# Application for authorization of MON 531 × 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) Notification number**

Not available 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 × MON 1445. In countries where MON 531 × MON 1445 is being cultivated, packages of this cotton are marketed under the name of the hybrid variety, in association with both trademarks Bollgard®[1] and Roundup Ready®[1], indicating clearly to growers that the hybrid is protected from specific lepidopteran insect pests and tolerant to Roundup®[1] herbicide, containing the active ingredient glyphosate.

**d) Date of acknowledgement of notification**

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

**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 531 × MON 1445 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.

[1] Bollgard, Roundup ready and Roundup are registered trademarks 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**<br>*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 U.S. and Australia, MON 531 x MON 1445 is authorized for all uses, corresponding to the full range of used of traditional cotton and single trait authorization. The scope of the approvals already granted for these genetically modified organisms 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 531 × MON 1445 has been produced by the traditional breeding of MON 531 and MON 1445. Although genetic modification was used in the development of MON 531 and MON 1445, no additional genetic modifications were involved for the production of MON 531 × MON 1445.

MON 531 x MON 1445 as well as MON 531 and MON 1445, were developed by the Monsanto Company. MON 531 produces Cry1Ac protein which confers protection against certain lepidopteran insect pests. MON 1445 is tolerant to Roundup® herbicide (containing the active ingredient glyphosate).

Both the introduced traits from the parental cotton lines are inherited in MON 531 x 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 531 × MON 1445 for food and feed, specifically cottonseed oil and its constituents. The range of uses of this cotton for food and feed will be identical to the full range of equivalent uses of traditional cotton.

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

MON 531 × MON 1445 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 531 × MON 1445 is substantially equivalent to other cotton varieties except for its introduced (*i.e.* inherited) traits, namely protection from target lepidopteran pests and tolerance to glyphosate, which are traits of agronomic interest. This cotton was shown to be as safe and as nutritious as traditional cotton. Therefore MON 531 × MON 1445 and derived products will be stored, packaged, transported, handled and used in the same manner as the commercial cotton products. No specific conditions are warranted or required for the food and feed use of MON 531 × MON 1445.

**e) Any proposed packaging requirements**

MON 531 × MON 1445 is substantially equivalent to its parental cotton lines MON 531 and MON 1445, and to traditional cotton varieties (except for its protection from targeted lepidopteran insect pests and tolerance to glyphosate). Therefore, MON 531 × MON 1445 and derived products will be used in the same manner as other cotton and no specific packaging is foreseen. (For the labelling, *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.**

The scope of this application covers foods and feeds produced from MON 531 x 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 531 x MON 1445 shall ensure that those products are labelled 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 x MON 1445 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) N° 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 x MON 1445. Therefore, no further specific measures are to be taken by the applicants.

**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)**

MON-ØØ531-6 × MON-Ø1445-2

MON 531 × MON 1445 is uniquely identified using this combination of the unique identifiers for MON 531 (MON-ØØ531-6), and MON 1445 (MON-Ø1445-2).

**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 × MON 1445 is 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 531 × MON 1445 is unlikely, as the proposed food and feed uses for this cotton include the current food and feed uses of traditional cotton. MON 531 × MON 1445 hybrids are substantially equivalent to other cotton hybrids except for the introduced traits, which are traits of agronomic interest. This cotton has been shown to be as safe and as nutritious as traditional cotton. Therefore, any measures for waste disposal and treatment of MON 531 × 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 531 × MON 1445 for food and feed.

## 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**

*hirsutum* (4n = 52)

**d) Subspecies**

N/A

**e) Cultivar/breeding line**

MON 531 × 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 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.

## 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 × 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 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 an 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 such as atrazine, bromoxynil, glufosinate and paraquat. However, it should be noted that cultivation and import of whole seed of MON 531 x 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 whole seed of MON 531 x 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

### 1. Description of the methods used for the genetic modification

Not applicable, since MON 531 × MON 1445 is produced by the traditional cotton breeding cross of MON 531 with MON 1445. Genetic modification was used in the development of MON 531 and MON 1445.

### 2. Nature and source of the vector used

Not applicable since MON 531 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 531 × MON 1445 has been produced by the traditional breeding of MON 531 and MON 1445. The inserted DNA fragment from both parental lines are inherited in MON 531 x MON 1445.

The individual components and the size, source and function of these inherited DNA sequences are given in Tables 1 and 2. while schematic representations of those inserts are shown in Figures 1 and 2.

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

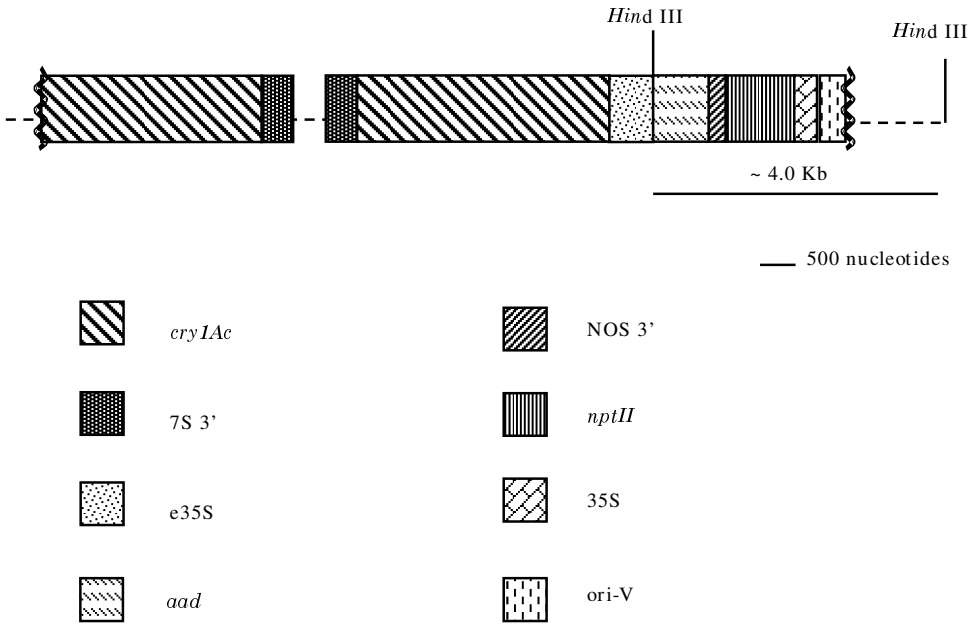
| Genetic element | Approximate size (Kb) | Description/source |
|---|---|---|
| ***cry1Ac cassette*** | | |
| **7S 3'** | 0.44 | 3' nontranslated region from soybean 7S seed storage protein gene which terminates transcription and directs polyadenylation of the *cry1Ac* mRNA |
| ***cry1Ac*** | 3.54 | Synthetic variant of the sequence coding for the Cry1Ab protein of *Bacillus thuringiensis* |
| **e35S** | 0.62 | Cauliflower mosaic virus (CaMV) promoter with the duplicated enhancer region. Drives expression of *cry1Ac* sequence |
| ***aad gene*** | | |
| ***aad*** | 0.79 | Bacterial gene coding for an aminoglycoside-modifying enzyme, 3'(9)-O-nucleotidyltransferase derived from the transposon Tn7 (GenBank accession X03043) |
| ***nptII cassette*** | | |
| **NOS 3'** | 0.24 | 3' nontranslated region of the nopaline synthase (NOS) coding sequence from *Agrobacterium tumefaciens* which terminates transcription and directs polyadenylation |
| ***nptII*** | 0.97 | DNA sequence isolated from Tn5 [Beck, 1982 #38] 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*) coding sequence 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 |

**Table 2. Summary of genetic elements of the insert in MON 1445**

| Genetic element | Approximate size (Kb) | Description/source |
|---|---|---|
| **Right Border** | 0.02 | DNA sequences derived from *Agrobacterium* containing the right border sequence 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, which functions to terminate 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 (*aro*A gene). |
| ***ctp2*** | 0.29 | DNA sequence coding for the N-terminal chloroplast transit peptide from *Arabidopsis thaliana* EPSPS gene. |
| **FMV** | 0.56 | 35S promoter from figwort mosaic virus. |
| ***aad gene*** | | |
| ***aad*** | 0.83 | Bacterial promoter and coding sequence for an aminoglycoside-modifying enzyme, 3'(9)-O-nucleotidyltransferase from the transposon Tn7 (GenBank accession X03043) |
| ***nptII cassette*** | | |
| **NOS 3'** | 0.25 | 3' nontranslated region of the nopaline synthase (NOS) gene from *Agrobacterium tumifaciens* which terminates transcription and directs polyadenylation. |
| ***nptII*** | 0.79 | DNA sequence isolated from 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. |
| **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 1 Schematic representation of the inserts in MON 531**

**a/ Functional *cry1Ac* insert**




**b/ Additional 242 bp insert**

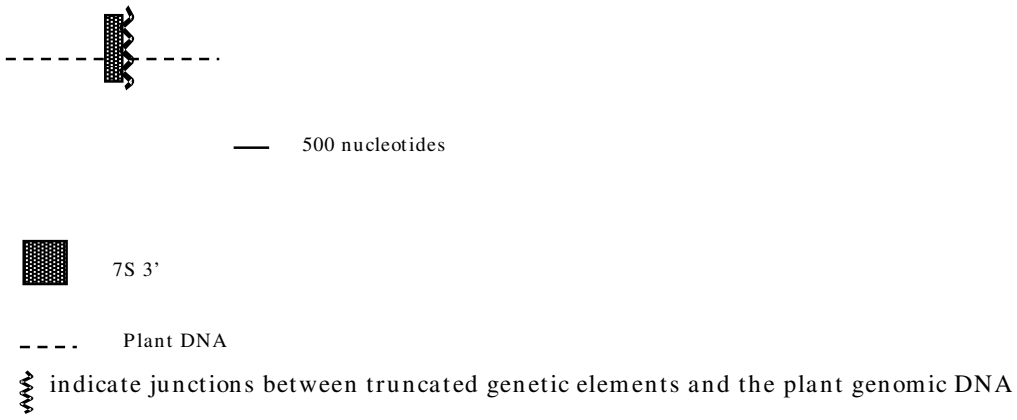
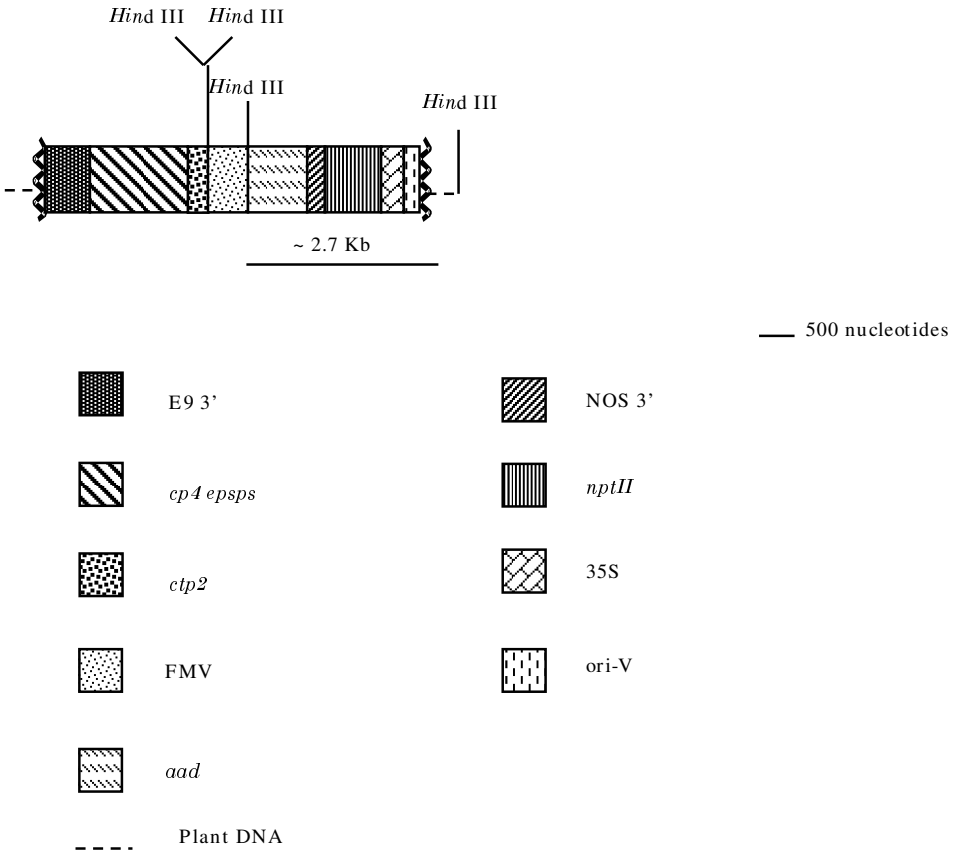


Figure 2 Schematic representation of the insert in MON 1445




indicate junctions between truncated genetic elements and the plant genomic DNA

## D. INFORMATION RELATING TO THE GM PLANT

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

MON 531 x MON 1445 has been produced by the traditional breeding of MON 531 and MON 1445. MON 531 x MON 1445 expresses the insect-protection trait found in MON 531, 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. 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

There is low likelihood of molecular interactions between the inserts from MON 531 and MON 1445 and, therefore, low likelihood of any changes in the molecular characteristics of the inherited inserts in MON 531 × MON 1445 (*e.g.* insert number, copy number, absence of backbone DNA and integrity of the individual inserts).

Southern blot analysis demonstrates that product-specific fingerprints for MON 531 and MON 1445 are present in MON 531 x MON 1445, and therefore that the structure, insert number and copy number are preserved.

Tables 1 and 2 summarize the genetic elements of the DNA inserts in MON 531 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

The traditionally bred MON 531 x MON 1445 contains the DNA inserts from both MON 531 and MON 1445 at separate sites in the nuclear genome, as they were inherited from the MON 531 and MON 1445 single trait material.

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

MON 531 x MON 1445 is the result of traditional breeding of MON 531 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 531 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 531 x MON 1445. In support to 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 cotton modified through biotechnology or traditional cottonseeds. Therefore, the following information related to the expression of the insert can be considered as mainly informative.

Studies were conducted to measure the amount of Cry1Ac, CP4 EPSPS and NPTII proteins in leaf and seed tissues collected from 1998 U.S. field trials. The test, MON 531 x MON 1445 expresses Cry1Ac, CP4 EPSPS and NPTII proteins. There were three types of control varieties used for this study consisting of 1) MON 531, that expresses Cry1Ac and NPTII proteins; 2) MON 1445, that expresses CP4 EPSPS and NPTII proteins; and 3) traditional cotton. The test and controls had similar background genetics representative of the test substances.

Enzyme-Linked Immunosorbent Assay (ELISA) methods were developed and validated to quantify the Cry1Ac, CP4 EPSPS and NPTII protein levels in seed and leaf tissues. Seeds were analyzed for all three proteins since these tissues are most relevant to food and feed product safety. Tissue samples were collected from four locations in 1998. The sites provided a variety of environmental conditions representative of regions where cotton is grown for commercial use.

Table 3 summarizes the mean levels of the Cry1Ac, CP4 EPSPS and NPTII proteins in seed samples across four field sites in 1998. All protein values are expressed as micrograms (µg) of the specific protein per gram (g) of tissue on a fresh weight (fw) basis. The range represents the minimum and maximum values from the analyses of samples across all sites.

In conclusion, Cry1Ac, CP4 EPSPS and NPTII protein levels between the test and MON 531, MON 1445 were generally very similar. 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 (*See* Section D.7.8), and the absence of detectable level of protein in oil.

**Table 3 Summary of Cry1Ac, CP4 EPSP and NPTII protein levels (µg/g fw[1]) measured in cotton varieties[2] Leaf and seed samples collected in 1998 U.S. field trials – Mean[3] (Range[4])**

| | Traditional controls | | | MON 531 | | | MON 1445 | | | MON 531 x MON 1445 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DP50 | DP5415 | DP5690 | DP50BG | DP5415BG | DP5690BG | DP50RR | DP5415RR | DP5690RR | DP50BGRR | DP5415BGRR | DP5690BGRR |
| ***Seed5*** | | | | | | | | | | | | |
| **Cry1Ac** | < 0.50 | < 0.50 | < 0.50 | 2.45 (0.84-3.02) | 3.70 (2.77-4.27) | 3.69 (2.77-4.66) | < 0.50 | < 0.50 | < 0.50 | 2.62 (1.75-3.24) | 3.09 (2.38-3.79) | 3.52 (3.37-3.67) |
| **CP4 EPSPS** | < 22.3 | < 22.3 | < 22.3 | < 22.3 | < 22.3 | < 22.3 | 255 (175-305) | 299 (235-335) | 276 (246-315) | 310 (280-339) | 313 (249-429) | 286 (250-327) |
| **NPTII** | < 2.54 | < 2.54 | < 2.54 | 11.9 (11.4-12.1) | 13.2 (10.0-15.9) | 10.9 (10.1-11.6) | 30.4 (25.2-42.1) | 35.1 (30.1-37.2) | 29.0 (22.7-33.3) | 51.8 (43.6-60.7) | 34.0 (27.5-40.8) | 40.8 (32.3-50.6) |

[1] Protein levels are expressed as microgram of protein per gram fresh weight of tissue and were corrected for assay bias which was calculated as follows: (% extraction efficiency)/100 x (% spike and recovery)/100.

[2] The limit of detection for Cry1Ac, CP4 EPSPS and NPTII assays in leaf and seed tissues are 0.63 and 0.50 µg/g, 0.06 and 22.3 µg/g and 0.30 and 2.54 µg/g, respectively.

[3] The mean was calculated from the analyses of plant samples, one from each of eight field sites except for tissues collected from single site.

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

[5] The sample was of tissue from up to six plants per plot from each site.

### 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 531 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 to 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 cotton modified through biotechnology or traditional cottonseeds.

MON 531 x MON 1445 expresses the insect protection protein Cry1Ac and the CP4 EPSPS protein that provides tolerance to glyphosate. The expression of these proteins in leaf and 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 531 and MON 1445 and traditional cotton have been conducted at multiple sites in the U.S. since development of these products began. Further, MON 531 and MON 1445 are currently registered and grown commercially in the U.S., Australia, South Africa and elsewhere. The extensive experience from commercial release 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 531 and MON 1445 compared to traditional cotton.

Based on the conclusions established for each single trait cotton, no differences are anticipated in the reproduction, dissemination or survivability of MON 531 x MON 1445 compared to MON 531 or MON 1445. A field study supports the conclusion that MON 531 x MON 1445 behaves similar agronomically to traditional cotton, with the exception of the protection against certain lepidopteran insect pests and the tolerance to glyphosate.

### b) Dissemination

The introduced traits have 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 the aid of human intervention. Field observations have demonstrated that MON 531 × MON 1445 has not been altered in its survivability when compared to its parental cotton lines (MON 531 and MON 1445) or compared to traditional cotton.

### d) Other differences

Comparative assessments in the field did not reveal any biologically significant differences between MON 531 × 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

*Molecular stability of the insert*
The presence of the parental inserts in MON 531 x MON 1445 was demonstrated using DNA material extracted at the 12$^{th}$ generation ($BC_4F_8$) 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.

*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 proteins, 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 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 531 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 531, MON 1445 or MON 531 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 531 x MON 1445 was 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**

Materials for the compositional analysis were produced in 1999 replicated field trials at four sites in the U.S. The four replicated trials, Loxley (Alabama), Newport (Arkansas), Hawkinsville (Georgia) and Stoneville (Mississippi), provided a variety of environmental conditions representative of regions in the U.S. where cotton is grown commercially. At each site, MON 531 x MON 1445, the control and the single trait references were planted in four replicates. The reference lines (four/site) were commercial varieties planted in non-replicated plots. A randomized complete block design has been used.

**b) the baseline used for consideration of natural variations**

For the compositional study, a total of 245 statistical comparisons were made between the test MON 531 x MON 1445 and the control cotton. For all the significant differences ($p<0.05$) observed for the combined site analysis, the range of the values for MON 531 x MON 1445 were within the 99% tolerance interval. 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.

The results of the compositional analyses conducted for MON 531 x MON 1445 in comparison to control cotton, as well as the results from similar analyses previously conducted for MON 531 and MON 1445 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 531 x MON 1445 has:

- 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 531 x MON 1445 has 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 531 × MON 1445 actually comprises all traditionally bred hybrid cotton varieties produced by the combination of MON 531 and MON 1445.

As MON 531 x MON 1445 is the result of a traditional cross of MON 531 and MON 1445, it contains the respective DNA inserts from both single trait cotton products. Therefore, MON 531 x MON 1445 is detectable using either the event-specific PCR method for detecting the introduced DNA present in MON 531 or the equivalent method for MON 1445.

### *7.6 Effect of processing*

As MON 531 x MON 1445 is substantially equivalent and as safe and nutritious as traditional cotton, the use of MON 531 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 531 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 or derived products for use as or in food or feed as a result of the addition of MON 531 × MON 1445 varieties to the traditional cotton supply. MON 531 × MON 1445 is 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 evaluation of newly expressed proteins*

The scope of the current application covers cottonseed oil and its constituents produced from MON 531 x MON 1445. In support to 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. Therefore, the following information related to the assessment of newly expressed proteins can be considered as mainly informative.

Updated bioinformatic analyses have been performed on Cry1Ac, CP4 EPSPS and NPTII expressed in MON 531 x MON 1445. 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.

MON 531 x MON 1445 is produced by the traditional crossing of MON 531 and MON 1445. The introduced traits present in MON 531 and MON 1445 are inherited in the MON 531 x MON 1445, thus resulting in the expression of these traits. No risk specific to the expression of the new traits in the same plant can be anticipated since they 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 two proteins, Cry1Ac 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 531 x MON 1445 is substantially equivalent to traditional cotton, with the exception of the introduced insect-protection and glyphosate-tolerance traits.

In support to 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 cotton modified through biotechnology or traditional cottonseeds. Additionally, the human and animal safety of the Cry1Ac 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 531 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 531 x MON 1445 is considered. Any interactions between Cry1Ac and CP4 EPSPS are highly unlikely for the reasons set out below.

- ⇨ There is no conceivable mechanism to expect that the combined presence of the insect-protection and glyphosate-tolerance traits in MON 531 x MON 1445 and, therefore, the expression of the Cry1Ac and CP4 EPSPS proteins in the same plant, would alter the conclusions of the risk assessments made for the individual proteins. Since the proteins have very different and well-documented modes of action, were shown to be safe in their individual safety assessments and are present at very low quantities in MON 531 x MON 1445, it is extremely unlikely that they would interact to produce adverse effects.
- ⇨ The Cry1Ac and CP4 EPSPS proteins have a history of safe use, *i.e.* through consumption of MON 531 and MON 1445, individually and in grain mixtures, but also from their combined use in MON 531 x MON 1445 cultivated in countries outside the E.U. since 1997. All these products and derivatives thereof have been handled and consumed without any reports of adverse health effects.

## *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 531 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 and CP4 EPSPS proteins expressed in MON 531 × MON 1445 has previously been demonstrated for the single-trait parental lines containing either MON 531 or MON 1445.

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 531 × 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 531 × MON 1445 expresses the introduced traits of insect-protection and glyphosate tolerance, which are agronomic traits, and are not intended to change any nutritional aspects of this cotton. Hence this cotton is 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 531 × MON 1445, and no nutritional imbalances are expected as a result of the use of MON 531 × 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 this cottonseed and traditional cottonseed, and as a consequence, no further nutritional assessments of MON 531 x MON 1445 seed for use as or in feed are considered necessary.

Considering the compositional equivalence of MON 531 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 531 x MON 1445 is equivalent to cottonseed oil produced from traditional cotton and has the same nutritional properties.

### *7.11 Post-market monitoring of GM food/feed*

The assessment of the human and animal safety of MON 531 × 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 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 conclude that oil produced from MON 531 x MON 1445 is not different from oil produced from traditional cottonseed.

There are no intrinsic hazards related to MON 531 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 531 x MON 1445 is based on the pre-market risk characterizations of both MON 531 and MON 1445. These pre-market risk assessments have demonstrated that the risks of consumption of foods and feeds produced from both MON 531 and 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 531 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 531 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 531 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 531 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)

Not applicable as neither the environmental release nor import of whole seed of MON 531 x MON 1445 in the E.U. is within 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 Case-specific GM plant monitoring (approach, strategy, method and analysis)*

Please see question **D.11.**

### *11.3 General surveillance of the impact of the GM plant (approach, strategy, method and analysis)*

Please see question **D.11.**

### *11.4 Reporting the results of monitoring*

Please see question **D.11.**

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

As MON 531 × MON 1445 is the result of a traditional cross of MON 531 and MON 1445, it contains both inserts. Therefore, MON 531 × MON 1445 is detectable using either the event-specific PCR method for detecting the introduced DNA present in MON 531 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 531 × MON 1445 in mixed consignments will require single seeds to be subjected to detection methods for both MON 531 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

Since its commercial introduction in the U.S. (1997) and in Australia (2001), MON 531 x MON 1445 has been grown on more than 9 million hectares.

**a) Release country**

Not applicable

**b) Authority overseeing the release**

Not applicable

**c) Release site**

Not applicable

**d) Aim of the release**

Not applicable

**e) Duration of the release**

Not applicable

**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**<br>No stable insect resistance has been detected. |
| **i)** | **Results of the release in respect to any risk to human health and the environment**<br>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**<br>The JRC websites http://gmoinfo.jrc.it/gmc_browse.asp and http://gmo-crl.jrc.it/statusofdoss.htm provide publicly accessible links to up-to-date databases on the regulatory progress of notifications under Directive 2001/18/EC and Regulation (EC) No 1829/2003. |
| **b)** | **Assessment Report of the Competent Authority (Directive 2001/18/EC)**<br>Not applicable |
| **c)** | **EFSA opinion**<br>No EFSA opinion is available at the time of this application. |
| **d)** | **Commission Register (Commission Decision 2004/204/EC)**<br>The exact link to the publicly accessible part of the Commission Register is yet to be released at the time of this application. |
| **e)** | **Molecular Register of the Community Reference Laboratory/Joint Research Centre**<br>Information on detection protocols is likely to be posted at http://gmo-crl.jrc.it/ |
| **f)** | **Biosafety Clearing-House (Council Decision 2002/628/EC)**<br>Not applicable |
| **g)** | **Summary Notification Information Format (SNIF) (Council Decision 2002/812/EC)**<br>Not applicable |