Application for renewal of the authorisation for continued marketing of existing food additives, feed materials and feed additives produced from MON 531 cotton that were notified according to Articles 8(1)(b) and 20(1)(b) of Regulation (EC) No 1829/2003 on genetically modified food and feed

Part II

Summary

April 2007

A. GENERAL INFORMATION

1. Details of application

a)	**Member State of application** Not applicable
b)	**Notification number** Not known at the time of application
c)	**Name of the product (commercial and other names)** The Monsanto development code for this genetically modified cotton is: MON 531. In countries where MON 531 is being cultivated, packages of this cotton are marketed under the name of the variety, in association with the Bollgard® trademark, indicating clearly to growers that this cotton is protected from specific lepidopteran insect pests.
d)	**Date of acknowledgement of notification** Not known 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
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))** Food additives, feed materials and feed additives produced from MON 531 will continue to 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 cotton.

® Bollgard is a registered trademark of Monsanto Technology LLC.

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 *yes*, 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 *no*, 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 Cottonseed oil produced from MON 531 has been notified under Regulation (EC) No 258/97 in July 2002, on the basis of an opinion of substantial equivalence delivered by the UK Advisory Committee on Novel Foods and Processes (ACNFP). Previously, in 1998, MON 531 had also received a favourable opinion by the Scientific Committee on Plants (SCP).	

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

Yes (x)	No ( )
If *yes*, specify Cultivation of MON 531 is lawful in Argentina, Australia/New Zealand, Brazil, China, Colombia, India, Mexico, South Africa and the U.S.A. Import of derived foods and feeds is lawful in Canada, Japan, Korea, Mexico, the Philippines and Singapore.	

8. General description of the product

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

MON 531 was produced through genetic modification. MON 531 expresses the Cry1Ac protein, which carries an insect-protection trait, as well as a trait for selection on kanamycin containing media. The insect protection trait provides effective control of target lepidopteran insects, which are economically damaging pests in most cotton growing regions.

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

The scope of this renewal application is for food additives, feed materials and feed additives produced from MON 531, which are lawfully placed on the market in the E.U., as listed in the Community register of GM Food and Feed[1]. The range of uses of these MON 531-derived products will be identical to the full range of equivalent uses of current commercial cotton derived products.

c) Intended use of the product and types of users

Food additives, feed materials, and feed additives produced from MON 531 will continue to be traded and used in the E.U. in the same manner as equivalent products from current commercial cotton and by the same operators currently involved in the trade and use of cotton.

[1] http://ec.europa.eu/food/dyna/gm_register/index_en.cfm

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

MON 531 is substantially equivalent to other cotton varieties except for its introduced trait, namely protection from target lepidopteran pests, which is a trait of agronomic interest. This cotton was shown to be as safe and as nutritious as conventional cotton. Therefore, MON 531-derived food additives, feed materials and feed additives will be stored, packaged, transported, handled and used in the same manner as products derived from current commercial cotton. No specific conditions are warranted or required for the food additives, feed materials and feed additives produced from MON 531.

e) Any proposed packaging requirements

MON 531 is substantially equivalent to conventional cotton varieties (except for its protection from targeted lepidopteran insect pests). Therefore, MON 531-derived food additives, feed materials and feed additives will continue to be used in the same manner as other equivalent cotton derived products and no specific packaging is required. [For labeling, *see* question 8.(f)].

f) A proposal for labeling in accordance with Articles 13 and 25 of Regulation (EC) 1829/2003. In the case of GMOs, food and/or feed containing, consisting of GMOs, a proposal for labeling 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.

In accordance with Regulations (EC) N° 1829/2003 and 1830/2003, a labelling threshold of 0.9 % is applied for the placing on the market of MON 531 seed and derived products.

According to Regulation (EC) No. 1829/2003, Articles 13 and 25, the operators placing on the market food and feed products produced from MON 531 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.

Operators handling or using foods and feeds produced from MON 531 in the E.U. are required to be aware of the legal obligations regarding traceability and labelling of these products.

Given that explicit requirements for the traceability and labelling of GMOs and derived foods and feeds are laid down in Regulations (EC) No. 1829/2003 and 1830/2003, and that authorized foods and feeds shall be entered in the Community Register, operators in the food and feed chain will be fully aware of the traceability and labelling requirements for foods and feeds produced from MON 531. Therefore, no further specific measures are to be taken by the applicant.

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

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 531 food additives, feed materials and feed additives are suitable for 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 food additives, feed materials and feed additives produced from MON 531 is unlikely, as the proposed uses for this cotton are included in the current food and feed uses of conventional cotton. MON 531 is substantially equivalent to other cotton except for the introduced insect-protection trait, which is a trait of agronomic interest. This cotton has been shown to be as safe and as nutritious as conventional cotton. Therefore, all measures for waste disposal and treatment of MON 531-derived products are the same as those for conventional cotton. No specific conditions are warranted or required for the placing on the market of MON 531-derived food additives, feed materials, and feed additives.

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

1. Complete name

a) Family name

Malvaceae

b) Genus

Gossypium

c) Species

spp.

d) Subspecies

N/A

e) Cultivar/breeding line

MON 531

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 normally 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. Rainfall, temperature, sunshine and spring warming all impact optimal growth.

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 531.

Gene transfer to cultivated genotypes

In as much as similar cotton genotypes are fully sexually 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. Cross-pollination decreased from five to less than one percent from one to seven meters, respectively, away from the source plot. Regardless, outcrossing with cultivated *Gossypium* varieties is not expected in the context of this renewal application.

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 E.U. No genera in the *Gossypieae* tribe occur naturally in these countries.

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 favorable. 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 and import of whole seed of MON 531 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. However, the current renewal application does not include the cultivation of MON 531 varieties in the E.U. but only the continued use of existing food and feed products derived from MON 531.

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

The major type of cotton being grown commercially around the world is the upland cotton *G. hirsutum*. There are, however, other two minor categories of cotton grown globally: the long staple cotton, *G barbadense* (commonly known as Pima or Egyptian cotton) and the Asiatic cotton, including *G. arboreum* and *G. herbaceum*.

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 produce 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.

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

1. Description of the methods used for the genetic modification

The *cry1Ac, nptII,* and *aad* coding sequences were stably transferred into the genome of cotton cells using *Agrobacterium tumefaciens* mediated transformation.

2. Nature and source of the vector used

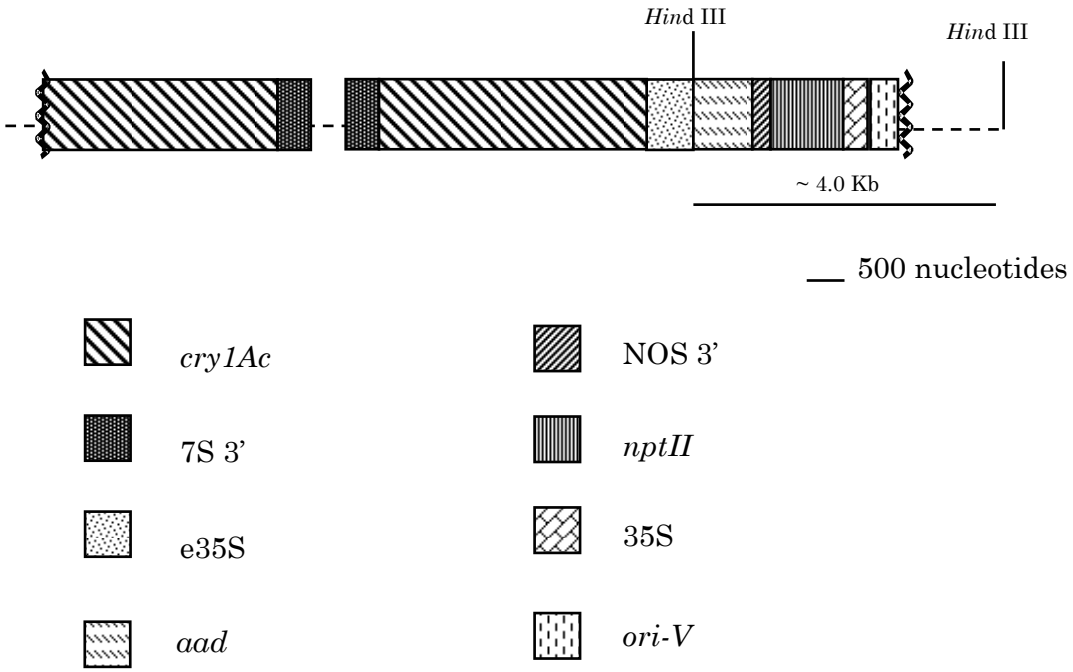
The vector used for the transformation was PV-GHBK04 from *Agrobacterium tumefaciens*. PV-GHBK04 is an 11.4 kb single-border, binary transformation vector. It contains well-characterized DNA segments required for selection and replication of the plasmid in bacteria as well as a right border for initiating the transfer of this DNA into the plant genomic DNA.

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

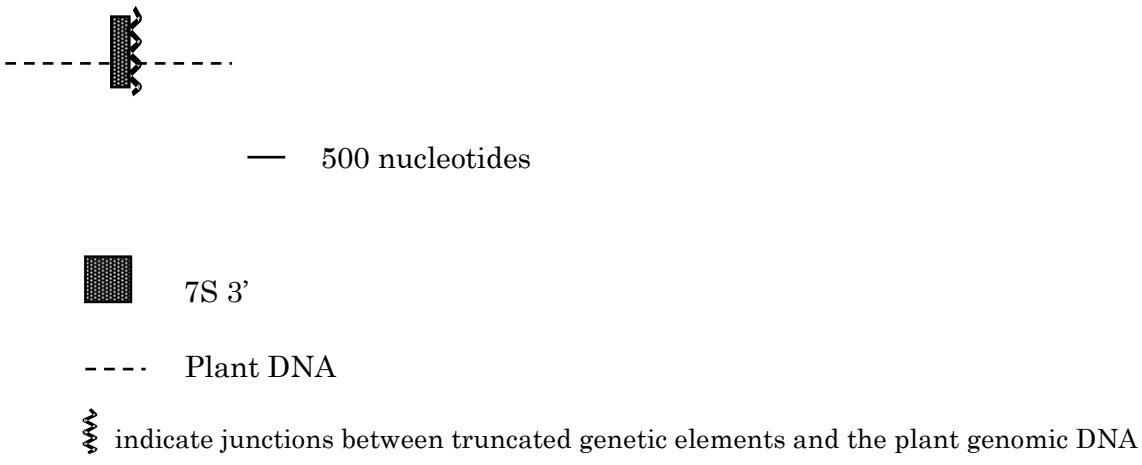
The individual components and the size, source and function of these inserted DNA sequences are given in Table 1. A schematic representation of the insert is shown in Figure 1.

Table 1. Summary of genetic elements in the T-DNA containing the full length *cry1Ac* coding region from MON 531

Genetic Element	Approximate size (bp)	Function (reference)
7S 3'	441	3′ nontranslated region from soybean 7S seed storage protein gene, which terminates transcription and directs polyadenylation of the *cry1Ac* mRNA.
cry1Ac	3537	Synthetic variant of the Cry1Ac protein of *Bacillus thuringiensis.*
e35S	621	Cauliflower mosaic virus (CaMV) promoter with the duplicated enhancer region used to drive expression of the *cry1Ac* coding region.
aad	789	Bacterial promoter and coding sequence for an aminoglycoside-modifying enzyme, 3′(9)-O-nucleotidyltransferase from the transposon Tn7 (GenBank accession X03043).
NOS 3′	241	3′ nontranslated region of the nopaline synthase (*NOS*) coding sequence from *Agrobacterium tumifaciens,* which terminates transcription and directs polyadenylation.
nptII	968	Gene isolated from Tn5, which codes for neomycin phosphotransferase type II. Expression of this gene 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	324	Cauliflower mosaic virus (CaMV) promoter.
ori-V	394	Origin of replication for *Agrobacterium* derived from the broad host range plasmid RK2.



a/ Functional *cry1Ac* insert



b/ Additional 242 bp insert

Figure 1. Schematic representation of the insert DNA in MON 531

D. INFORMATION RELATING TO THE GM PLANT

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

MON 531 was produced by genetic modification. MON 531 expresses the Cry1Ac protein, which is an insect-protection trait, as well as a trait for selection on kanamycin containing media. The insect protection trait provides effective control of lepidopteran insects, which are economically damaging pests in most cotton growing regions. The trait and characteristics introduced in MON 531 have been described previously in a notification pursuant to Regulation (EC) No 258/97.

2. Information on the sequences actually inserted or deleted

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

Molecular and immunochemical characterization of MON 531 was initially conducted using various techniques, including Southern blot analyses, western blot analyses and ELISA. Results from these analyses led to the conclusion that MON 531 contained two linked T-DNA insertions in the plant genome arranged in a head-to-tail configuration. The primary insert was shown to be approximately 8.2 kb in size and consisted of transformation plasmid DNA, PV-GHBK04 that extended from the right border through the *ori-V* genetic element. The insert included one functional *cry1Ac* cassette, which consists of an e35S promoter, *cry1Ac* coding sequence, and a 7S 3′ transcript termination sequence

Following this, additional molecular characterization was performed using cosmid cloning, PCR, DNA sequencing, Southern blotting and genome walking. These experiments have provided additional details regarding the inserted DNA and the genomic DNA sequences flanking the 5′ and 3′ ends of the insertion(s) in MON 531. It was demonstrated that the T-DNA containing the 3′ portion of the *cry1Ac* coding region, was adjacent to, but in the opposite orientation with respect to the T-DNA containing the full length *cry1Ac* coding region. Together, these two T-DNAs, arranged as an inverted repeat, make up the functional insert in MON 531.

Table 1 summarizes the genetic elements of the DNA insert in MON 531. A schematic representation of the T-DNA insert in MON 531 is shown in Figure 1.

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

At the insertion site of the MON 531 functional insert, an 85 bp genomic DNA region was deleted. However, the compositional and agronomic studies indicate that this fragment may not be essential for any plant function.

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

Stable integration and maintenance of the functional insert in the nuclear genome of MON 531 was evident from Southern blot data (R_0 generation), segregation ratios from selfed progeny (R_1) and four generations of backcrossed derivatives of MON 531, and Southern blot of R_5 and R_6 generations of MON 531 as well as two commercial cotton lines containing MON 531.

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

The organization of elements within the T-DNA inserts in MON 531 was demonstrated by PCR amplification of genomic DNA and Southern blot. Furthermore, sequencing of these PCR fragments confirmed that MON 531 contained the T-DNA that was intended to be inserted by transformation. The consensus sequence representing the T-DNA containing the full-length *cry1Ac* coding region was generated by compiling numerous sequencing reactions performed on the overlapping PCR products. Additionally, PCR products containing DNA at the 5′ and 3′ ends of the functional insert, as well as the cotton genomic DNA flanking the 5′ and 3′ ends of the functional insert in MON 531 were amplified by PCR and sequenced. PCR analyses were performed using genomic DNA from MON 531, negative control line Coker 312, or no DNA template.

From the Southern analyses, it was evident that: 1) The functional insert in MON 531 consisted of two T-DNAs that were closely linked; 2) These two linked T-DNAs were arranged as an inverted repeat at the functional insertion site of MON 531; 3) MON 531 contained a second 242 bp insertion that contained a portion of the 7S 3′ transcriptional termination sequence; and 4) This 242 bp insert was not closely associated with the functional insert. Genomic DNA template from MON 531 samples generated the expected size PCR products of ~1400 bp for the 5′ flanking sequence and ~600 bp for the 3′ flanking sequence. These PCR products were sequenced and the resulting consensus sequences corresponded to the 5′ and 3′ ends, respectively, of the functional insert in MON 531.

To summarize, MON 531 has only one intact functional copy of the *cry1Ac* coding region and three copies of the 7S 3′ transcriptional termination sequence as well as a truncated copy of the *cry1A*c coding region. Additionally, the intactness of the genetic elements within the functional insert of MON 531 was confirmed using PCR analyses that amplified six overlapping regions of DNA throughout the T-DNA containing the full-length *cry1Ac* coding region.

3. Information on the expression of the insert

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

Studies were conducted to measure the amount of Cry1Ac and NPTII proteins in various cotton tissues collected from 1992 U.S. field trials. The field trial sites provided a variety of environmental conditions representative of regions where cotton is grown for commercial use. Enzyme-Linked Immunosorbent Assay (ELISA) methods were developed and validated to quantify the Cry1Ac and NPTII protein levels in cotton tissues.

Mean levels of the Cry1Ac and NPTII proteins in cotton tissue samples collected from six field sites in 1992 are presented below.

The Cry1Ac and NPTII proteins were produced at extremely low and relatively consistent levels across all six field sites. MON 531 contained 0.857 µg/g fresh weight of Cry1Ac and 2.451 µg/g fresh weight of NPTII in seed tissues.

b) Parts of the plant where the insert is expressed

The levels of Cry1Ac and NPTII proteins were assessed in leaf, seed, whole plant, pollen and nectar, using validated enzyme-linked immunosorbent assays (ELISA). Results for seed tissue are the most relevant for the evaluation of the food and feed safety of MON 531 x MON 1445 and are, therefore, presented in this summary.

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 531 and conventional cotton have been conducted at multiple sites in the U.S.A. since development of this product began. The extensive experience from commercial release of this product has demonstrated that, except for the insect protection trait, there are no biologically significant differences in the reproductive capability, dissemination or survivability of MON 531 compared to conventional cotton.

A field study supports the conclusion that MON 531 behaves similarly

agronomically to conventional cotton, with the exception of the protection against certain lepidopteran insect pests.

Regardless, it should be noted that the scope of the current renewal application does not include the cultivation of MON 531 varieties in the E.U. but only the renewal of the authorisation for the continued marketing of existing MON 531-derived food additives, feed materials, and feed additives, entered in the Community Register of GM Food and Feed, in the E.U.

b) Dissemination

The introduced trait has no influence on cotton reproductive morphology and hence no changes in seed dissemination are to be expected.

c) Survivability

Cotton is known to be a weak competitor in the wild, which cannot survive outside cultivation without human intervention. Field observations have demonstrated that MON 531 has not been altered in its survivability when compared to conventional cotton.

d) Other differences

Comparative assessments in the field did not reveal any biologically significant differences between MON 531 and conventional cotton, except for the introduced trait that is of agronomic interest.

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

Molecular stability of the insert

The data from the molecular analysis of MON 531 demonstrated that a single copy and an inactive partial copy of the *cry1Ac* coding sequence were inserted at two tightly linked sites into cotton. The coding sequence for *cry1Ac* segregated in a manner consistent with a single insertion of the intact *cry1Ac* sequence that is stably transferred via traditional breeding methods. The data from the selfed crosses further demonstrated the stability of the insert during transfer from generation to generation. The structural and local maintenance of the inserted coding sequences was demonstrated over four generations.

No increased hazard is to be expected from potential recombination

Recombination is unlikely to occur. This fact is supported by the stability of the genetic elements over generations. Due to the safety properties associated with the introduced protein, the hazard arising from a hypothetical recombination event is negligible.

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 531 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

The scope of the current renewal application does not include the cultivation of MON 531 varieties in the E.U. but only the renewal of the authorisation for continued marketing of existing MON 531-derived food additives, feed materials and feed additives, entered in the Community Register of GM Food and Feed, in the E.U.Therefore, plant to plant gene transfers 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 531, 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 531 was compared with a conventional 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

Materials for the compositional analysis were produced in 1992 U.S.field trials at six sites with replicated plots. The six trials, located in the states of MS; LA; TX; GA; AZ and AL), provided a variety of environmental conditions representative of regions in the U.S.A. where cotton is grown commercially. At each site, MON 531 and the Coker 312 conventional control were planted.

b) the baseline used for consideration of natural variations

For the compositional studies, statistical comparisons were made between the test, MON 531, and the control cotton,. For statistically significant differences ($p<0.05$) observed for the combined site analyses, the range of the values were compared to

established ranges cited in the pertinent literature. These values fell within the literature ranges and were therefore not considered to be biologically relevant. For amino acids, only one literature reference could be cited and, therefore, the literature range for amino acids is, although indicative, not considered fully representative of the baseline variability of these components in cotton.

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.

The results of the compositional analyses conducted for MON 531 in comparison to conventional control cotton demonstrated equivalence and did not indicate a need for further analysis of selected compounds in this cotton product.

7.4 Agronomic traits

The scope of this application is limited to the renewal of the authorisation for continued marketing of existing MON 531-derived food additives, feed materials and feed additives in the E.U., but does not include the cultivation of MON 531 varieties in the E.U. The observations from previous environmental releases provide additional evidence to confirm the absence of any significant unintended or unanticipated effects of the genetic modifications present in this cotton. MON 531 varieties have been commercially grown in the U.S.A. since 1996, where variety development was based on agronomic field data.

7.5 Product specification

MON 531- derived food additives, feed materials and feed additives are currently imported into the EU in mixed shipments of cotton products, produced in other world areas. These products are handled by operators that have traditionally been involved in the commerce, processing and use of cotton and cotton derived products in the European Union.

MON 531 comprises all traditionally bred cotton produced by the combination of MON 531 and conventional cotton. The presence of the lepidopteran-protection trait in MON 531-derived products can be detectable using the insert-specific PCR method for detecting the introduced DNA present in MON 531. The event specific method of detection of MON 531 will be published on the Community Reference Laboratory (CRL) website[2] once they complete the process of validation

[2] http://gmo-crl.jrc.it/statusofdoss.htm

7.6 Effect of processing

The compositional analyses of MON 531 have demonstrated that MON 531 is substantially equivalent to conventional cotton, except for the MON 531 trait and the resulting expression of Cry1Ac, which was shown to be safe for human and animal health.

As MON 531 is substantially equivalent to and as safe and nutritious as conventional cotton, the use of MON 531 for the production of foods and feeds is no different from that of conventional cotton. Consequently, any effects of the production and processing of MON 531 foods and feeds are not expected to be any different from the production and processing of the equivalent foods and feeds, originating from conventional cotton.

7.7 Anticipated intake/extent of use

Food additives, feed materials and feed additives produced from MON 531 were first placed on the E.U. market in 1996 . In 2004, these products were notified to the European Commission, following Articles 8(1)(b) and 20(1)(b) of Regulation (EC) No 1829/2003, in order to allow for their continued marketing in the E.U. given that they had been lawfully placed on the market before Regulation (EC) No 1829/2003 came into force, on 18 April 2004.

MON 531-derived food additives, feed materials and feed additives replace a portion of current commercial cotton products, such that the dietary intake and/or extent of use of of current commercial cotton products is not expected to be altered upon renewal of the authorisation of existing MON 531-derived products.

7.8 Toxicology

7.8.1 Safety evaluation of newly expressed proteins

The scope of this renewal application covers food additives, feed additives, and feed materials produced from MON 531.

The introduced Cry1Ac and NPTII proteins in MON 531 are present at low levels and were demonstrated to be safe for animal and human health. The conclusion these proteins are safe to humans and animals was based upon the following findings: 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.

Updated bioinformatics analyses have been performed on Cry1Ac and NPTII proteins expressed in MON 531. The results of these bioinformatics analyses confirm the initial conclusions: none of these proteins share significant sequence similarity with protein toxins relevant to animal or human health.

7.8.2 Testing of new constituents other than proteins

Compared to its conventional counterpart, MON 531 does not contain new toxicants. The data presented as part of the compositional comparison of the two lines, lead to the conclusion that foods and feeds derived from MON 531 and Coker 312 are substantially equivalent.

The introduced coding sequences are not intended to produce new constituents other than the two proteins, Cry1Ac and NPTII. Since cotton is known as a common source of food and feed products, with a long history of safe use and consumption globally, and as MON 531 was shown to be substantially equivalent to conventional cotton, toxicological testing of any constituents, other than the introduced proteins, is not indicated.

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 and meal, 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 531 is substantially equivalent to conventional cotton, with the exception of the introduced insect-protection trait.

7.9 Allergenicity

7.9.1 Assessment of allergenicity of the newly expressed protein

The scope of the current application covers food additives, feed additives and feed materials produced from MON 531. In support of our notification for MON 531 under Regulation (EC) No 258/97, it has been demonstrated that there is no detectable level of protein in refined cottonseed oil produced from genetically modified or conventional cottonseeds. Similarly, highly processed products such as food additives produced from cottonseed are unlikely to contain proteins at detectable levels. Therefore, the information related to the assessment of allergenicity of the newly expressed proteins can be considered as mainly informative.

The Cry1Ac and NPTII 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. Therefore, the potential allergenicity of the Cry1Ac and NPTII proteins has been evaluated and it is concluded that the proteins do not pose significant allergenic concerns.

7.9.2 Assessment of allergenicity of the whole GM plant or crop

MON 531 is substantially equivalent to conventional cotton. Furthermore, highly processed foods (such as cottonseed oil) are unlikely to contain detectable levels of protein. In addition, studies of the introduced proteins in MON 531 do not reveal any allergenic potential. The use of food or feed products produced from MON 531 is unlikely to lead to an increased risk for allergenic reaction compared to the equivalent range of food and feed uses from conventional cotton.

7.10 Nutritional assessment of GM food/feed

7.10.1 Nutritional assessment of GM food

MON 531 expresses the introduced trait of insect protection that is of agronomic interest and is not intended to change any nutritional aspects of this cotton. The presence of this trait is not expected to alter patterns or volumes of cotton food product consumption. Compared to any other cotton food products, no differences in intake or extent of use are therefore expected for MON 531.

In conclusion, cottonseed produced from MON 531 is nutritionally equivalent to conventional cottonseed. Hence, this cottonseed is not expected to be more or less attractive for use in producing food products. The dietary intake of foods produced from cottonseed is not expected to be altered by the continued commercial use of MON 531, and no nutritional imbalances are expected as a result of the use of MON 531 seed for food processing.

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 this cottonseed and conventional cottonseed, and as a consequence, no further nutritional assessments of MON 531 for use in feed is considered necessary.

7.11 Post-market monitoring of GM food/feed

The assessment of the human and animal safety of MON 531 was conducted on the basis of the product's substantial equivalence to conventional cotton (except for the introduced trait) and by extensive characterization of the introduced trait, which is of agronomic interest, resulting in the expression of the Cry1Ac protein.

Based on compositional comparisons of cottonseeds, it can be concluded that food additives, feed additives, and feed materials produced from MON 531 are not different from their counterpart products produced from conventional cottonseed.

There are no intrinsic hazards related to MON 531 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 531 has demonstrated that the risks of consumption of foods and feeds produced from MON 531 are negligible and no different than the risks associated with the consumption of conventional cotton-derived products. Therefore, as previously stipulated in the Community Register of GM food and feed, no specific risk management measures are indicated for MON 531 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 renewal application under Regulation (EC) No 1829/2003 does not cover the deliberate release of cottonseeds into the environment.but only the food additives, feed materials and feed additives produced from MON 531 cottonseed.

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

Not applicable as neither the GMO, nor the food and feed containing or consisting of the GMO, are within the scope this renewal application under Regulation (EC) No 1829/2003. This renewal application includes only food additives, feed materials and feed additives produced from MON 531 cottonseed.

10. Potential interactions with the abiotic environment

Not applicable as neither the GMO, nor the food and feed containing or consisting of the GMO, are within the scope this renewal application under Regulation (EC) No 1829/2003. This renewal application includes only food additives, feed materials and feed additives produced from MON 531 cottonseed.

11. Environmental monitoring plan (not if application concerns only food and feed produced from GM plants, or containing ingredients produced from GM plants)

Not applicable as neither the GMO, nor the food and feed containing or consisting of the GMO, are within the scope this renewal application under Regulation (EC) No 1829/2003. This renewal application includes only food additives, feed materials and feed additives produced from MON 531 cottonseed.

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

MON 531 is detectable using an event-specific PCR method for detecting the introduced DNA present in MON 531. The validated method prepared by the Community Reference Laboratory (CRL) will be published on the CRL website once completed.

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, as the environmental release of MON 531 is not in scope of this application.

b) Conclusions of post-release monitoring

Not applicable, as the environmental release of MON 531 is not in scope of this application.

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, as the environmental release of MON 531 is not in scope of this application.

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

a)	**Release country** MON 531 has been commercially introduced in the U.S.A. (1996), Argentina, Australia, Brazil, China, Colombia, India, and South Africa. Globally, the production of MON 531 has grown rapidly since its introduction in the various production markets. In the past four years, the single trait, MON 531, was grown on more than six million hectares, globally.
b)	**Authority overseeing the release** United States: Environmental Protection Agency (EPA), Food and Drug Administration (FDA), and the United States Department of Agriculture (USDA). Argentina: National Service for Agri-Food Safety and Quality (SENASA), National Advisory Committee on Agricultural Biosafety (CONABIA), Market Risk Assessment Department (DNMA), and Secretary of Agriculture. Australia: Office of the Gene Technology Regulator (OGTR), Food Standards Australia and New Zealand (FSANZ). Brazil: National Technical Commission on Biotechnology (CTN-Bio). China: Ministry of Agriculture (MOA). Colombia: The National Institute for the Surveillance of Drugs and Food (INVIMA), Colombian Institute of Agriculture (ICA), and Ministry of Health (MOH). India: Genetic Engineering Approval Committee (GEAC). Mexico: Ministry of Health (MOH) and Ministry of Agriculture (MOA). South Africa: National Department of Agriculture.
c)	**Release site** Commercial fields for MON 531 production, as for any other cotton.
d)	**Aim of the release** Commercial release in the U.S, Argentina, Australia, Brazil, China, Colombia, India, Mexico, and South Africa: all uses of conventional cotton.
e)	**Duration of the release** Please see question E.2.(a)
f)	**Aim of post-releases monitoring** Extensive pre-market risk assessment did not provide evidence of adverse effects potentially associated with the cultivation, handling or use of MON 531, indicating that post-release monitoring would not be

necessary.

In addition, the commercialisation of MON 531 is accompanied by stewardship programmes to ensure correct handling of this cotton by downstream stakeholders (implementation of good agricultural practice for cultivation; ensure a channel of communication in the unlikely event that unanticipated adverse effects might occur).

No unanticipated effects have been observed during field testing or since commercialization of MON 531.

g) Duration of post-releases monitoring

Please see question E.2.(f)

h) Conclusions of post-release monitoring

Please see question E.2.(f).

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

Field-testing and post-marketing experience provided no significant evidence that cottonseed or derived products from MON 531 are likely to cause adverse effects to human health, animal health or 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.europa.eu/en/science/gmo/gm_ff_applications.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://ec.europa.eu/food/dyna/gm_register/index_en.cfm

e)	**Molecular Register of the Community Reference Laboratory/Joint Research Centre** The CRL is currently on step 5 (Reporting) of the validation process for MON 531. Once completed, they will post in their website http://gmo-crl.jrc.it/ the relevant information on detection protocols and validated methods.
f)	**Biosafety Clearing-House (Council Decision 2002/628/EC)** The publicly accessible portal site of the Biosafety Clearing-House (BCH) can be found at http://bch.biodiv.org/
g)	**Summary Notification Information Format (SNIF) (Council Decision 2002/812/EC)** This public summary for the renewal application of MON 531 under Regulation (EC) No 1829/2003 will be posted at http://www.efsa.europa.eu/en/science/gmo/gm_ff_applications.html