20: RISK ASSESSMENT

Risk Assessment of stacked product Bt11 x GA21

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

Country Taking Decision:	South Africa
Title:	Risk Assessment of stacked product Bt11 x GA21 in South Africa. This risk assessment forms part of the Syngenta SA application for General Release of all seed and derived products from the genetically modified Bt11 x GA21 maize in South Africa. Bt11 maize has already conditional General Release approval in South Africa.
Contact details:	Name and Address and Contact details of the Importer[18] Commodity imports are performed by various grain traders on the international market, depending on the local need in South Africa. Name and Address and contact details of the Applicant[19] 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	
Name and identity of the living modified organism:	The genetically modified maize product Bt11 x GA21 is resistant to Lepidoptera insects and tolerant to normal applications of herbicide products containing glyphosate and glufosinate. The genetically modified maize event Bt11 is resistant to certain Lepidoptera insects and tolerant to normal applications of products containing glufosinate ammonium. Formulations of glufosinate ammonium are currently not approved for use on maize in South Africa, hence Bt11 x GA21 will not locally be marketed for this trait. The genetically modified maize event GA21 is tolerant to normal applications of products containing glyphosate

[18] An Importer is any natural or legal person by whom and on whose behalf a notification is made.

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

Unique identification of the living modified organism:	Bt11 x GA21: Unique identifier: SYN-BTØ11-1 x MON-ØØØ21-9 Bt11 : Unique identifier: SYN- BTØ11-1 GA21 Unique identifier: MON-ØØØ21-9
Transformation event:	Maize event Bt11 x GA21 produced from conventional breeding of the transformation events Bt11 and GA21 maize.
Introduced or Modified Traits:	Altered growth, development and product quality - Insect resistance and herbicide tolerance
Techniques used for modification:	Bt11 x GA21 Stacked maize product Bt11 x GA21 was produced through traditional breeding of Bt11 and GA21 maize. Bt11: Maize event Bt11 was transformed using a protoplast transformation /regeneration system (Negrutiu *et al.*1987) GA21: Maize event GA21 was produced through micro-projectile bombardment of maize suspension culture cells. This is described in the International Patent PCT/US98/06640 (pages 75-77; Spencer *et al.*, 1998). Using this method, the *Not*I restriction fragment of the pDPG434 plasmid DNA was introduced into maize cells. .
Description of gene modification:	Bt11 x GA21 Stacked maize product Bt11 x GA21 was produced through traditional breeding of Bt11 and GA21. Bt11 Maize event Bt11 was genetically modified using a protoplast transformation/regeneration system, to expresses a truncated Cry1Ab protein for control of certain lepidopteran pests and a phosphinothricin acetyl transferase (PAT) protein that confers tolerance to herbicide products containing glufosinate ammonium. GA21 Maize event GA21 was genetically modified to express a modified maize *mepsps* gene, coding for the 5-enolpyruvylshikimate-3-phosphate synthase enzyme (mEPSPS) that confers tolerance to herbicide products containing glyphosate
Vector characteristics	Bt11 x GA21: Stacked maize product Bt11 x GA21 was produced through traditional breeding of Bt11 and GA21 Bt11: The *Not*I restriction fragment of vector pZO1502 was used for the transformation of maize event Bt11. Vector pZO1502 is a derivative of the plasmid pUC18, which is commercially available. GA21: The *Not*I fragment of vector pDPG434 was used for the transformation (Spencer *et al.*, 1998). The vector is derived from a pSK-vector, which is commonly used in molecular biology and is derived from pUC19 (Short *et al.*, 1988).

Insert or inserts (Annex III.9(d)):	Bt11 x GA21 Stacked maize product Bt11 x GA21 was produced through traditional breeding of Bt11 and GA21 and contains the following gene: *cry1Ab*, *pat* and *mepsps*. Bt11 The *cry1Ab* gene from *Bacillus thuringiensis* for control of certain lepidoptera pests. The *pat* gene encoding a phosphinothricin acetyl transferase, which imparts tolerance to herbicides containing glufosinate ammonium. Formulations of glufosinate ammonium are not currently approved for use on maize in South Africa, hence it is not our immediate intention to market Bt11 x GA21 maize locally for this trait. Bt11 x GA21 will only locally be marketed for this trait after chemical approval of the herbicide for use on maize under South African Act 36. GA21; The modified maize 5-enolpyruvylshikimate-3-phosphate synthase (*mepsps)* gene confers tolerance to herbicide products containing glyphosate.
Recipient organism or parental organisms (Annex III.9(a)):	
Taxonomic name/status of recipient organism or parental organisms:	Family name: Poaceae Genus: *Zea* Species: *Zea mays* L. Subspecies: *mays*
Common name of recipient organism or parental organisms:	Maize/corn.
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).
Characteristics of recipient organism or parental organisms related to biosafety:	*Zea mays* reproduces 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 and Jarosz *et al.*, 2005). 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) and there are no wild relatives of maize in SA. 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 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). 3. Dissemination: (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). (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 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. <u>4. Geographical distribution of the plant.</u> Maize is the world's most widespread cereal and is grown on in the region of 156 million hectares worldwide[20] with a yield

[20] http://www.fas.usda.gov/wap/circular/2009/09-05/productionfull05-09.pdf (assessed March 2010)

	recorded in March 2010 for 2009/2010 season at 1,100,179 thousand metric tons[21]. 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. 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).

[21] https://www.fas.usda.gov/grain/circular/2010/03-10/graintoc.asp (assessed March 2010)

	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.
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).
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).
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. 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)):	
Taxonomic name/status of donor organism(s)	Bt11: *Bacillus thuringiensis* var. *kurstaki* strain HD-1 (Btk) and *Streptomyces viridochromogenes* strain Tu494. GA21: Family name: Poaceae Genus: *Zea* Species: *Zea mays* L. Subspecies: *mays*
Common name of donor organism(s):	Bt11: Bacteria or Micro-organisms: *Bacillus thuringiensis* and *Streptomyces viridochromogenes*. GA21: Maize/corn.
Point of collection or acquisition of donor organism(s):	Bt11: *Bacillus thuringiensis* and *Streptomyces viridochromogenes* bacteria are widely prevalent in the environment. GA21: Maize originates from the Mesoamerican region, i.e. Mexico and Central America region (CFIA, 2003).

Characteristics of donor organism(s) related to biosafety:	Bt11: *B.thuringiensis* and *S.viridochromogenes* are widespread in the environment. GA21: 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	
Intended use of the LMO (Annex III 9(g)):	General Release of Bt11 x GA21 maize in South Africa. Bt11 maize has conditional General Release approval in SA since 2003. Bt11 and GA21 maize have Commodity Clearance approval in SA (2002) and can be used as food, feed or processing
Receiving environment (Annex III.9(h)):	The environment into which the Bt11 x GA21 maize plants will be introduced is a typically agricultural environment in the country. Bt11 maize has conditional General Release approval in SA since 2003.
Risk assessment summary	
Detection/Identification method of the LMO (Annex III.9(f)):	Stacked maize product Bt11 x GA21 was produced through traditional breeding of maize events Bt11 and GA21. Quantitative event-specific detection methods to detect and quantify maize event Bt11 and GA21 have been validated by the European Commission Directorate General - Joint Research Centre Community Reference Laboratory (DG-JRC CRL) and can be found on the DG-JRC CRL website. http://gmo-crl.jrc.ec.europa.eu/summaries/Bt11_CRLVL1007_Validated_Method%20doc.pdf and http://gmo-crl.jrc.ec.europa.eu/summaries/Bt11_CRLVL1007_Val_Report.pdf. http://gmo-crl.jrc.ec.europa.eu/summaries/GA21Syng_validated_Method.pdf and http://gmo-crl.jrc.ec.europa.eu/summaries/GA21Syng_val_report.pdf Detection of the single events could also confirm presence of the stacked product. The use of the Bt11 and GA21 event-specific detection methods have been evaluated and verified for use on Bt11 x GA21 by the DG-JRC CRL. http://gmo-crl.jrc.ec.europa.eu/summaries/Bt11xGA21_val_report.pdf
Evaluation of the likelihood of adverse effects (Annex III.8(b)):	Maize is planted and harvested as an annual crop. Wild populations with which it could cross-pollinate are uncommon, and not prevalent in South Africa. 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.

	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). Because of the structure of the maize 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). 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). Bt11 maize has a history of safe use in South Africa and has been cultivated in South Africa since its approval in 2003 A substantial weight of evidence indicates there are no harmful unintended changes to Bt11 x GA21 maize as a result of the transformation and production of Cry1Ab, PAT and mEPSPS proteins. This has taken into account the absence of known allergenicity of the source organisms; the history of safe use of maize; no significant amino acid homology to known or putative allergenic protein sequences that are biologically relevant or have implications for allergenic potential; and, readily digestion under typical mammalian gastric conditions in *in vitro* digestibility studies. Taking into consideration the level of expression of the Cry1Ab, PAT and mEPSPS proteins, a dietary exposure assessment for Bt11 x GA21 maize in South Africa was conducted taking a worst case assumption that 100% of the maize consumed will be Bt11 x GA21 maize. Based on an average maize consumption of 248.1 g/person/day[22], the theoretical daily intake for Cry1Ab, PAT and mEPSPS proteins produced by Bt11 x GA21 maize was calculated, based on a bodyweight of 60kg. In addition, margins of exposure have been calculated by comparing the no-observed-

[22] WHO cluster diet in South Africa, http://www.who.int/foodsafety/chem/gems/en/index1.html

	effect-level (NOEL) from the acute oral toxicity study of Cry1Ab, PAT and mEPSPS proteins to the expected intake level. Expected levels of intake of Cry1Ab, PAT and mEPSPS through consumption of Bt11 x GA21 maize in South Africa will be very low. Margins of exposure exceed 36 000 for the proteins, supporting the conclusion that no unacceptable risk is posed to consumers Studies comparing the composition and whole food safety of Bt11 x GA21 maize plants and non-transgenic maize lead to the conclusion that this maize is substantially equivalent to conventional maize. Bt11 x GA21 maize is highly unlikely to have adverse effects on human health or the environment; that is to say, the effects of Bt11 x GA21 maize on human health or the environment are unlikely to be different from those of non-transgenic maize. Bt11 x GA21 maize is highly unlikely to have environmental effects through interactions with non-target organisms. In the event that kernels of maize event Bt11 x GA21 maize 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 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.
Evaluation of the consequences (Annex III.8(c)):	Agronomic performance and the reproductive and vegetative fitness described above as part of the risk assessment summary, shows no selective advantage to the transgenic plants. Tolerance to glyphosate herbicides and resistance to lepidoptera insects are the specific and the intended effect of the modification. Bt11 maize has a history of safe use in South Africa and has been cultivated in South Africa since its approval in 2003 No specific risk management measures would be required for General Release of Bt11 x GA21 maize in SA. Bt11 x GA21 maize expresses Cry1Ab, PAT and mEPSPS proteins. The intended effect of production of Bt11 x GA21 maize is to enhance the resistance of maize to certain lepidoptera insect pests (s.a. *Busseola fusca* and *Chilo partellus)* and tolerance to herbicides containing glyphosate. Persistence and invasiveness of maize is limited by dispersal and seedling survival, not by sensitivity to glyphosate; therefore, presence of the Cry1Ab, PAT and mEPSPS proteins in Bt11 x GA21 maize is likely to have no effect on its persistence or invasiveness. Bt11 x GA21 maize is unlikely to become more persistent, weedy or invasive than maize varieties currently cultivated in South Africa. No biologically significant unintended changes were observed in Bt11 x GA21 maize when compared with non-transgenic, near-isogenic lines in agronomic field trials. Agronomic trials indicate no biologically significant unintended changes in seed dispersal or other traits that might affect the ability of maize to survive without human intervention

Overall risk (Annex III.8(d)):	Bt11 maize has conditional General Release approval and GA21 maize has Commodity Clearance approval in SA. The overall risk for potential adverse effects on human and animal health or the environment is negligible in the context of the intended uses of Bt11 x GA21 maize relative to: Persistence and invasiveness Selective advantage or disadvantage Potential for gene transfer Interactions between the GM plant and target organisms Interactions of the GM plant with non-target organisms Effects on human health Effects on animal health Effects on biogeochemical processes Impacts of the specific cultivation, management and harvesting techniques Potential interactions with the abiotic environment.
Recommendation (Annex III.8(e)):	Full compliance with permit condition and other risk management conditions imposed by the Competent National Authority.
Actions to address uncertainty regarding the level of risk (Annex III.8(f)):	Not applicable.
Additional information	
Availability of detailed risk assessment information:	More information on the stacked product and the assessment of risk can be obtained from the application.
Any other relevant information:	No
Attach document:	Not applicable
Notes:	Not applicable.

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