RISK ASSESSMENT OF STACKED PRODUCT Bt11 x MIR162 x MIR604 x 1507 x 5307 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 MIR162 x MIR604 x 1507 x 5307 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 1507 x 5307 x GA21 maize in South Africa.
Contact details:	**Name and Address and Contact details of the Importer**[2] 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**[3] 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 combined maize trait product, Bt11 x MIR162 x MIR604 x TC1507 x 5307 x GA21 maize (hereafter referred to as 'Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21 maize') is a stacked genetically modified (GM) product that has been produced by conventional breeding crosses of: • Event Bt11 maize (Bt11 maize) which produces a truncated Cry1Ab protein for control of certain lepidopteran pests and a phosphinothricin acetyltransferase (PAT) protein that confers tolerance to herbicide products containing glufosinate ammonium. • Event MIR162 maize (MIR162 maize) is a GM product which expresses a Vip3Aa20 protein for control of certain lepidopteran pests and a phosphomannose isomerase (PMI) protein, which acts as a selectable marker enabling transformed plant cells to utilize mannose as the only primary carbon source. • Event MIR604 maize (MIR604 maize) is a GM product which expresses a modified Cry3A (mCry3A) protein for control of certain coleopteran pests and a phosphomannose isomerase (MIR604 PMI) protein, which acts as a selectable marker enabling transformed plant cells to utilize mannose as a primary carbon source.

[2] An Importer is any natural or legal person by whom and on whose behalf a notification is made.
[3] 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.

	• Event TC1507 maize[4] (hereafter referred to as 1507 maize) expressing the Cry1F protein which confers protection against certain lepidopteran pests and a phosphinothricin acetyltransferase (PAT) protein that confers tolerance to herbicide products containing glufosinate ammonium. • Event 5307 maize (5307 maize) has been genetically modified to produce the proteins eCry3.1Ab for control of certain coleopteran pests and PMI which acts as a selectable marker enabling transformed plant cells to utilize mannose as the only primary carbon source. • Event GA21 maize (GA21 maize) which produces a modified maize 5-enolpyruvylshikimate-3-phosphate synthase enzyme (mEPSPS) that confers tolerance to herbicide products containing glyphosate.
Unique identification of the living modified organism:	Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21: SYN-BTØ11-1 x SYN-IR162-4 x SYN-IR6Ø4-5 x DAS-Ø15Ø7-1 x SYN-Ø53Ø7-1 x MON-ØØØ21-9 Bt11 : SYN- BTØ11-1 MIR162: SYN-IR162-4 MIR604: SYN-IR6Ø4-5 1507: DAS-Ø15Ø7-1 5307: SYN-Ø53Ø7-1 GA21: MON-ØØØ21-9
Transformation event:	The Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21 maize is a GM maize that is produced by conventional breeding crosses using different combinations of the GM maize events: Bt11, MIR162, MIR604, 1507, 5307 and GA21 maize.
Introduced or Modified Traits:	Insect resistance and herbicide tolerance
Techniques used for modification:	Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21: Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21 was produced by combining Bt11, MIR162, MIR604, 1507, 5307 and GA21 maize through conventional breeding. Bt11: Bt11 maize was transformed using a protoplast transformation / regeneration system (Negrutiu *et al.*1987)

[4] Event TC1507 may be referred to as maize line 1507 in South Africa. It is normally referred to as Event TC1507 in applications submitted by Dow AgroSciences LLC to most countries other than South Africa.

	MIR162: 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). MIR604: MIR604 maize was transformed using immature maize embryos derived from a proprietary *Z. mays* line, *via A. tumefaciens* - mediated transformation using the method described by Negrotto *et al.* (2000). 1507: 1507 maize was produced by insertion of a DNA fragment into the maize genome using particle acceleration technology on embryogenic maize cells. 5307: 5307 maize was produced by transformation of immature maize embryos derived from a proprietary *Z. mays* line *via A. tumefaciens*-mediated transformation (Negrotto *et al.*, 2000). GA21: GA21 maize 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:	Stacked maize product Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21 was produced by combining Bt11, MIR162, MIR604, 1507, 5307 and GA21 maize through conventional breeding. Therefore these maize plants express the traits present in Bt11, MIR162, MIR604, 1507, 5307 and GA21 maize plants through the production of: • a truncated Cry1Ab protein for control of certain lepidopteran pests like the common maize pests: *Ostrinia nubilalis* (European corn borer; ECB), *Sesamia nonagrioides* (Mediterranean corn borer; MCB), *Sesamia calamintis* (Pink stemborer; PSB), *Busseola fusca* (Fuller) (African stemborer; ASB) and *Chilo partellus* (Swinhoe) (Spotted stemborer; SSB) • a PAT protein that confers tolerance to herbicide products containing glufosinate ammonium. • a Vip3Aa protein (designated Vip3Aa20) for control of certain lepidopteran pests like *Heliothis zea* (corn earworm), *Agrotis ipsilon* (black cutworm; BCW), *Spodoptera frugiperda* (fall armyworm; FAW), and *Striacosta albicosta* (western bean cutworm). • a PMI protein, that acts as a selectable marker trait enabling transformed plant cells to utilize mannose as the only primary carbon source. • 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 maize cells to utilize mannose as the only primary carbon source while maize cells lacking this protein fail to grow. • an engineered *ecry3.1Ab* gene encoding the eCry3.1Ab protein for control of certain coleopteran pests like *Diabrotica virgifera virgifera* (WCRW) and related *Diabrotica* species. • a Cry1F protein for control of certain lepidopteran pests such as *Ostrinia nubilalis* (ECB) and *Sesamia nonagrioides* (MCB). It is also highly effective against *S. frugiperda* (FAW), *A. ipsilon* (BCW) and *Diatraea grandiosella* (Southwestern corn borer). • a modified mEPSPS enzyme that confers tolerance to herbicide products containing glyphosate.
Vector characteristics	Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21: No vector was used for the production of Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21 maize. Stacked maize product Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21 was produced by combining Bt11, MIR162, MIR604, 1507, 5307 and GA21 maize through conventional breeding. 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. MIR162: The plasmid pNOV1300 was used for transformation of MIR162 maize. MIR604: The vector used for the transformation was pZM26 1507: The PHI8999A fragment of vector PHP8999 was used for the transformation of 1507 maize. 5307: Plasmid pSYN12274 was used to generate 5307 maize 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 MIR162 x MIR604 x 1507 x 5307 x GA21 The Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21 maize is a GM maize

	that is produced by conventional breeding crosses using different combinations of the GM maize events: Bt11, MIR162, MIR604, 1507, 5307 and GA21 maize. No further genetic modification to produce this stack has taken place. Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21 maize therefore contains the *cry1Ab* and *pat* genes from Bt11 maize, the v*ip3Aa20* and *pmi* genes from MIR162 maize, the *cry3A (mcry3A)* and *pmi* genes from MIR604 maize, the *cry1F* and *pat* genes from 1507 maize, the *ecry3.1Ab* and *pmi* genes from 5307 and the *mepsps* gene from GA21 maize. The foreign genes in Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21 maize are not different from the foreign genes of the individual events Bt11, MIR162, MIR604, 1507, 5307 and GA21 maize. <u>Bt11</u> The *Not*I restriction fragment of vector pZO1502, a derivative of plasmid pUC18, was used for the transformation. The *Not*I fragment contains a truncated Bt gene which has been derived from the *cry1Ab* gene of *Bacillus thuringiensis (B. thuringiensis)*; this gene is under the control of the 35S promoter from the cauliflower mosaic virus (CaMV), including the intron sequence IVS6 from maize, and the nopaline synthase (NOS) terminator from *Agrobacterium tumefaciens (A. tumefaciens)*. It contains the *pat* gene from *Streptomyces viridochromogenes (S. viridochromogenes)* encoding a phosphinothricin acetyl transferase; this gene is under the control of the 35S promoter from the CaMV, including the intron IVS2 from maize and the NOS terminator from *A. tumefaciens*. It also contains the *Escherichia coli (E. coli)* origin of replication. The *Not*I fragment does not contain the *E. coli amp* gene present on pZO1502 which confers resistance of bacterial cells to ampicillin. <u>MIR162</u> The plasmid pNOV1300 was used for transformation. Replication of pNOV1300 in LBA4404 is made possible via homologous recombination with an "acceptor vector" pSB1 (Komari *et al.*, 1996). The region intended for insertion contains the *vip3Aa19* gene[5], a modified version of the native *vip3Aa1* gene from *B. thuringiensis*; this gene is under the control of the maize polyubiquitin promoter, the intron #9 from the maize phosphoenolpyruvate carboxylase gene and the 35S terminator from the cauliflower mosaic virus (CaMV). It also contains the *pmi* gene from *E. coli* encoding a phosphomannose isomerase; this gene is under the control of the maize polyubiquitin promoter and the nopaline synthase (NOS) terminator from *A. tumefaciens*. <u>MIR604</u> MIR604 maize was produced by transformation of immature maize embryos derived from a proprietary *Z. mays* line *via A. tumefaciens*-mediated transformation. The plasmid pZM26 was used for transformation. The region

[5] The gene conferring protection against lepidopteran insect pests present on the plasmid pNOV1300 is *vip3Aa19*. The gene inserted in MIR162 maize differs from the *vip3Aa19* gene by two nucleotides. These transformation-induced nucleotide changes in the *vip3Aa19* coding sequence resulted in one single amino acid change in the encoded protein. Therefore the gene expressed in MIR162 maize was designated *vip3Aa20* and the encoded protein Vip3Aa20.

	intended for insertion contains a modified *cry3A (mcry3A)* gene from *B. thuringiensis*; this gene is under the control of the maize MTL promoter and the NOS terminator from *A. tumefaciens*. It also contains the *pmi* gene from *E.coli* encoding a phosphomannose isomerase; this gene is under the control of the maize polyubiquitin promoter and the NOS terminator from *A. tumefaciens*. 1507 The PHI8999A fragment of vector PHP8999 was used for the transformation. The region intended for insertion (PHI8999A) contains the *cry1F* gene from *B. thuringiensis* var. *aizawai*; this gene is under the control of the ubiquitin promoter from maize (Ubi1ZM), and a terminator sequence from *A. tumefaciens* (ORF25PolyA). It also contains the *pat* gene from *S. viridochromogenes* encoding a phosphinothricin acetyl transferase; this gene is under the control of the 35S promoter and terminator from the CaMV. The PHI8999A fragment does not contain the *nptII* gene present on PHP8999 which confers resistance of bacterial cells to kanamycin. 5307 Plasmid pSYN12274, a vector used for *Agrobacterium* mediated plant transformation, was used to generate 5307 maize. The region intended for insertion contains the engineered *ecry3.1Ab* gene, based on the *cry1Ab* and the *mcry3A* genes derived from B. *thuringiensis*; the *ecry3.1Ab* gene is under the control of the CMP promoter from Cestrum Yellow Leaf Curling Virus and the NOS terminator from *A. tumefaciens*. It also contains the *pmi* gene from *E. coli* encoding a phosphomannose isomerase; this gene is under the control of the maize polyubiquitin promoter and the NOS terminator from *A. tumefaciens*. GA21 The *Not*I restriction fragment of vector pDPG434 was used for the transformation. The vector pDPG434 is derived from a pSK- vector, which is derived from pUC19 (Short *et al.*, 1988). The *Not*I fragment contains the modified 5-enolpyruvylshikimate-3-phosphate synthase (*mepsps*) gene from maize; this gene is under the control of the rice actin promoter (containing the first intron and exon), the optimised chloroplast transit peptide (CTP) based on CTP sequences from sunflower and maize and the nopaline synthase (NOS) terminator from *A. tumefaciens*. The *Not*I fragment does not contain the origin of replication, the *bla* (*amp*) gene or the partial *lacZ* sequence.
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, 1994).
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, 1994) 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.

4. Geographical distribution of the plant.

Maize is the world's most widespread cereal and is grown on approximately 158 million hectares worldwide[6] with a yield estimation for 2010/11 season of

[6] www.fas.usda.gov/wap/circular/2010/10-10/productionfull10-10.pdf (accessed October 2010)

	809 million tons[7]. 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, 1994). 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, 1994). 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, 1994). 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, 1994). 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, 1994). *Tripsacum*-annual teosinte hybrids have not been produced.
Centre(s) of origin of recipient organism or	Maize originates from the Mesoamerican region, i.e. Mexico and Central America region (CFIA, 1994).

[7] http://www.isaaa.org/resources/publications/pocketk/16/default.asp (accessed April 2011)

parental organisms:	
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, 1994).
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, 1994). Please refer to information provided above regarding geographical distribution of the maize plant. 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)	<u>*Bacillus thuringiensis*</u> The source of native *cry1Ab, vip3Aa1, mcry3A* and *cry1F* genes is *Bacillus thuringiensis* (Bt). The species is a member of the genus *Bacillus*, a diverse group of rod-shaped, gram-positive, facultative anaerobic, spore forming bacteria. Bt occurs naturally and ubiquitously in the environment. It is a common component of the soil microflora and has been isolated from most terrestrial habitats (Glare and O'Callaghan, 2000). Several subspecies of Bt have been described; many of them have been extensively studied and used in commercial insecticide preparations. Insecticidal products using Bt have been used for several decades and have a long history of safe use (US EPA, 2001). <u>*Escherichia coli*</u> The source of the *pmi* gene is the common bacterium *E. coli,* K-12 strain. *E. coli* belongs to the Enterobacteriaceae, a relatively homogeneous group of rod-shaped, gram-negative, facultative bacteria. Members of the genus *Escherichia* are ubiquitous in the environment and found in the digestive tract of vertebrates, including humans. The vast majority of *E. coli* strains are harmless to humans, although some strains can cause diarrhoea and urinary infections. However, this particular group of pathogenic *E. coli* are distinct from the strains that are routinely used in the laboratory and from which the *pmi* gene was obtained. The K-12 strain from *E. coli* has a long history of safe use and is commonly used as a protein production system in many commercial applications. <u>*Streptomyces viridochromogenes*</u> The source of the *pat* gene is the aerobic bacterium *S. viridochromogenes* strain Tu494, a gram-positive, sporulating, soil inhabiting bacterium widespread in the environment and with a long history of safe use (OECD, 1999). <u>*Zea mays*</u> The source of the *epsps* gene is maize (*Zea mays*). Maize is the world's most widespread cereal. It is grown between latitudes 50° north and 50° south and from below sea level to altitudes of more than 3000m. Maize is an important crop in the world with an estimated production of 809 million tons for the

	2010/11 season[8]. Maize is believed to have been domesticated about 6000 to 10000 years ago from teosinte (Doebley, 2004). Maize is now a main commodity crop used for food and feed with a long history of safe use (OECD, 2003). *B. thuringiensis*, *E. coli* and *S. viridochromogenes* are bacteria and therefore not related to the recipient organism maize. The donor organism for the *mepsps* gene is maize, the same organism as the recipient.
Common name of donor organism(s):	Bacteria or Micro-organisms: *B. thuringiensis, S. viridochromogenes, E. coli* Maize/corn: *Z. mays*
Point of collection or acquisition of donor organism(s):	*B. thuringiensis*, *E. coli* and *S. viridochromogenes* bacteria are widely prevalent in the environment. Maize originates from the Mesoamerican region, i.e. Mexico and Central America region (CFIA, 1994).
Characteristics of donor organism(s) related to biosafety:	*B. thuringiensis, S. viridochromogenes* and *E. coli* are widespread in the environment. The donor organism, maize (*Z. 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, 1994).
Intended use and receiving environment	
Intended use of the LMO (Annex III 9(g)):	Commodity clearance (i.e. full food, feed and processing approval) of Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21 maize in South Africa.
Receiving environment (Annex III.9(h)):	The intended use of Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21 maize does not currently include cultivation in South Africa.
Risk assessment summary	
Detection/Identification method of the LMO (Annex III.9(f)):	Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21 maize is a GM maize that is produced by conventional breeding crosses using different combinations of the GM maize events: Bt11, MIR162, MIR604, 1507, 5307 and GA21 maize. No further genetic modification to produce this stack has taken place. The detection methods developed for the single events will detect also the individual events in Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21 maize. For specific detection of Bt11 maize genomic DNA, a real-time quantitative TaqMan® PCR method has been developed. This method has been validated for use by the DG-JRC-EURL and can be found on the DG-JRC-EURL 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

[8] http://www.isaaa.org/resources/publications/briefs/42/executivesummary/default.asp (accessed April 2011)

	For specific detection of MIR162 maize genomic DNA, a real-time quantitative TaqMan® PCR method has been developed. One of the oligonucleotide primers is located within the maize specific flanking sequence and the other is located in the insert. This method has been validated for use by the DG-JRC-EURL and can be found on the DG-JRC-EURL website http://gmo-crl.jrc.ec.europa.eu/summaries/MIR162_validated_Method.pdf and http://gmo-crl.jrc.ec.europa.eu/summaries/MIR162_val_report.pdf For specific detection of MIR604 maize genomic DNA, a real-time quantitative TaqMan® PCR method has been developed by Syngenta. One of the oligonucleotide primers is located within the maize specific flanking sequence and the other is located in the insert. This method has been validated for use by the DG-JRC-EURL and can be found on the DG-JRC EURL website http://gmo-crl.jrc.ec.europa.eu/gmomethods/entry.do?db=gmometh&query=ac%3ASYN-IR604-5&id=qt_zm_013 For specific detection of 1507 maize genomic DNA, a real-time quantitative TaqMan® PCR method has been developed. This method has been validated for use by the DG-JRC-EURL and can be found on the DG-JRC-EURL website http://gmo-crl.jrc.ec.europa.eu/summaries/TC1507-WEB-Protocol-Validation.pdf and http://gmo-crl.jrc.ec.europa.eu/summaries/TC1507-report_mm.pdf For specific detection of 5307 maize genomic DNA, a real-time quantitative TaqMan® PCR method has been developed by Syngenta. One of the oligonucleotide primers is located within the maize specific flanking sequence and the other is located in the insert. This detection method has been submitted for validation to the DG JRC-EURL as part of the application EFSA-GMO-DE-2011-95. For specific detection of GA21 maize genomic DNA, a real-time quantitative TaqMan® PCR method has been developed. This method has been validated for use by the DG-JRC-EURL and can be found on the DG-JRC-EURL website http://gmo-crl.jrc.ec.europa.eu/summaries/GA21Syngenta_validated_Method_correctedVersion1.pdf http://gmo-crl.jrc.ec.europa.eu/summaries/GA21Syng_val_report_correctedVersion1.pdf
Evaluation of the likelihood of adverse effects (Annex III.8(b)):	Cultivation of maize derived from Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21 maize in South Africa is not within the scope of Syngenta's Application for Commodity Clearance of Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21 maize. There are no wild relatives of maize 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 humid conditions. 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). Thus, even in the rare event that small amounts of maize kernels of the stacked product could accidentally find their way into the environment during importation of this product by grain traders, their survival would be very unlikely as maize is highly domesticated and cannot survive without human intervention (Niebur, 1993; Owen, 2005). In addition, 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. Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21 maize is unlikely to become more persistent, weedy or invasive than maize varieties currently cultivated in South Africa, as the expression of the Cry1Ab, PAT, Vip3Aa20, PMI, mCry3A, MIR604 PMI, eCry3.1Ab, Cry1F and mEPSPS proteins does not affect the overall agronomic characteristics or weediness potential. Maize 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, 1994) and maize is not considered as a major allergenic food (EFSA, 2007; Metcalfe *et al.*, 2003). The Cry1Ab, PAT, Vip3Aa20, PMI, mCry3A, MIR604 PMI, eCry3.1Ab, Cry1F and mEPSPS proteins expressed in Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21 maize are not derived from a source known to produce allergenic proteins; have no significant amino acid homology to known mammalian protein toxins or to known or putative allergenic protein sequences that are biologically relevant or have implications for allergenic potential; they are readily degraded in *in vitro* digestibility assays; and, they show no acute oral toxicity in mammalian studies. There is no reason to anticipate that conventional breeding of Bt11, MIR162, MIR604, 1507, 5307 and GA21 maize would result in stacked products that differ in toxicity to humans or animals. None of the proteins expressed by Bt11, MIR162, MIR604, 1507, 5307 and GA21 maize are known to be toxic to humans or animals and there are no known precedents where interactions between non-toxic proteins lead to toxic effects (FIFRA SAP, 2004). In addition, compositional analysis and broiler feeding studies, have confirmed that the Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21 maize is equivalent in composition to conventional maize and is as safe and nutritious as conventional maize. A dietary exposure was assessed taking a worst case assumption that 100% of

	the maize consumed in South Africa is Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21 maize. Taking into consideration the level of expression of the Cry1Ab, PAT, Vip3Aa20, PMI, mCry3A, MIR604 PMI, eCry3.1Ab, Cry1F and mEPSPS proteins, based on an average maize consumption of 248.1 g/person/day[9], the theoretical daily intake for protein produced by Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21 maize was calculated, based on a bodyweight of 60kg. In addition, margins of exposure have been calculated by comparing the no-observed-effect-level (NOEL) from the acute oral toxicity study with each protein to the expected intake level. The results indicate that the expected levels of intake of the proteins Cry1Ab, PAT, Vip3Aa20, PMI, mCry3A, MIR604 PMI, eCry3.1Ab, Cry1F and mEPSPS through consumption of Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21 maize in South Africa will be very low. Margins of exposure exceed a factor of at least 5720, supporting the conclusion that the risk to consumers is negligible and confirming the results previously obtained. Studies comparing the composition and whole food safety of Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21 maize plants and non-transgenic maize lead to the conclusion that this maize is substantially equivalent to conventional maize. Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21 maize is highly unlikely to have adverse effects on human or animal; that is to say, the effects of Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21 maize on human or animal health are unlikely to be different from those of non-transgenic maize. 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). However, the importation and use as food, feed or for processing of grain from stacked Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21 maize is highly unlikely to have environmental effects through interactions with non-target organisms.
Evaluation of the consequences (Annex III.8(c)):	Cultivation of maize derived from Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21 maize in South Africa is not within the scope of Syngenta's Application for Commodity Clearance of Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21 maize. The importation and use as food, feed or for processing of grain from stacked Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21 maize is not expected to have any adverse consequences on human or animal health or the South African environment. As discussed above, the conclusion obtained from the detailed evaluation of the characteristics of Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21 maize and the analysis of the likelihood of any adverse effects is that this maize is substantially equivalent to conventional maize and that it is highly unlikely to have any adverse effects on human or animal health or the South African

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

	environment. Therefore, no adverse consequences will result from the importation and use as food, feed or for processing of grain from stacked Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21 maize in South Africa.
Overall risk (Annex III.8(d)):	The overall 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 1507 x 5307 x GA21 is negligible. None of the components introduced into Bt11, MIR162, MIR604, 1507, 5307 and GA21 maize are considered to be dangerous to human health or the environment. There is no reason to anticipate that conventional breeding of Bt11, MIR162, MIR604, 1507, 5307 and GA21 maize would result in stacked products that differ in toxicity to humans or animals. None of the proteins expressed by Bt11, MIR162, MIR604, 1507, 5307 and GA21 maize are known to be toxic to humans or animals and there are no known precedents where interactions between non-toxic proteins lead to toxic effects (FIFRA SAP, 2004). In addition, compositional analysis and broiler feeding studies, have confirmed that the Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21 maize is equivalent in composition to conventional maize and is as safe and nutritious as conventional maize. The overall risk for potential adverse effects on human and animal health or the environment as discussed in this document, is thus negligible in the context of the intended uses of Bt11 x MIR162 x MIR604 x 1507 x 5307 x GA21 maize.
Recommendation (Annex III.8(e)):	Full compliance with permit conditions and other risk management conditions imposed by the South African Authorities.
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