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# RISK ASSESSMENT
# MON 15985

(In accordance with Annex III of the Cartagena Protocol on Biosafety)

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| **Risk assessment details** | | |
| 1. | Country Taking Decision: | South Africa |
| 2. | Title: | Regulatory Affairs Manager: Sub Saharan Africa. |
| 3. | Contact details: | Monsanto South Africa (PTY) Ltd.<br>Building No. 4.<br>Fourways Office Park<br>Fourways ,Randburg.<br>Gauteng.<br>R.S.A. |
| **LMO information** | | |
| 4. | Name and identity of the living modified organism: | MON 15985 (Bollgard®[1] II) |
| 5. | Unique identification of the living modified organism: | MON-15985-7 |
| 6. | Transformation event: | MON 15985 |
| 7. | Introduced or Modified Traits: | Choose the trait from the following list:<br>3. Altered growth, development and product quality<br>- Insect resistance |
| 8. | Techniques used for modification: | Microparticle bombardment of plant cells or tissue |
| 9. | Description of gene modification: | MON 15985 was developed to produce the Cry2Ab2 insect control protein, which provides effective season-long control of key lepidopteran insect pests. This product was produced by re-transformation of Bollgard® I (MON 531), which produces the Cry1Ac insect-control protein and the NPTII selectable marker protein. Therefore, Bollgard II cotton produces two proteins for effective control of the major lepidopteran insect pests of cotton, including the cotton bollworm, tobacco budworm, pink bollworm, and armyworm. Bollgard II cotton also produces the β-D-glucuronidase (GUS) marker protein. |

[1] ® Bollgard is a registered trademark of Monsanto Co., St. Louis, MO

| Characteristics of modification | | |
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| 10. Vector characteristics (Annex III.9(c)): | MON 15985 was generated by re-transformation of cotton meristems of MON 531, variety DP50B. A particle acceleration plant transformation procedure was used to insert the *cry2Ab2* insect control coding sequence and the *uidA* marker coding sequence into the Bollgard I cotton genome. The purified plasmid vector, PV-GHBK11, is a 8.7Kb high copy number based plasmid containing well-characterized DNA elements for selection and replication of the plasmid in bacteria. The purified, linear DNA was inserted into the Bollgard I cotton genome.<br><br>The linear plasmid fragment only contains two plant gene expression cassettes, each using separate controlling DNA elements essential for production in the cotton plant cells and does not contain the *nptII* selectable marker gene or origin of replication. The first cassette contains a copy of the *cry2Ab2* gene encoding the *B.t.* insecticidal protein Cry2Ab2 and the second cassette contains the *uidA* gene encoding the β-D-glucuronidase (GUS) marker protein to facilitate selection of Cry2Ab2-producing plants. The GUS protein serves no other purpose and has no known insect control properties. | |
| 11. Insert or inserts (Annex III.9(d)): | The molecular characterization of Bollgard II cotton demonstrated that there is one DNA *cry2Ab2* insert. The single DNA insert in Bollgard II cotton contains one copy of the *cry2Ab* and *uidA* gene cassettes from the linear DNA PV-GHBK11 used for transformation containing:<br>- the complete *cry2Ab* coding region and cassette, although the restriction site following the NOS 3' polyadenylation sequence in the cassette is not present; and<br>- the complete *uidA* coding region and cassette, except that 260 bp of the 5' end of the enhanced CaMV 35S promoter is not present; however, the truncated promoter is functional as demonstrated by production of the GUS protein.<br>PCR and DNA sequencing verified the 5' and 3' ends of the insert and confirmed that the DNA flanking the insert was native to cotton. Production of the full-length Cry2Ab2 and GUS proteins was confirmed by western blot analysis. | |
| **Recipient organism or parental organisms (Annex III.9(a)):** | | |
| 12. Taxonomic name/status of recipient organism or parental organisms: | Common name:<br>Family name:<br>Genus:<br>Species: | Cotton<br>Malvaceae<br>*Gossypium*<br>(*Gossypium hirsutum* Linnaeus) |
| 13. Common name of recipient organism or parental organisms: | Cotton | |

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| 14. Point of collection or acquisition of recipient or parental organisms: | The original transformations that produced MON 15985 used privately owned germplasm acquired for this purpose. |
| 15. Characteristics of recipient organism or parental organisms related to biosafety: | Cotton is a perennial crop by nature but is seeded and harvested annually for its fiber and seed. Cultivated cotton is not able to establish itself without the intervention of man who creates agronomically the ideal germination and sustainable growth conditions. Cultivated cotton cannot compete with natural field grasses.<br><br>Pollen grains are large, heavy and somewhat sticky, dissemination by wind is absent or negligible (McGregor, 1976; Umbeck *et al*., 1991; Borém *et al*., 2003). Although cotton is mostly self-pollinating, in the presence of suitable insect pollinators it is also cross-pollinating at generally low levels, which improves yields (McGregor, 1976; Tanda, 1984; Mamood *et al*., 1990; Rhodes, 2002; Sanchez and Malerbo-Souza, 2004; Llewellyn *et al*., 2007).<br><br>The diploid *Gossypium herbaceum* race *africana* is the only wild type of the genus Gossypium, tribe Gossypieae of the Family Malvacea, Order Malvales that occurs in South Africa. Cultivated cotton (*Gossypium hirsutum*) is an allotetraploid and in the highly unlikely event of pollen being transferred to *G herbaceum* the resulting offspring would be sterile. To date there is no recorded incidence of conventional cotton hybridizing with *G. herbaceum* after decades of cultivation side by side where these wild types occur. |
| 16. Centre(s) of origin of recipient organism or parental organisms: | Cotton is of the genus *Gossypium* of the tribe Gossypieae of the family Malvaceae of the order Malvales (Fryxell, 1979)). The genus *Gossypium* is comprised of 39 very diverse species that occur in widely separated parts of the world, typically in relatively arid parts of the tropics and subtropics (Fryxell, 1984).<br><br>Worldwide, four species of cotton are of agronomic importance: the two diploid Old World (or Asiatic) species, *G. arboreum* and *G. herbaceum*; and the two allotetraploid New World species, *G. barbadense* and *G. hirsutum*. Although the old world species remain important in restricted areas of India, Africa and Asia, the two new world species account for about 98% of the world's cotton fiber production. Of this amount *G. hirsutum* accounts for 90% while *G. barbadense* accounts for 8% (Lee, 1984).<br><br>The original native habitat of *Gossypium hirsutum* is considered to involve central Mesoamerica (Hutchinson, 1951; Stephens, 1958; Brubaker and Wendel, 1994). |
| 17. Centres of genetic diversity, if known, of recipient organism or parental organisms: | Please refer to the response in section 16. |

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| 18. Habitats where the recipient organism or parental organisms may persist or proliferate: | The genus *Gossypium* occurs in widely separated parts of the world, typically in relatively arid parts of the tropics and subtropics (Fryxell, 1984). The natural distribution of *G. hirsutum* as a wild species is particularly obscure because of millennia of early use, domestication and expanded cultivation. |
| **Donor organism or organisms (Annex III.9(b)):** | |
| 19. Taxonomic name/status of donor organism(s) | *Bacillus thuringiensis* subsp. *kurstaki* |
| 20. Common name of donor organism(s): | Entomocidal bacterium (spore producing) |
| 21. Point of collection or acquisition of donor organism(s): | Organisms are ubiquitous in nature. |
| 22. Characteristics of donor organism(s) related to biosafety: | The donor organisms are ubiquitous in nature, and *Bt* protein products have been used as bio-pesticides around the world for the last 40 years. |
| **Intended use and receiving environment** | |
| 23. Intended use of the LMO (Annex III 9(g)): | MON 15985 is approved for commercial use in South Africa. |
| 24. Receiving environment (Annex III.9(h)): | The receiving environments are varied and represent the entire cotton producing area in South Africa. |
| **Risk assessment summary** | |
| 25. Detection/Identification method of the LMO (Annex III.9(f)): | An event specific detection method for detection of MON 15985 DNA has been validated by the European Commission Joint Research Centre (EU JRC), and is available on the EU JRC website at: http://gmo-crl.jrc.ec.europa.eu/summaries/MON15985_validated_Method.pdf |
| 26. Evaluation of the likelihood of adverse effects (Annex III.8(b)): | MON 15985 was approved for commercial cultivation in South Africa in 2003. MON 15985 cotton is therefore considered as safe as conventional cotton, based on the following –<br>• The inserted genes in Bollgard II cotton are stably integrated and the line is phenotypically and genetically stable over several generations, and in various environments.<br>• Safety assessments of the Cry2Ab2 and GUS proteins produced in Bollgard II included protein characterization, |

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| | demonstration of the lack of similarity to known allergens and toxins, the long history of safe consumption of similar proteins, *in vitro* digestibility, and the lack of acute oral toxicity in mice.<br>• Bollgard II cottonseed is compositionally equivalent to the parental variety and conventional cotton varieties. Processing is unlikely to alter the compositional components of cotton and, therefore, products derived from cottonseed will also be compositionally equivalent to and as safe as current cotton-derived products.<br>• Cultivated cotton (*Gossypium hirsutum*) is an allotetraploid and in the highly unlikely event of pollen being transferred to *G herbaceum* the resulting offspring would be sterile. To date there is no recorded incidence of conventional cotton hybridizing with *G. herbaceum* after decades of cultivation side by side where these wild types occur.<br>• Cotton is not considered to have weediness characteristics, such as seed dormancy, soil persistence, germination under diverse environmental conditions, rapid vegetative growth, a short life cycle or high seed output and dispersal.<br>• Studies demonstrate that the Cry2Ab2 protein is safe to non-target organisms, including humans, animals, and beneficial insects. |
| 27. Evaluation of the consequences (Annex III.8(c)): | Considering the safety assessment conducted for MON 15985, the potential risk of adverse consequences is considered to be negligible. |
| 28. Overall risk (Annex III.8(d)): | MON 15985 cotton obtained general release approval in South Africa in 2003; the overall risk of cultivating MON 15985 is therefore considered to be the same as the risk of cultivating conventional cotton. |
| 29. Recommendation (Annex III.8(e)): | Except for the requirement of an insect resistance management strategy, no other risk management measures are required. |
| 30. Actions to address uncertainty regarding the level of risk (Annex III.8(f)): | There is no uncertainty regarding the risk profile. |
| **Additional information** | |
| 31. Availability of detailed risk assessment information: | All the current information is contained in the application (Part I) that was approved by the Executive Council. |
| 32. Any other relevant information: | None. |
| 33. Attach document: | *Not applicable to applicant* |
| 34. Notes: | See references below. |

**References:**

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