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PART II

Risk Assessment of stacked maize product Bt11 x MIR604 x GA21 South Africa

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

Risk assessment details	
1. **Country Taking Decision:**	South Africa
2. **Title:**	Risk Assessment of stacked maize product Bt11 x MIR604 x GA21 in South Africa. This risk assessment is in support of the Syngenta SA Application for commodity clearance of stacked maize product Bt11 x MIR604 x GA21 in South Africa.
3. **Contact details:**	**Name and Address and Contact details of the Exporter**[1] Commodity imports are done by various grain traders on the international market, depending on the local need in SA. **Name and Address and contact details of the importer**[2] Syngenta SA (Pty) Ltd. Building 10, Thornhill Office Park 94 Bekker Street Midrand, 1685 Tel: +27 11 541 4000 Fax: +27 11 541 4072
LMO information	
4. **Name and identity of the living modified organism:**	Stacked maize product Bt11 x MIR604 x GA21 is resistant to certain lepidopteran and coleopteran pests, and tolerant to herbicides containing glufosinate ammoinium and herbicides containing glyphosate. Formulations of glufosinate ammonium are however currently not approved for use on maize in South Africa.
5. **Unique identification of the living modified organism:**	Unique identifier: SYN- BTØ11-1 x SYN-IR6Ø4-5 x MON-ØØØ21-9
6. **Transformation event:**	Stacked maize product Bt11 x MIR604 x GA21

[1] Exporter is any natural or legal person by whom and on whose behalf a notification is made.

[2] An importer is any natural or legal person, under the jurisdiction of a Party or non-Party, who arranges for a GMO to be imported.

Risk assessment details	
7. Introduced or Modified Traits:	A: <u>Altered growth, development and product quality</u> - Insect resistance and herbicide tolerance
8. Techniques used for modification:	Stacked maize product Bt11 x MIR604 x GA21 was produced through conventional breeding of maize events Bt11, MIR604 and GA21. Bt11-maize was transformed using a protoplast transformation /regeneration system. Transformation of maize event MIR604 was conducted using immature maize embryos derived from a proprietary *Zea mays* line, *via Agrobacterium*-mediated transformation using the method described by Negrotto *et al*. (2000). GA21 maize was produced through microprojectile bombardment of maize suspension culture cells. This is described in the International Patent PCT/US98/06640 (pages 75-77; Spencer *et al*., 1998a). Using this method, the *Not*1 restriction fragment of the pDPG434 plasmid DNA was introduced into corn cells.
9. Description of gene modification:	Stacked maize product Bt11 x MIR604 x GA21 was produced through conventional breeding of maize event Bt11, maize event MIR604 and maize event GA21, and contains 5 transgenes, viz. *cry1Ab*, *pat*, *mcry3A*, MIR604 *pmi* and *mepsps.*
10. Vector characteristics	Stacked maize product Bt11 x MIR604 x GA21 was produced through conventional breeding. The vector used for the transformation of Bt11 was pZO1502. The vector used for the transformation of MIR604 was pZM26. Vector pDPG434 was used for the transformation of GA21 (see Spencer *et al*., 1998b and Spencer *et al*., 1998a). The vector is derived from a pSK-vector, which is commonly used in molecular biology and is derived from pUC19 (Short *et al*., 1988).

Risk assessment details	
11. Insert or inserts (Annex III.9(d)):	Stacked maize product Bt11 x MIR604 x GA21 is genetically modified to express five proteins – - a truncated Cry1Ab protein for control of certain lepidopteran pests. Bt11 maize has been successfully grown in SA since its general release approval in 2003. - a phosphinothricin acetyltransferase (PAT) protein that confers tolerance to herbicide products containing glufosinate ammonium. Formulations of glufosinate ammonium is not approved for use on maize in South Africa, hence Bt11 is not locally marketed for this specific trait. - a modified Cry3A (mCry3A) protein for control of certain coleopteran pests like *Diabrotica virgifera virgifera* (Western corn rootworm; WCRW). *Diabrotica* spp are not regarded as a maize pest in South Africa. - a phosphomannose isomerase (MIR604 PMI) protein as a selectable marker. PMI allows transformed corn cells to utilize mannose as a sole carbon source while maize cells lacking this protein fail to grow. - a modified maize 5-enolpyruvylshikimate-3-phosphate synthase enzyme (mEPSPS) that confers tolerance to herbicide products containing glyphosate.
Recipient organism or parental organisms (Annex III.9(a)):	
12. Taxonomic name/status of recipient organism or parental organisms:	Family name: Poaceae Genus: *Zea* Species: *Zea mays* L. Subspecies: *mays*
13. Common name of recipient organism or parental organisms:	Maize/corn.
14. Point of collection or acquisition of recipient or parental organisms:	Maize originates from the Mesoamerican region, i.e. Mexico and Central America region (CFIA, 2003).
15. Characteristics of recipient organism or parental organisms related to biosafety:	*Zea mays* reproduce sexually via the production of seed. Although maize is an allogamous species (capable of cross-fertilzation), both self-fertilization and cross-fertilization are usually possible. Most maize varieties are protoandrous so pollen shedding precedes silk emergence by up to five days. Pollen dispersal is limited by several factors, including large size (0.1 mm diameter), rapid settling rate and short survivability. Greater than 98% of

the pollen settles to the ground within a maximum distance of 25-50 meters of its source (EEA, 2002). Shed pollen typically remains viable for 10 to 30 minutes, but may remain viable longer under refrigerated and humid conditions (Coe *et al.*, 1988 Herrero and Johnson, 1980; Hoekstra *et al.*, 1989; Jones and Newel, 1948). Fertilization is affected by a number of complicating factors, such as genetic sterility factors and differential growth rates of pollen tubes.

1. Sexual compatibility with other cultivated or wild plant species, including the distribution in South Africa of the compatible species.

As there are no wild relatives of maize in South Africa, the potential for genetic transfer and exchange with other organisms is limited to other maize plants. Maize is wind pollinated and pollen distribution and viability depends on prevailing wind patterns, humidity, and temperature. The frequency of cross-pollination and fertilization depends on the co-availability of fertile pollen and receptive plants. Wild *Zea* species have no pronounced weedy tendencies (CFIA, 2003).

2. Survivability

(a) ability to form structures for survival or dormancy;

Maize is an annual crop. Seeds are the only survival structures; they cannot be dispersed without mechanical disruption of the cobs and show little or no dormancy. Natural regeneration from vegetative tissue is not known to occur.

(b) specific factors affecting survivability, if any.

Survival of maize is dependent upon temperature, seed moisture, genotype, husk protection and stage of development. Maize cannot persist as a weed. Maize seed can only survive under a narrow range of climatic conditions. Volunteers are killed by frost or easily controlled by current agronomic practices including cultivation and the use of selective herbicides (Niebur, 1993; Owen, 2005). Maize is incapable of sustained reproduction outside of domestic cultivation and is non-invasive of natural habitats (OECD, 2003).

3. Dissemination:

(a) ways and extent (e.g. an estimation of how viable pollen and/or seeds declines with distance) of dissemination;

Maize dissemination can only be accomplished through seed dispersal which does not occur naturally due to the structure of the ear (OECD, 2003). Natural regeneration from vegetative tissue in the field is not known to occur.

Risk assessment details

Maize is predominantly wind pollinated. Plants produce pollen for 10-13 days according to the genotype. Shed pollen typically remains viable only a short time but may remain viable longer under refrigerated and humid conditions. Reports range from a few minutes (CFIA, 2003) to a few days (EEA 2002).

98% of pollen settles to the ground within a maximum distance of 25-50 meters of its source (EEA, 2002).

(b) specific factors affecting dissemination, if any.

Maize has a polystichous (arranged in many rows) female inflorescence (flower) called the ear on a stiff central spike (cob) enclosed in husks (modified leaves). Because of the structure of the ears, seed dispersal of individual kernels does not occur naturally. Maize is non-invasive of natural habitats (OECD, 2003).

Pollen dispersal is influenced by wind and weather conditions and is limited by several factors, including large size (0.1 mm diameter), rapid settling rate, short survivability, and physical barriers. Greater than 98% of the pollen settles to the ground within a maximum distance of 25-50 meters of its source (EEA, 2002). The pollen grain has a relatively thin outer membrane that gives little environmental protection, consequently shed pollen typically remains viable only for 10 to 30 minutes, but may remain viable longer under refrigerated and humid conditions (Coe *et al.*, 1988 Herrero and Johnson, 1980; Hoekstra *et al.*, 1989; Jones and Newel, 1948).

Pollen release can be prevented by detasselling and genetic sterility.

<u>4. Geographical distribution of the plant.</u>

Maize, which has very diverse morphological and physiological traits, is grown on approximately 140 million hectares worldwide (FAO, 2007). It is distributed over a wide range of conditions: from 56° N Lat to 40° S Lat, below sea level of the Caspian plains up to 3000 m in the Andes Mountains and from semi-arid regions to arid regions (Russell and Hallauer, 1980). The greatest maize production occurs where the warmest month isotherms range between 21° and 27° C and the freeze-free season lasts 120-180 days.

<u>5. Other potential interactions, relevant to the GMO, of the plant with organisms in the ecosystem where it is usually grown, or elsewhere, including information on toxic effects on humans, animals and other organisms.</u>

Risk assessment details

Maize is known to interact with other organisms in the environment including insects, birds, and mammals. It is susceptible to a range of fungal diseases and insect pests, as well as to competition from surrounding weeds (OECD, 2003, Appendix C).

Maize is extensively cultivated and has a history of safe use for human food and animal feed. .

No significant native toxins are reported to be associated with the genus Zea (CFIA, 2003).

As there are no wild relatives of maize in South Africa, the potential for genetic transfer and exchange with other organisms is limited to other maize plants. Maize is wind pollinated and pollen distribution and viability depends on prevailing wind patterns, humidity, and temperature. The frequency of cross-pollination and fertilization depends on the co-availability of fertile pollen and receptive plants.

All maize can cross-fertilize, except for certain popcorn varieties and hybrids that have one of the gametophyte factors of the allelic series *Ga* and *ga* allelleic series on chromosome 4 (OECD, 2003).

7. Wild plant species

Wild *Zea* species have no pronounced weedy tendencies (CFIA, 2003).

The only wild species with which *Zea mays* ssp. *mays* is sexually compatible are annual teosinte (*Zea mays* ssp. *mexicana*, formerly *Euchlaena mexicana*) and *Tripsacum*. No wild *Zea* or *Tripsacum* species are present in South Africa.

Annual teosinte is a wind-pollinated grass. Out-crossing and gene exchange between *Z. mays* ssp. *mexicana* and *Z. mays* ssp. *mays* do occur, but hybrids have reduced seed dispersal and often reduced viability (OECD, 2003). The natural distribution of *Z. mays* ssp. *mexicana* is limited to Mexico and Central America (CFIA, 2003).

Although some *Tripsacum* species (*Tripsacum dactyloides, Tripsacum floridanum, Tripsacum lanceolatum, and Tripsacum pilosum*) can be crossed with *Z. mays* ssp *mays*, hybrids have a high degree of sterility and are genetically unstable. Out-crossing of maize and Tripsacum species is not known to occur in the wild (OECD 2003).

Tripsacum species are geographically restricted to the Americas (CFIA, 2003). Only two species are known to be

Risk assessment details	
	found north of Mexico: *Tripsacum floridanum* which is native to the southern tip of Florida, USA; and *Tripsacum dactyloides* (Eastern gammagrass), which can be found in the northern US. The center of diversity for *Tripsacum* is the western slopes of Mexico, the same area where teosinte is frequently found (CFIA, 2003). *Tripsacum*-annual teosinte hybrids have not been produced.
16. Centre(s) of origin of recipient organism or parental organisms:	Maize originates from the Mesoamerican region, i.e. Mexico and Central America region (CFIA, 2003).
17. Centres of genetic diversity, if known, of recipient organism or parental organisms:	Maize originates from the Mesoamerican region, i.e. Mexico and Central America region (CFIA, 2003).
18. Habitats where the recipient organism or parental organisms may persist or proliferate:	Maize originates from the Mesoamerican region, i.e. Mexico and Central America region (CFIA, 2003). Kindly refer to paragraph 15 for more information.
Donor organism or organisms (Annex III.9(b)):	
19. Taxonomic name/status of donor organism(s)	Bt11: *Bacillus thuringiensis* var. *kurstaki* strain HD-1 (Btk) and *Streptomyces viridochromogenes* strain Tu494. MIR604: The *mcry3A* gene is a modified gene encoding a modified Cry3A protein, related to the Cry3A protein from *Bacillus thuringiensis* The donor organism for the *pmi* gene is *Escherichia coli.* GA21: The donor organism is maize (see section 12 for details)
20. Common name of donor organism(s):	Bt11 and MIR604: Bacteria or Micro-organisms GA21: Maize/corn.

Risk assessment details	
21. Point of collection or acquisition of donor organism(s):	Bt11and MIR604: These bacteria are widely prevalent in the environment. GA21: Maize originates from the Mesoamerican region, i.e. Mexico and Central America region (CFIA, 2003).
22. Characteristics of donor organism(s) related to biosafety:	Bt11: The donor organism for the *cry1Ab* gene is *Bacillus thuringiensis* var. *kurstaki* strain HD-1 (Btk). Btk is a spore forming soil micro-organism which produces crystal proteins. These crystal proteins are effective as insecticides after ingestion by specific sensitive insects. The crystal proteins, also called "protoxins" are solubilised by the alkaline gut juice and proteolytically cleaved into a smaller active toxic fragment, the core fragment (Höfte and Whiteley, 1989). The activated protein binds to brush border membrane vesicules in the insect midgut, inducing the formulation of pores, which affects the osmotic balance. The cells swell and lyse, leading to eventual death of the insect. The donor organism for the *pat* gene is *Streptomyces viridochromogenes* strain Tu494. *S. viridochromogenes* is a gram-positive, sporulating, soil-inhabiting bacterium. It produces an enzyme, phosphinothricin acetyl-transferase, which protects itself from a tripeptide, phosphinothricin-alanyl-aline (Ptt), which the bacterium also produces, but which chows broad spectrum toxicity to plants. The gene which encodes the enzyme has been designated *pat*. Glufosinate ammonium inhibits the glutamine synthetase of plants, resulting in an accumulation of ammonia in plant tissue, which lead to the death of the plant. MIR604: *B. thuringiensis* has been described above. *E. coli* is widespread in the environment. GA21: The donor organism, maize, is widespread in the environment or human and animal diets. No known associated toxicity or pathogenicity are associated with maize.
Intended use and receiving environment	
23. Intended use of the LMO (Annex III 9(g)):	Food, feed and processing (commodity clearance)
24. Receiving environment (Annex III.9(h)):	Stacked maize product Bt11 x MIR604 x GA21 will not be cultivated in SA.
Risk assessment summary	

Risk assessment details	
25. Detection/Identification method of the LMO (Annex III.9(f)):	Bt11: A quantitative event-specific method to detect and quantify Bt11 maize has been validated by the European Union Joint Research Centre (JRC) and could be used for detection of Bt11. MIR604: A quantitative event-specific method to detect and quantify MIR604 maize was developed by Syngenta and has been validated by the European Union Joint Research Center (JRC) and could be used for detection of MIR604. GA21: A quantitative event-specific method to detect and quantify GA21 maize was developed by Syngenta and has been validated by the European Union Joint Research Centre (JRC) and could be used for detection of GA21. Stacked maize product Bt11 x MIR604 x GA21: The methods developed for detection of maize events Bt11, MIR604 and GA21 should also be utilised to detect stacked maize product Bt11 x MIR604 x GA21.
26. Evaluation of the likelihood of adverse effects (Annex III.8(b)):	Cultivation of maize derived from stacked maize Bt11 x MIR604 x GA21 in South Africa is not within the scope of Syngenta's Application for Commodity Clearance. In the rare event that small amounts of maize kernels of the stacked product could accidentally find their way into the environment their survival would be very unlikely as maize is highly domesticated and cannot survive without human intervention (Niebur, 1993; Owen, 2005), especially under normal South African climatic conditions. In the rare event that these maize plants were to survive they could be easily controlled using any of the current agronomic measures taken to control other commercially available maize. The expression of the Cry1Ab, PAT, mCry3A, PMI and mEPSPS proteins does not affect the agronomic characteristics or weediness potential of stacked maize product Bt11 x MIR604 x GA21.
27. Evaluation of the consequences (Annex III.8(c)):	The probability of stacked maize Bt11 x MIR604 x GA21 plants becoming more persistent than the recipient or parental plants in agricultural habitats or more invasive in natural habitats as a result of importing maize kernels of stacked maize Bt11 x MIR604 x GA21 into South Africa can be considered negligible.
28. Overall risk (Annex III.8(d)):	Commodity clearance of stacked maize product Bt11 x MIR604 x GA21 is not likely to have significant long-term effects on the environment, human or animal health.
29. Recommendation (Annex III.8(e)):	Full compliance with permit conditions and other risk management conditions imposed by the Competent National Authority.

Risk assessment details	
30. Actions to address uncertainty regarding the level of risk (Annex III.8(f)):	Not applicable.

Additional information	
31. Availability of detailed risk assessment information:	More information on the event and the assessment of risk can be obtained from the application.
32. Any other relevant information:	Not applicable.
33. Attach document:	Not applicable to applicant.
34. Notes:	Not applicable.