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PART II

Risk Assessment of Bt11 x MIR162 x MIR604 x GA21 maize 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 Bt11 x MIR162 x MIR604 x GA21 maize in South Africa. This risk assessment is in support of the Syngenta SA Application for commodity clearance of Bt11 x MIR162 x MIR604 x GA21 maize 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:	Bt11 x MIR162 x MIR604 x GA21 maize is resistant to certain lepidopteran and coleopteran pests, and tolerant to herbicides containing glufosinate ammonium and herbicides containing glyphosate.
5. Unique identification of the living modified organism:	Unique identifier: SYN- BTØ11-1 x SYN-IR162-4 x SYN-IR6Ø4-5 x MON-ØØØ21-9
6. Transformation event:	Bt11 x MIR162 x MIR604 x GA21 maize
7. Introduced or Modified Traits:	A. Altered growth, development and product quality - Insect resistance and herbicide tolerance
8. Techniques used for modification:	Bt11 x MIR162 x MIR604 x GA21 maize was produced through conventional breeding of maize events Bt11, MIR162, MIR604 and GA21. Bt11 maize was transformed using a protoplast transformation /regeneration system.

[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.

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	MIR162 maize was produced by transformation of immature maize embryos derived from a proprietary *Zea mays* line *via Agrobacterium tumefaciens*-mediated transformation (Negrotto *et al.*, 2000; Hoekema *et al.*, 1983). Transformation of MIR604 maize 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.*, 1998).
9. Description of gene modification:	Bt11 x MIR162 x MIR604 x GA21 maize was produced by conventional breeding crosses of insect resistant Bt11, MIR162 and MIR604 maize, and herbicide tolerant GA21 maize. Bt11 x MIR162 x MIR604 x GA21 maize therefore contains the *cry1Ab* and *pat* genes from Bt11 maize, the *vip3Aa20* and *pmi* genes from MIR162 maize, the *mcry3A* and *pmi* genes from MIR604 and the *mepsps* gene from GA21 maize. The genes in Bt11 x MIR162 x MIR604 x GA21 maize are not different from the genes of the individual events Bt11, MIR162, MIR604 and GA21 maize.
10. Vector characteristics	Bt11 x MIR162 x MIR604 x GA21 maize was produced through conventional breeding. The *Not*I fragment of vector pZO1502 was used for the transformation of Bt11 maize. The vector used for the transformation of MIR162 maize was pNOV1300. The vector used for the transformation of MIR604 maize was pZM26. The *Not*I fragment of vector pDPG434 was used for the transformation of GA21 maize.
11. Insert or inserts (Annex III.9(d)):	Bt11 x MIR162 x MIR604 x GA21 maize expresses the traits present in Bt11, MIR162, MIR604 and GA21 maize plants through the production of: • a truncated Cry1Ab protein for control of certain lepidopteran pests • a phosphinothricin acetyl transferase (PAT) protein that confers tolerance to herbicide products containing glufosinate ammonium. • a Vip3Aa20 protein for control of certain lepidopteran pests of the family Noctuidae. • a phosphomannose isomerase (PMI) protein as a selectable marker. PMI allows transformed maize cells to utilize mannose as the only primary carbon source while maize cells lacking this protein fail to grow. • a modified Cry3A (mCry3A) protein for control of certain coleopteran pests. • a phosphomannose isomerase (MIR604 PMI) protein as a selectable marker. PMI allows transformed maize cells to utilize mannose as the only primary carbon source while maize cells lacking this protein fail to grow. • a modified maize 5-enolpyruvylshikimate-3-phosphate synthase

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	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-fertilization), 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, Jarosz *et al.*, 2005 and Devos *et al.*, 2008). 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. <u>1. Sexual compatibility with other cultivated or wild plant species, including the distribution in South Africa of the compatible species.</u> 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) and there are no wild relatives of maize in SA. <u>2. Survivability</u> (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.

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	(b) Specific factors affecting survivability, if any. Survival of maize is dependent upon temperature, seed moisture, genotype, husk protection and stage of development. Maize seed can only survive under a narrow range of climatic conditions. The biology of maize means that other than deliberate cultivation, the only means by which it can persist in the environment is accidental dispersal of kernels into disturbed ground during harvest or transport; maize cannot reproduce vegetatively (OECD, 2003). Maize kernels spilled in fields during harvest may germinate immediately and seedlings may be killed by frost (Miedema, 1982; OECD, 2003); however, maize can occur as a volunteer weed in areas with mild winters, or when seeds germinate in the spring (OECD, 2003). Volunteers are easily controlled with herbicides or other agronomic practices (Owen, 2005). Maize kernels spilled into disturbed ground outside agriculture can germinate to give occasional feral plants; however, even in areas with mild winters, persistent or invasive populations of feral maize are not observed in South Africa, presumably because of low seed dispersal and seedling survival due to retention of kernels on the ear (Doebley, 2004; Warwick and Stewart, 2005, OECD, 2003). <u>3. Dissemination:</u> (a) ways and extent (e.g. an estimation of how viable pollen and/or seeds declines with distance) of dissemination; Maize dissemination may be accomplished through seed dispersal. Seed dispersal does not occur naturally due to the structure of the ear (OECD, 2003). Maize has a large ear with 500 or more kernels attached to its central axis. The kernels are naked and easily digested (cannot survive through the digestive tracts of birds and mammals) and so cannot be dispersed by animals. As ears of maize do not shatter, any ears left on the plant fall to the ground with all the kernels attached; when the hundreds of seeds on the ear germinate, the emerging plants are unable to obtain adequate light and soil to grow and reproduce (Doebley, 2004). Dissemination may also occur via pollen and pollen flow. 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, Jarosz *et al.*, 2005 and Devos *et al.*, 2008). (b) specific factors affecting dissemination, if any. Maize has a polystichous (arranged in many rows) female inflorescence (group of flowers), 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). The rate of dissemination via pollen will be influenced by the size of pollen, wind direction and speed, other weather conditions such as

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rainfall, the presence of barriers and the degree of synchrony of flowering. Maize pollen is large and heavy and tends to be deposited close to the source plant. In addition, most maize varieties are protoandrous so pollen shedding precedes silk emergence by up to five days.

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. 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.

4. Geographical distribution of the plant.

Maize, which has very diverse morphological and physiological traits, is grown on approximately 157 million hectares worldwide[3]. It is distributed over a wide range of conditions: from 50° N Lat to 50° S Lat, below sea level of the Caspian plains up to 3000m 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.

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.

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). 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).

[3] FAO FAOSTAT Data updated June 2008. Downloaded January 2009. http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567 .

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	6. Wild plant species Wild *Zea* species have no pronounced weedy tendencies (CFIA, 2003). The only wild taxa known to hybridise spontaneously with maize are species of teosinte (OECD, 2003; Owen, 2005). 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). No *Tripsacum* species are present in South Africa. *Tripsacum* species are geographically restricted to the Americas (CFIA, 2003). Only two species are known to be 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). Refer to paragraph 15 for more information. Maize is incapable of sustained reproduction outside domestic cultivation and is non-invasive of natural habitats (OECD, 2003).
Donor organism or organisms (Annex III.9(b)):	
19. Taxonomic name/status of donor organism(s)	Bt11 x MIR162 x MIR604 x GA21 maize was produced through conventional breeding of maize events Bt11, MIR162, MIR604 and GA21. Bt11 maize: The donor organisms are *Bacillus thuringiensis* var. *kurstaki* strain HD-

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	1 (Btk) and *Streptomyces viridochromogenes* strain Tu494. MIR162 maize: The donor organisms are *Bacillus thuringiensis* strain AB88 and *Escherichia coli.* MIR604 maize: The donor organisms are *Bacillus thuringiensis* and *Escherichia coli* GA21 maize: The donor organism is *Zea mays* (maize)
20. Common name of donor organism(s):	Bt11 x MIR162 x MIR604 x GA21 maize was produced through conventional breeding of maize events Bt11, MIR162, MIR604 and GA21. Bt11, MIR162 and MIR604 maize: Bacteria or Micro-organisms GA21 maize: Maize/corn.
21. Point of collection or acquisition of donor organism(s):	Bt11 x MIR162 x MIR604 x GA21 maize was produced through conventional breeding of maize events Bt11, MIR162, MIR604 and GA21. Bt11, MIR162 and MIR604 maize: These bacteria are widely prevalent in the environment. GA21 maize: 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 x MIR162 x MIR604 x GA21 maize was produced through conventional breeding of maize events Bt11, MIR162, MIR604 and GA21. Bt11 maize: 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 shows broad spectrum toxicity to plants. The gene which encodes the enzyme has been designated *pat*. Glufosinate ammounium acts by inhibiting the plant enzyme glutamine synthetase, the only enzyme in plants that detoxifies ammonia by incorporating it into glutamine. Inhibition of this enzyme leads to an accumulation of ammonia in the plant tissues, leading to death of the plant. PAT catalyses the acetylation of the herbicide phosphinothricin and thus detoxifies glufosinate ammonium into an inactive compound.

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	<u>MIR162 maize:</u> *B. thuringiensis* and *E. coli* is widespread in the environment. <u>MIR604 maize:</u> *B. thuringiensis* and *E. coli* is widespread in the environment <u>GA21 maize:</u> The donor organism, maize (*Zea mays*), is widespread in the environment or human and animal diets. No significant native toxins are reported to be associated with the genus *Zea* (CFIA, 2003).
Intended use and receiving environment	
23. Intended use of the LMO (Annex III 9(g)):	Commodity clearance (i.e. full food, feed and processing approval)
24. Receiving environment (Annex III.9(h)):	Bt11 x MIR162 x MIR604 x GA21 maize will not be cultivated in SA.
Risk assessment summary	
25. Detection/Identification method of the LMO (Annex III.9(f)):	Bt11 x MIR162 x MIR604 x GA21 maize was produced through conventional breeding of maize events Bt11, MIR162, MIR604 and GA21. <u>Bt11 maize:</u> A quantitative event-specific method to detect and quantify Bt11 maize has been validated by the European Commission Joint Research Centre (JRC) and could be used for detection of Bt11 maize. <u>MIR162 maize:</u> A quantitative event-specific method to detect and quantify MIR162 maize has been submitted to the European Commission Joint Research Center (JRC) for validation. <u>MIR604 maize:</u> A quantitative event-specific method to detect and quantify MIR604 maize has been validated by the European Commission Joint Research Center (JRC) and could be used for detection of MIR604 maize. <u>GA21 maize:</u> A quantitative event-specific method to detect and quantify GA21 maize has been validated by the European Commission Joint Research Centre (JRC) and could be used for detection of GA21 maize. <u>Bt11 x MIR162 x MIR604 x GA21 maize:</u> The detection methods provided for Bt11, MIR162, MIR604 and GA21 maize will unambiguously detect the single events, but also the stacked product in a mixture of seed/grain by using single seed analysis and the detection methods for each of the single events. It is therefore possible to differentiate the stacked maize product from the single events in mixtures of Bt11, MIR162, MIR604, GA21 and Bt11 x MIR162 x MIR604 x GA21 maize with the detection methods described above.
26. Evaluation of the	Cultivation of maize derived from Bt11 x MIR162 x MIR604 x GA21

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likelihood of adverse effects (Annex III.8(b)):	maize in South Africa is not within the scope of Syngenta's Application for Commodity Clearance of Bt11 x MIR162 x MIR604 x GA21 maize. 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, Vip3Aa20, PMI, mCry3A, MIR604 PMI and mEPSPS proteins does not affect the agronomic characteristics or weediness potential of Bt11 x MIR162 x MIR604 x GA21 maize.
27. Evaluation of the consequences (Annex III.8(c)):	The probability of stacked maize Bt11 x MIR162 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 Bt11 x MIR162 x MIR604 x GA21 maize into South Africa can be considered negligible.
28. Overall risk (Annex III.8(d)):	The risk of potential adverse effects from importation of and use as food, feed or for processing of grain from stacked maize Bt11 x MIR162 x MIR604 x GA21 is negligible.
29. Recommendation (Annex III.8(e)):	Full compliance with permit conditions and other risk management conditions imposed by the Competent National Authority.
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.