COMMON FORMAT FOR Risk Assessment
(In accordance with Annex III of the Cartagena Protocol ion Biosafety)

Risk assessment
details

1. Country Taking Decision: South Africa

2. Title: Mr. Wally Green. Biotechnology Regulatory Manager.

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LMO
information

4.Name and identity of the living modified organism:

MON 88017 × MON 810.

The Monsanto development code for this product is MON 88017 × MON 810. In countries where MON 88017 × MON 810 is being cultivated, seed is marketed under the name of the hybrid, in association with the trademark YieldGard®[1] VT Triple™, indicating clearly to growers that the maize is protected from specific coleopteran and lepidopteron insect pests as well as tolerant to Roundup herbicide formulations.

5. Unique identification of the living modified organism:
MON-88Ø17-3 × MON-ØØ81Ø-6

6. Transformation events:

MON 88017 × MON 810 is produced by conventional breeding techniques

7. Introduced or Modified Traits:

Introduced traits provide resistance to certain insects belonging to two different Orders, the Coleoptera and the Lepidoptera as well as herbicide tolerance.

8. Techniques used for modification:

No novel method of genetic modification is utilized in the production of MON 88017 × MON 810. Instead, conventional maize breeding techniques are used to cross parental inbred maize plants of MON 888017and MON 810. MON 88017 × MON 810 is therefore, considered an extension of the use of the parental double and single trait lines, combining three events in one maize plant.

While the MON 88017 × MON 810 hybrids results from traditional breeding, genetic modification was used in the development of the double and single-trait parents, MON 88017 and MON 810:

Transformation event MON 88017(,YieldGard® VT Rootworm/RR2 maize)

MON 88017 was produced by *Agrobacterium sp.*-mediated transformation of corn with plasmid vector PV-ZMIR39, which contains both the *cp4 epsps* and *cry3Bb1* gene expression cassette. MON 88017 contains one copy of the introduced DNA at a single integration locus.

MON 88017 contains a single insertion of the stably integrated DNA cassette, which is inherited as a single dominant gene in a Mendelian fashion.

Transformation event MON 810, inherited from YieldGard® Corn Borer maize (MON 810)

MON 810 maize was produced by particle acceleration. MON 810 contains a single functional copy of the *cry1Ab* coding sequence in the maize genome, which expresses the insecticidally active Cry1Ab protein. The *cry1Ab* coding sequence from *Bacillus thuringiensis* subsp. HD-1 was modified to increase the levels of the Cry1Ab protein in plants. The enhanced cauliflower mosaic virus *(CaMV) 35S* promoter and *hsp70* maize intron regulate the expression of the *cry1Ab* coding sequence.

MON 810 contains a single insertion of the stably integrated DNA cassette, which is inherited as a single dominant gene in a Mendelian fashion.

Inherited traits in MON 88017 × MON 810.

The F1 MON 88017 × MON 810 seed inherits the introduced coleopteran insect-protection trait from MON 88017, as well as herbicide tolerance. The lepidopteran insect-protection trait is inherited from MON 810 The expression of the Cry3Bb1 protein protects the maize plant from certain coleopteran insect pests, including *Diabrotica* spp. The insecticidal activity of the Cry3Bb1 protein is specific to predation by the larvae of the targeted coleopteran insects.

The expression of the Cry1Ab protein protects the maize plant from certain lepidopteran insect pests, including the European Corn Borer (*Ostrinia nubilalis*) and pink borers (*Sesamia* spp.). The insecticidal activity of the Cry1Ab protein is specific to predation by the larvae of the targeted lepidopteran insects.

The expression of glyphosate-tolerant CP4 EPSPS enzymes in maize plants imparts tolerance to glyphosate (N-phosphonomethyl-glycine) to the plant. Glyphosate is the active ingredient in the non-selective, foliar-applied, broad-spectrum, post-emergent Roundup family of agricultural herbicides. The use of MON88017 x MON810 will, therefore, enable the grower to utilise Roundup agricultural herbicides for effective control of weeds during the growing season and to take advantage of the favourable environmental and safety characteristics of Roundup herbicide. It will also enable the grower to effectively control these maize pests, ensuring maximum realization of yield potential, while removing the environmental burden of the production, packaging and transport of chemical insecticides previously used to control *Diabrotica* spp., *Ostrinia nubilalis* and *Sesamia* spp.

MON 88017 × MON 810 was demonstrated to be substantially equivalent in composition and agronomics to traditional maize, with the exception of the introduced insect-protection and herbicide tolerant traits.

Choose the trait from the following list:

B. Altered growth, development and product quality

Chemical tolerance

- Herbicide tolerance

Pest resistance

- Insect resistance
- Insect resistance (both traits)

9. Description of gene modification:

Genes derived from *Bacillus thuringiensis* subsp. *kumamotoensis* and Agrobacterium spp. Strain CP4 was used to develop MON 88017. The genes express the Cry protein Cry3 Bb1 and protein enzyme CP4 EPSPS respectively. These proteins act as endo toxins in certain families of coleopteran larvae commonly called corn root worms while the CP4 epsps gene expresses the protein enzyme CP4EPSPS that provides the maize plant with tolerance to applications of Roundup herbicide formulations..
A Gene derived from *Bacillus thuringiensis* subsp. *kurstaki* was used to develop MON810, commercialised as Yieldgard®. Maize parental lines each containing one of the events MON88017 and MON810 were then crossed using conventional breeding techniques to produce hybrids containing three different traits.

Characteristics of modification

10. Vector characteristics

Plasmid vector PV-ZMIR39 derived from *a pUC based plasmid.*
Plasmid vector PV- ZMBK07 derived from a *pUC based plasmid.*
Vector characteristics are not relevant as this application concerns a product derived from traditional breeding.

11. Insert or inserts

The following inserts: MON 88017 plasmid vector PV-ZMIR39 contains a *cry 3ABb1* gene expression cassette that produces the Cry3Bb1 protein and the CP4 epsps gene expression cassette that produces the CP4 EPSPS protein. MON810 plasmid vector PV-ZMBK07 contains a *cry1Ab* gene expression cassette that produces the Cry 1Ab protein. The *cry* endo toxin proteins must be ingested and processed by enzymes in the midguts of specific Lepidopteron and Coleopteran larvae to produce an active endotoxin. The CP4 *epsps* gene that expresses the CP4 EPSPS protein enzyme provides tolerance to Roundup Ready herbicide formulations.

Recipient organism or parental organisms

12. Taxonomic name/status of recipient organism or parental organisms:

Common name:	Maize
Family name:	*Gramineae*
Genus:	*Zea*
Species:	*mays*(2n+20)

13. Common name of recipient organism or parental organisms:
Maize, corn

14. Point of collection or acquisition of recipient or parental organisms:

The original transformations that produced MON 88017 and MON 810 used privately owned germplasm acquired for this purpose.

15. Characteristics of recipient organism or parental organisms related to biosafety:

Maize is the world's third leading cereal, following rice and wheat, in terms of production and area harvested. It has a long history of safe use as a raw material for processed products, and direct uses as a human food or animal feed. Today, maize is produced on every continent except Antarctica, and is exported and imported as viable grain for use as foods or feeds, or directly in processing, without risk to the environment.

According to OECD [Consensus Document on the Biology of *Zea mays* subsp. *mays* (Maize), 2003], "Maize has lost the ability to survive in the wild due to its long process of domestication, and needs human intervention to disseminate its seed." In addition, "maize is incapable of sustained reproduction outside of domestic cultivation", and "maize plants are non-invasive in natural habitats." Despite the fact that maize frequently appears as a volunteer plant in a subsequent rotation, is has no inherent ability to persist or propagate. In all regions of the world, volunteer plants are managed with herbicides, tillage, or manual removal of plants. As such, maize is not considered a pest anywhere in the world. When it occurs outside of cultivation, it has no impact on the conservation and sustainable use of biological diversity.

Gene flow from maize occurs through dispersal of seed and pollen mediated exchange of genes to sexually compatible plants. Since maize has no biological mechanism to scatter seed, low-level, incidental dispersal of viable grain occurs as a result of human-based activities such as transport and harvesting operations. As was noted by OECD, the few plants that might result from incidental release will not persist or meaningfully reproduce without human intervention. Gene flow via pollen is only possible to other maize plants throughout the world except in Mexico and Guatemala where wild relatives occur (*See* Section g). Maize reproduces sexually, is a wind-pollinated, monoecious species with separate staminate (tassels) and pistillate (silk) flowers, which encourages natural cross-pollination between maize plants. The distance that viable pollen can travel depends on prevailing wind patterns, humidity, and temperature. Generally, the pollen dissemination period lasts three to seven days. Because incidental release of maize during importation occurs at very low levels, and because maize in not competitive, pollen mediated gene flow between local maize and rare volunteers has had no effect on the conservation and sustainable use of biological diversity.

16. Centre(s) of origin of recipient organism or parental organisms:

Maize is thought to have its origin in Mexico, from where it spread northward to Canada and southward to Argentina. Although secondary centres of origin in South America are possible, the oldest archaeological evidence of domesticated maize (5000 B.C.) was discovered in the valley of Tehuacan in Mexico (Benson and Pearce, 1987). Several theories on the origin of maize have been proposed; the two theories most adhered to being that either teosinte (a wild relative of maize that is endemic to parts of Mexico and Guatemala) or a wild pod maize that is now extinct was the wild ancestor of maize (Benson and Pearce, 1987; Brown *et al.*, 1984).

Maize is a member of the genus *Zea,* which is broken into 2 sections: ZEA and LUXURIENTES. The section ZEA includes one species (*mays*), which includes three subspecies: ssp. *mays,* ssp. *mexicana* (formerly *Euchlaena mexicana),* and ssp. *parviglumis.* The former subspecies is known as maize while the latter comprise a portion of the complex known as teosinte. Furthermore, ssp. *mexicana* and ssp. *parviglumis* are further separated into several races (OECD, 2003). Section LUXURIENTES encompasses 3 species: an annual *Z. luxurians,* and perennials *Z. diploperennis* and *Z. perennis.* While the classification of *Zea* continues to be modified, teosintes are the only know wild relatives of maize capable of forming hybrids in nature. Outcrossing and gene exchange between teosinte and maize has been reported with annual teosinte (*Zea mays* ssp. *mexicana*) (2n = 20) and maize (*Zea mays L.*) (2n = 20). A frequency of one F1 hybrid (maize × teosinte) for every 500 maize plants or 20 to 50 teosinte plants in the Chalco region of the Valley of Mexico was reported. However, newer information shows that annual teosintes may be a separate species because of the level of genetic isolation and that hybrids that do form are highly unsuccessful in introgressing genetic material (OECD, 2003). Regardless, Mexico and parts of Central America are regarded as the center of genetic diversity for maize. The natural distribution of teosinte is limited to the seasonally dry, subtropical zone with summer rain along the western escarpment of Mexico and Guatemala and the Central Plateau of Mexico.

The belief that Central America and southern Mexico are both the center of origin and a center of diversity for maize was supported by (Vavilov, 1992).

17. Centres of genetic diversity, if known, of recipient organism or parental organisms:
See question 16 above.

18. Habitats where the recipient organism or parental organisms may persist or proliferate:

As noted by OECD (2003), maize is not invasive of natural habitats, does not persist or disperse anywhere in the world without the human intervention. Early domestication and diversification through selection occurred in Meso-America. Maize is grown across a wide range of ecological conditions including soil types, altitude and rainfall. Currently, maize is grown over a wide range of conditions because of its many divergent types that have been bred for this purpose.

The bulk of the maize is produced between latitudes 30° and 55°, with relatively little grown at latitudes higher than 47° latitude anywhere in the world. The greatest maize production occurs where the warmest month isotherms range between 21 and 27° C and the frost-free season lasts 120 to 180 days. A summer rainfall of 15 cm is approximately the lower limit for maize production without irrigation.

Experience with maize imported for use as foods or feeds, or directly in processing, has demonstrated that stable populations do not establish, persist or proliferate as a result of this practice.

Donor organism or organisms (Annex III.9(b)):

19. Taxonomic name/status of donor organism(s):

Bacillus thuringiensis subsp. *kurstaki*
Bacillus thuringiensis subsp. *kumamotoensisi*
Agrobacterium species strain CP4

20. Common name of donor organism(s):

MON 88017 results from Agrobacterium mediated transformation using genes derived from *Bacillus thuringiensis* and CP4 *Agrobacterium* species that commonly occur in soil and on plant material. MON810 containing the *cry1Ab* coding sequence was derived from the common soil bacterium *Bacillus thuringiensis* subsp. *kurstaki* and encodes the insecticidally active Cry1Ab protein.

21. Point of collection or acquisition of donor organism(s):
See question 20 above.

22. Characteristics of donor organism(s) related to biosafety:

Not applicable seeing that the donor organisms are ubiquitous in nature and that the same organisms and their protein products have been used as bio-pesticides around the world for the last 40 years. Similarly crops such as cotton and soybeans with the herbicide tolerant gene CP4 epsps have been commercially planted for the last ten years are also derived from a donor organism that is ubiquitous in nature.

Intended use and receiving environment

23. Intended use of the LMO

Possible importation in the form of grain for Feed and Food production.

24. Receiving environment:

Should South Africa need to import maize grain from the USA and should this grain contain a % of kernels derived from hybrids that contain these events, then the receiving environment would be feed and food processor' facilities, who would crush the grain for Feed/Food manufacturing purposes. (No longer LMO's)

Risk assessment summary

25. Detection/Identification method of the LMO.

Event specific PCR, ELISA and diagnostic strip technology can be used. These are specific for DNA sequence, identification and quantitative protein and protein presence, respectively.

26. Evaluation of the likelihood of adverse effects (Annex III.8(b)):

See question 24 above. The receiving environment (Feed and Food processors) will change the LMO status of the grain through crushing prior to the production of feed or food products.

27. Evaluation of the consequences (Annex III.8(c)):

The consequences are that the grain kernels that might contain these events are no longer viable as seed and therefore will have no environmental impact.

28. Overall risk (Annex III.8(d)):

This section presents a risk assessment report consistent with Annex III of the Cartagena Protocol on Biosafety as required by Annex II.j. The information was collected following the general principles and methodology described in Annex III. Specifically, the country(ies) that has (have) approved intentional introduction of the GM crop into the environment have conducted risk assessments that meet the objectives outlined in Annex III, which was "to identify and evaluate the potential adverse effects of living modified organisms on the conservation and sustainable use of biological diversity in the likely potential receiving environment, taking into account risks to human health." General principles outlined in Annex III paragraphs (3), (4), (5), and (6) were utilized in the risk assessment including: scientific soundness, transparency, consistency with international guidance and expert advice, and comparison of the non-modified recipient or parental organism within the likely receiving environment. The assessment was carried out considering the intended use and likely receiving environment. The framework underlying the evaluation of MON 88017 × MON 810 is consistent with guidance established by the United Nations World Health Organization (WHO) and the Food and Agriculture Organization (FAO) (OECD, 1993; WHO, 1995; WHO, 1996), the U.S.A., Canada, Japan, the EU and other countries.

The conclusion of the risk assessment conducted herein demonstrate the MON 88017 x MON 810 poses no increased risk to the conservation and sustainable use of biological diversity in the likely potential receiving environment, taking into account risks to human health under the intended direct use as food or feed, or for processing.

29. Recommendation (Annex III.8(e)):

See Question 28 above.

30. Actions to address uncertainty regarding the level of risk (Annex III.8(f)):

There is no uncertainty regarding the risk profile based on the fact that the application only concerns the importation of grain and is not for production purposes.

Additional information

31. Availability of detailed risk assessment information:

Further information is available in the dossier in which we are making application for a commodity clearance permit number

32. Any other relevant information:
None.

33. Attach document: Not *applicable to applicant*

34. Notes: <Text entry>