced DNA present in MON 15985 or the equivalent method for MON 1445. However, as for all plants in which one or more genetically modified traits are combined by traditional breeding, unambiguous detection of MON 15985 x MON 1445 can only occur with seeds from the MON 15985 x MON 1445, by using a combination of the provided PCR methods on a single seed.

7.6 *Effect of processing*

As MON 15985 and MON 15985 x MON 1445 are substantially equivalent and as safe and nutritious as traditional cotton, the use of MON 15985 and MON 15985 x MON 1445 seed for the production of foods and feeds is no different from that of traditional cotton. Consequently, any effects of the processing of MON 15985 and MON 15985 x MON 1445 are not expected to be any different from the processing of the equivalent foods and feeds, originating from traditional cottonseed.

7.7 Anticipated intake/extent of use

There are no anticipated changes in the intake and/or extent of use of cotton-derived foods or feeds as a result of the addition of MON 15985 and MON 15985 × MON 1445 varieties to the traditional cotton supply. MON 15985 and MON 15985 × MON 1445 are expected to replace a portion of current cotton such that its intake or use will represent some fraction of the total products derived from cotton.

7.8 Toxicology

7.8.1 Safety assessment of newly expressed proteins

The scope of the current application covers cottonseed oil and its constituents produced from MON 15985 and MON 15985 x MON 1445. In support of notifications for MON 531 and MON 1445 under Regulation (EC) No 258/97, it has been demonstrated that there is no detectable level of protein in refined cottonseed oil produced from transgenic or non-transgenic cottonseeds. Therefore, the following information related to the assessment of newly expressed proteins can be considered as mainly informative.

MON 15985

MON 15985 produces the Cry2Ab2 and GUS E377K proteins as well as the Cry1Ac and NPTII proteins. The safety assessments of the Cry1Ac and NPTII proteins have been previously discussed in the notification for MON 531 pursuant to Regulation (EC) No 258/97. Several studies, including characterization of the introduced proteins, digestion in simulated gastric and intestinal fluids, and bioinformatics analyses, were performed with Cry2Ab2 and GUS. Additionally, acute oral toxicity studies have been conducted in mice using the Cry2Ab2 and GUS proteins. Analyses from these multiple studies support the conclusion that the Cry2Ab2 and GUS E377K proteins are not toxic to mammals and present no unacceptable risk to human safety. Finally, exposure to the introduced proteins were also considered to be extremely reduced, if at all existing; expression studies show that those proteins are present at very low levels in MON 15985 cottonseed and are unlikely to remain in highly processed cotton food products.

Regarding the potential interactivity of Cry2Ab2 with the Cry1Ac protein, which is also expressed in MON 15985, to date, there is no evidence to support the hypothesis that the presence of the Cry1Ac protein would affect the activity of the Cry2Ab2 protein in MON 15985, and thus affect the safety assessment of the Cry2Ab2 protein.

MON 15985 x MON 1445

MON 15985 x MON 1445 was produced by the traditional crossing of MON 15985 and MON 1445. The introduced traits present in MON 15985 and MON 1445 are inherited in MON 15985 x MON 1445. The safety of the introduced traits present in MON 15985 x MON 1445 have been assessed. No evidence of toxic or other harmful effects on human health have been identified and no risks specific to the expression of the new proteins in the same plant can be anticipated since the proteins have specific and independent targets.

7.8.2 Testing of new constituents other than proteins

The introduced genes are not intended to produce new constituents other than the proteins, Cry1Ac, NPTII, Cry2Ab2, GUS and CP4 EPSPS.

Since cotton is known as a common source of food and feed products with a centuries-long history of safe use and consumption around the world, and as MON 531 × MON 1445 was shown to be substantially equivalent to traditional cotton, no toxicological testing of any constituents, other than the introduced proteins is warranted.

7.8.3 Information on natural food and feed constituents

Cotton is known as a common source of human food and feed products, with a long history of safe use and consumption around the world. All cotton contains cyclopropenoid fatty acids (CPFA) and gossypol, natural compounds that are considered to be undesirable and anti-nutritional. The steps taken during cottonseed processing, in order to produce cottonseed oil, detoxify gossypol and greatly reduce the CPFA content. No other particular natural constituents of cotton are considered to be of significant concern to require additional information or further risk assessment.

7.8.4 Testing of the whole GM food/feed

Compositional analyses and comparative phenotypic assessments have demonstrated that MON 15985 and MON 15985 x MON 1445 are substantially equivalent to traditional cotton, with the exception of the introduced insect-protection or the introduced insect-protection and glyphosate-tolerance traits.

In support of notifications for MON 531 and MON 1445 under Regulation (EC) No 258/97, it has been demonstrated that there is no detectable level of protein in refined cottonseed oil produced from cotton modified through biotechnology or traditional cottonseeds. Additionally, the human and animal safety of the Cry1Ac, Cry2Ab2 and CP4 EPSPS proteins was demonstrated on the basis of a) an extensive characterization of each protein,

b) comparison of these proteins to known protein toxins and allergens, c) their digestion in simulated gastric and intestinal fluids, and d) the assessment of each protein for evidence of any acute toxicity in oral gavage studies in rodents. All these studies confirmed the absence of any toxic effects associated to the introduced proteins and confirmed the absence of any unanticipated or pleiotropic effects of the genetic modification. The introduced proteins in MON 15985 and MON 1445 have shown no evidence of adverse effects on human or animal safety.

The conclusions of the safety assessments for the individual proteins are unaffected when their combined expression in MON 15985 x MON 1445 is considered.

7.9 Allergenicity

7.9.1 Assessment of allergenicity of the newly expressed protein

The scope of the current application covers cottonseed oil and its constituents produced from MON 15985 and MON 15985 x MON 1445. In support of our notifications for MON 531 and MON 1445 under Regulation (EC) No 258/97, it has been demonstrated that there is no detectable level of protein in refined cottonseed oil produced from transgenic or non-transgenic cottonseeds.

Absence of any allergenic potential associated with the introduced Cry1Ac, Cry2Ab2 and CP4 EPSPS proteins expressed in MON 15985 and MON 15985 × MON 1445 has previously been demonstrated.

These proteins were assessed for their potential allergenicity by a variety of tests, including a) whether the genes came from allergenic or non-allergenic sources, b) sequence similarity to known allergens, and c) pepsin stability of the protein in an *in vitro* digestion assay. In all cases, the proteins did not exhibit properties characteristic of allergens.

7.9.2 Assessment of allergenicity of the whole GM plant or crop

As the introduced proteins do not have any allergenic potential, it was concluded that the use of MON 15985 and MON 15985 × MON 1445 for food or feed does not lead to an increased risk for allergenic reactions compared to the equivalent range of food and feed uses of traditional cotton.

7.10 Nutritional assessment of GM food/feed

7.10.1 Nutritional assessment of GM food

MON 15985 and MON 15985 × MON 1445 expresses the introduced trait of insect-protection or of insect-protection and glyphosate tolerance, which are agronomic traits, and are not intended to change any nutritional aspects of this cotton. Hence both are not expected to be more or less attractive for use as food (or feed), for processing, or as a food (or feed) ingredient. Therefore, anticipated dietary intake of cotton-derived foods and feeds is not expected to be altered upon commercialisation of MON 15985 and MON 15985 × MON 1445, and no nutritional imbalances are expected as a result of the use of MON 15985 and MON 15985 × MON 1445.

7.10.2 Nutritional assessment of GM feed

Once compositional equivalence has been established in GM feed modified for agronomic input traits, nutritional equivalence can be assumed. The results of the compositional analyses have established the compositional equivalence of these cottonseed and traditional cottonseed, and as a consequence, no further nutritional assessments of MON 15985 and MON 15985 x MON 1445 for use in feed are considered necessary.

Considering the compositional equivalence of MON 15985 and MON 15985 x MON 1445 and the fact that refined cottonseed oil does not contain detectable level of DNA or protein, it can be concluded that cottonseed oil and its constituents, produced from MON 15985 and MON 15985 x MON 1445 are equivalent to cottonseed oil produced from traditional cotton and have the same nutritional properties.

7.11 Post-market monitoring of GM food/feed

The assessment of the human and animal safety of MON 15985 and MON 15985 × MON 1445 was conducted on the basis of these products substantial equivalence to traditional cotton (except for the introduced traits) and by extensive characterisation of the introduced traits, which are of agronomic interest, resulting in the expression of the Cry1Ac, Cry2Ab2 and CP4 EPSPS.

In the case of oil, protein and DNA are not present at detectable levels, and based on compositional comparisons of cottonseeds, it can be concluded that oil produced from MON 15985 and MON 15985 x MON 1445 is not different from oil produced from traditional cottonseed.

There are no intrinsic hazards related to MON 15985 and MON 15985 x MON 1445 as no signs of adverse or unanticipated effects have been observed in a number of safety studies. The pre-market risk characterization for food and feed from MON 15985 and

MON 15985 x MON 1445 is based on the pre-market risk characterizations of both MON 15985 and MON 1445. These pre-market risk assessments have demonstrated that the risks of consumption of foods and feeds produced from both MON 15985 and MON 15985 x MON 1445 are negligible and no different than the risks associated with the consumption of traditional cotton and cotton-derived products. Therefore, specific risk management measures are not warranted for MON 15985 and MON 15985 x MON 1445, and post-market monitoring of the use of this cotton for food and feed products is not considered appropriate.

8. Mechanism of interaction between the GM plant and target organisms (if applicable)

Not applicable as the scope of this application does not cover the GM plant but only food and feed products, specifically oil and its constituents, produced from MON 15985 and MON 15985 x MON 1445.

9. Potential changes in the interactions of the GM plant with the biotic environment resulting from the genetic modification

Not applicable as this application under Regulation (EC) No 1829/2003 includes food and feed, specifically oil and its constituents, produced from MON 15985 and MON 15985 x MON 1445 for uses equivalent to any other cotton and does not include the import of whole seeds and environmental release of this cotton in the E.U.

9.1 Persistence and invasiveness

Please see question **D.9.**

9.2 Selective advantage or disadvantage

Please see question **D.9.**

9.3 Potential for gene transfer

Please see question **D.9.**

9.4 Interactions between the GM plant and target organisms

Please see question **D.9.**

9.5 Interactions of the GM plant with non-target organisms

Please see question **D.9.**

9.6 Effects on human health

Please see question **D.9.**

9.7 Effects on animal health

Please see question **D.9.**

9.8 Effects on biogeochemical processes

Please see question **D.9.**

9.9 Impacts of the specific cultivation, management and harvesting techniques

Please see question **D.9.**

10. Potential interactions with the abiotic environment

Not applicable as this application under Regulation (EC) No 1829/2003 includes food and feed, specifically oil and its constituents, produced from MON 15985 and MON 15985 x MON 1445 for uses equivalent to any other cotton and does not include the import of whole seeds and environmental release of this cotton in the E.U.

11. Environmental monitoring plan (not if application concerns only food and feed produced from GM plants, or containing ingredients produced from GM plants and if the applicant has clearly shown that environmental exposure is absent or will be at levels or in a form that does not present a risk to other living organisms or the abiotic environment)

Not applicable as there will be no environmental release nor import of cottonseed of MON 15985 and MON 15985 x MON 1445 in the E.U. and these are excluded from the scope of this application under Regulation (EC) No 1829/2003. The scope of the current application only includes the use of this cotton as processed food and feed products.

11.1 General (risk assessment, background information)

Please see question **D.11.**

11.2 Interplay between environmental risk assessment and monitoring

Please see question **D.11.**

11.3 Case-specific GM plant monitoring (approach, strategy, method and analysis)

Please see question **D.11.**

11.4 General surveillance of the impact of the GM plant (approach, strategy, method and analysis)

Please see question **D.11.**

11.5 Reporting the results of monitoring

Please see question **D.11.**

12. Detection and event-specific identification techniques for the GM plant

MON 15985 contains MON 15947 insert and therefore is detectable using the event-specific PCR method for detecting the introduced DNA present from MON 15947.

As MON 15985 × MON 1445 is the result of a traditional cross of MON 15985 and MON 1445, it contains both inserts. Therefore, MON 15985 × MON 1445 is detectable using either the event-specific PCR method for detecting the introduced DNA present in MON 15985 or the equivalent method for MON 1445. However, as for all plants in which one or more events are combined by traditional breeding, the unambiguous detection of MON 15985 × MON 1445 in mixed consignments will require single seeds to be subjected to detection methods for both MON 15985 and MON 1445, and to test positive for both.

E. INFORMATION RELATING TO PREVIOUS RELEASES OF THE GM PLANT AND/OR DERIVED PRODUCTS

1. History of previous releases of the GM plant 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

Not applicable

b) Conclusions of post-release monitoring

Not applicable

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)

Not applicable

2. History of previous releases of the GM plant carried out outside the Community by the same notifier

a)	**Release country** Since their commercial introduction in the U.S. and in Australia, MON 15985 and MON 15985 x MON 1445 have been grown on more than 0.2 million hectares, respectively. Field tests of MON 15985 and MON 15985 x MON 1445 in countries such as the U.S. and Australia have been conducted since 1999.
b)	**Authority overseeing the release** MON 15985: U.S.: Environmental Protection Agency; Australia: Office of Gene Technology Regulator. MON 15985 x MON 1445: no oversight specific to the combined trait product where the single traits are approved.
c)	**Release site** Selected sites based on where MON 15985 and MON 15985 x MON 1445 would be grown.
d)	**Aim of the release** Since 2003, MON 15985 and MON 15985 x MON 1445 is grown commercially in the U.S. and in Australia.
e)	**Duration of the release** Please see question E.2.(a)
f)	**Aim of post-releases monitoring** Insect resistance management
g)	**Duration of post-releases monitoring** Insect resistance management is an annual condition of the registrations.
h)	**Conclusions of post-release monitoring** No stable insect resistance has been detected.
i)	**Results of the release in respect to any risk to human health and the environment** No evidence of any adverse effect to human or animal health and the environment.

3. Links (some of these links may be accessible only to the competent authorities of the Member States, to the Commission and to EFSA):

a)	**Status/process of approval** The EFSA website http://www.efsa.eu.int/science/gmo/gm_ff_applications/catindex_en.html provides information related to the applications submitted under Regulation (EC) No 1829/2003 on genetically modified food and feed.
b)	**Assessment Report of the Competent Authority (Directive 2001/18/EC)** Not applicable
c)	**EFSA opinion** No EFSA opinion is available at the time of this application.
d)	**Commission Register (Commission Decision 2004/204/EC)** http://europa.eu.int/comm/food/food/biotechnology/authorisation/commun_register_en.htm
e)	**Molecular Register of the Community Reference Laboratory/Joint Research Centre** Information on detection protocols is likely to be posted at http://gmo-crl.jrc.it/
f)	**Biosafety Clearing-House (Council Decision 2002/628/EC)** Not applicable
g)	**Summary Notification Information Format (SNIF) (Council Decision 2002/812/EC)** A notification and SNIF according to Directives 2001/18/EC and 2002/812/EC, respectively, have not been submitted for MON 15985 and MON 15985 x MON 1445. The EFSA website http://www.efsa.eu.int/science/gmo/gm_ff_applications/catindex_en.html does provide a link to this summary of the application for MON 15985 and MON 15985 x MON 1445 under Regulation (EC) No 1829/2003.