Part II

13 RISK ASSESSMENT

Risk assessment: Bt11 × MIR162 × MON 89034 × GA21 maize in accordance with Annex III of Cartagena Protocol on Biosafety	
Country Taking Decision:	South Africa
Title:	Risk Assessment of the stacked-event Bt11 × MIR162 × MON 89034 × GA21 maize product in SA. This risk assessment is in support of the Syngenta SA (Pty) Ltd. application for General Release.
Contact details:	**Name and Address and contact details of the Applicant** Syngenta SA (Pty) Ltd. Westend Office Park Building C 254 Hall street Centurion South Africa Tel: +27 11 541 4000 Fax: +27 11 541 4072
LMO information	
Name and identity of the living modified organism:	Bt11 × MIR162 × MON 89034 × GA21 maize is a variety developed by Syngenta using conventional breeding techniques. Bt11, MIR162, MON 89034 and GA21 maize were used to produce the breeding stack Bt11 × MIR162 × MON 89034 × GA21 maize. No further genetic modification to produce this stack has taken place. • Bt11 × MIR162 × MON 89034 × GA21 maize stably inherited the *cry1Ab, pat, vip3Aa20, pmi, cry1A.105, cry2Ab2,* and *mepsps* genes, retaining the hybridization patterns as predicted. • Maize plants derived from Bt11 maize contain the transgene *cry1Ab*, which encodes the insecticidal protein Cry1Ab, and the transgene *pat*, which encodes the enzyme PAT. • Maize plants derived from MIR162 maize contain the transgene *vip3Aa20*, and the transgene *pmi*. • Maize plants derived from MON 89034 maize produce the Cry1A.105 and Cry2Ab2 proteins. • Maize plants derived from GA21 maize contain the transgene *mepsps*, which encodes the enzyme mEPSPS.
Unique identification of the living modified organism:	SYN-BTØ11-1 x SYN-IR162-4 x MON-89Ø34-3 x MON-ØØØ21-9
Transformation event:	Stacked maize event Bt11 × MIR162 × MON 89034 × GA21

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Introduced or Modified Traits:	**Altered growth, development and product quality:** Insect control and herbicide tolerance
Techniques used for modification:	Bt11 × MIR162 × MON 89034 × GA21 maize is a variety developed by Syngenta using conventional breeding techniques. Bt11, MIR162, MON 89034 and GA21 maize were used to produce the breeding stack Bt11 × MIR162 × MON 89034 × GA21 maize. No further genetic modification to produce this stack has taken place. • Bt11 maize was produced using a protoplast transformation/regeneration system (Negrutiu *et al.*, 1987). • MIR162 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; Hoekema *et al.*, 1983). • MON 89034 maize was produced by *Agrobacterium*-mediated transformation of maize cells. • GA21 maize was produced via micro-projectile bombardment of maize suspension culture cells.
Description of gene modification:	Syngenta developed Bt11 × MIR162 × MON 89034 × GA21 by combining individual transformation events, Bt11, MIR162, MON 89034 and GA21 through conventional breeding. No further genetic modification to produce this stack has taken place. Bt11 × MIR162 × MON 89034 × GA21 maize produces the transgenic proteins Cry1Ab, PAT, Vip3Aa20, PMI, Cry1A.105, Cry2Ab2, and mEPSPS.
Vector characteristics:	Bt11 × MIR162 × MON 89034 × GA21 maize was developed by using conventional breeding techniques that combines transformation events Bt11, MIR162, MON 89034 and GA21. No vector was used for the production of Bt11 × MIR162 × MON 89034 × GA21 maize. • The *Not*I restriction fragment of vector pZO1502, a derivative of commercially available plasmid pUC18, was used for the transformation of Bt11. • The plasmid pNOV1300 was used for transformation of MIR162. • The binary plasmid PV-ZMIR245 was used for transformation of MON 89034. • The *Not*I fragment of vector pDPG434 was used for the transformation. The vector is derived from a pSK-vector, which is derived from pUC19.
Insert or inserts (Annex III.9(d)):	Bt11 × MIR162 × MON 89034 × GA21 maize was developed using conventional breeding techniques that combined Bt11, MIR162, MON 89034 and GA21 maize. Southern blot analyses confirmed that the DNA hybridization patterns for Bt11 × MIR162 × MON 89034 × GA21 maize corresponds to the hybridization bands observed for the single events.

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Insert or inserts	Thus, Bt11 × MIR162 × MON 89034 × GA21 maize produces the transgenic proteins, Cry1Ab, PAT, Vip3Aa20, PMI, Cry1A.105, Cry2Ab2, and mEPSPS present in the four individual transformation events. This indicates that the integrity of the transgenic inserts from the single events was preserved during conventional breeding to produce Bt11 × MIR162 × MON 89034 × GA21 maize.
Recipient organism or parental organisms (Annex III.9(a)):	
Taxonomic name/ status of recipient or parental organisms:	Family name: Poaceae Genus: *Zea* Species: *Z. mays* L. Subspecies: *Z. mays* ssp. *mays*
Common name of recipient or parental organisms:	Maize.
Point of collection of recipient organisms:	Maize originates from the Mesoamerican region, i.e. Mexico and Central America (CFIA, 2003).
Characteristics of recipient or parental organisms related to biosafety:	*Z. 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, therefore 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). 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 and differential growth rates of pollen tubes. **1. Sexual compatibility with other cultivated or wild plant species, including the distribution in SA of the compatible species.** As there are no wild relatives of maize in SA, 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 depend on prevailing wind patterns, humidity, and temperature. The frequency of cross-pollination and fertilization depends on 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.

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Characteristics of recipient or parental organisms related to biosafety:	**(b) Specific factors affecting survivability** 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 grain 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 SA, presumably because of low seed dispersal and seedling survival. (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. More 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** 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.

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Characteristics of recipient or parental organisms related to biosafety:	In addition, most maize varieties are protoandrous, therefore 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 with very diverse morphological and physiological traits; it is grown on approximately 185 million hectares worldwide (FAOSTAT, 2015). Maize is distributed over a wide range of environmental conditions: from latitudes 50° North to 50° South, 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°C 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.** Several other organisms in the environment, including insects, birds, and mammals, is known to interact with maize. Maize 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 SA, 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 depend 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. **6. Wild plant species** Wild *Zea* species have no pronounced weedy tendencies (CFIA, 2003). The only wild taxa known to hybridize 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 (*T. dactyloides, T. floridanum, T.*

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Characteristics of recipient or parental organisms related to biosafety:	*lanceolatum, and T. 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 SA. *Tripsacum* species are geographically restricted to the Americas (CFIA, 2003). Only two species are known to be found north of Mexico: *T. floridanum* which is native to the southern tip of Florida, USA; and *T. 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 or parental organisms:	Maize originates from the Mesoamerican region, i.e. Mexico and Central America (CFIA, 2003).
Centres of genetic diversity	Maize originates from the Mesoamerican region, i.e. Mexico and Central America (CFIA, 2003).
Habitats where the recipient or parental organisms may persist or proliferate:	Maize originates from the Mesoamerican region, i.e. Mexico and Central America (CFIA, 2003). Please refer to information provided above regarding geographical distribution. 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)	The donor organisms of the single events are: • ***Bacillus thuringiensis*** The source of the native *cry1Ab, vip3Aa1, cry1A.105* and *cry2Ab2* genes is *B. thuringiensis*. The species is a member of the genus *Bacillus*, a diverse group of rod-shaped, gram-positive, facultative anaerobic, spore forming bacteria. *B. thuringiensis* 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). • ***Streptomyces viridochromogenes*** 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). • ***Escherichia coli*** 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

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Taxonomic name/ status of donor organism(s)	history of safe use and is commonly used as a protein production system in many commercial applications. • ***Zea mays*** The source of the *epsps* gene is maize (*Z. mays*). Maize is the world's most widely planted cereal. It is grown between latitudes 50° North and 50° South and from below sea level to altitudes of more than 3000m. 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).
Common name of donor organism(s):	*B. thuringiensis, S. viridochromogenes* and *E. coli* are bacteria. Maize is a Commodity crop.
Point of collection/ acquisition of donor organism(s):	*B. thuringiensis, S. viridochromogenes, E. coli* and maize are widespread in the environment.
Characteristics of donor organism(s) related to biosafety:	*B. thuringiensis, S. viridochromogenes, E. coli* and maize are widely prevalent in the environment.
Intended use and receiving environment	
Intended use of the LMO:	General release of Bt11 × MIR162 × MON 89034 × GA21 maize in SA.
Receiving environment:	All maize growing areas in South Africa
Risk assessment summary	
Detection/ Identification method of the LMO:	Bt11 × MIR162 × MON 89034 × GA21 maize contains all the transgenes of the individual events and produces the following proteins: Cry1Ab, PAT, Vip3Aa20, PMI, Cry1A.105, Cry2Ab2, and mEPSPS. For the verification of the presence of Bt11, MIR162, MON 89034 and GA21, DNA samples consisting of Bt11 × MIR162 × MON 89034 × GA21 DNA and non-transgenic, near-isogenic DNA were provided to the European Union Reference Laboratory for Genetically Modified Food and Feed (EU-RL GMFF, Ispra). The detection methods developed for the single events will also detect the individual events in Bt11 × MIR162 × MON 89034 × GA21 maize. For specific detection of Bt11, MIR162, MON 89034 and GA21 maize genomic DNA, real-time quantitative TaqMan® PCR methods have been developed using the taxon specific target sequence (*Adh*1) and the Bt11, MIR162, MON 89034 and GA21 target sequences. These methods have been validated for use by the EU-RL GMFF and can be found on the EU-RL GMFF website. Bt11 maize: http://gmo-crl.jrc.ec.europa.eu/summaries/Bt11_CRLVL1007_Validated_Method%20doc.pdf http://gmo-crl.jrc.ec.europa.eu/summaries/Bt11_CRLVL1007_Val_Report.pdf

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Detection/ Identification method of the LMO:	MIR162 maize: http://gmo-crl.jrc.ec.europa.eu/summaries/MIR162_validated_Method.pdf http://gmo-crl.jrc.ec.europa.eu/summaries/MIR162_val_report.pdf MON 89034 maize Detection methods for detection of MON 89034 DNA will be submitted to DALRRD (Department of Agriculture, Land Reform and Rural Development) by Bayer. GA21 maize: 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: **Evaluation of the likelihood of adverse effects:**	The comparative assessment of composition, whole food safety, phenotypic and agronomic traits conducted for Bt11 × MIR162 × MON 89034 × GA21 maize and near-isogenic non-transgenic maize has shown that the Bt11 × MIR162 × MON 89034 × GA21 maize does not contain altered agronomic and phenotypic characteristics apart from the intended modification, which is insect control and herbicide tolerance. It leads to the conclusion that this maize is substantially equivalent to non-transgenic maize. The persistence or invasiveness of the Bt11 × MIR162 × MON 89034 × GA21 maize when compared to non-transgenic maize has not increased. There are no data indicating that Cry1Ab, PAT, Vip3Aa20, PMI, Cry1A.105, Cry2Ab2, and mEPSPS protein expression results in altered seed dormancy, over wintering capacity, or other characteristics that would alter the prevalence of volunteer maize in subsequent growing seasons. Maize has a long history of safe use and the crop itself causes few health problems. Expression of the proteins Cry1Ab, PAT, Vip3Aa20, PMI, Cry1A.105, Cry2Ab2, and mEPSPS in food and feed products derived from Bt11 × MIR162 × MON 89034 × GA21 maize is unlikely to cause adverse effects through toxicity or allergenicity based on the following information: • Well-characterized specificity of the biological activity of Cry1Ab, PAT, Vip3Aa20, PMI, Cry1A.105, Cry2Ab2, and mEPSPS proteins. • No known adverse effects of prior exposure to Cry1Ab, PAT, Vip3Aa20, PMI, Cry1A.105, Cry2Ab2, and mEPSPS proteins in food or feed. • Cry1Ab, PAT, Vip3Aa20, PMI, Cry1A.105, Cry2Ab2, and mEPSPS proteins have no significant sequence identity to known toxins with known adverse effects in humans or animals.

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	• Cry1Ab, PAT, Vip3Aa20, PMI, Cry1A.105, Cry2Ab2, and mEPSPS proteins have no detectable acute toxicity in mice at high doses. • Cry1Ab, PAT, Vip3Aa20, PMI, Cry1A.105, Cry2Ab2, and mEPSPS proteins have no significant amino acid homology to known or putative allergenic protein sequences that are biologically relevant or have implications for allergenic potential. • Cry1Ab, PAT, Vip3Aa20, PMI, Cry1A.105, Cry2Ab2, and mEPSPS proteins are degraded in simulated gastric fluid. • Very low dietary exposure to Cry1Ab, PAT, Vip3Aa20, PMI, Cry1A.105, Cry2Ab2, and mEPSPS proteins. In addition, margins of dietary exposure have been calculated by comparing the NOAEL from the acute oral toxicity studies of Cry1Ab, PAT, Vip3Aa20, PMI, Cry1A.105, Cry2Ab2, and mEPSPS protein to the expected intake level. The results indicate that expected levels of intake of Cry1Ab, PAT, Vip3Aa20, PMI, Cry1A.105, Cry2Ab2, and mEPSPS through consumption of Bt11 × MIR162 × MON 89034 × GA21 maize in SA will be very low. Margins of dietary exposure for the Cry1Ab, PAT, Vip3Aa20, PMI, Cry1A.105, Cry2Ab2, and mEPSPS proteins, supports the conclusion that no unacceptable risk is posed to consumers. The conclusion reached from the detailed evaluation of the characteristics of Bt11 × MIR162 × MON 89034 × GA21 maize and the likelihood of any adverse effects, is that this maize is substantially equivalent to non-transgenic maize and that it is highly unlikely to have any adverse effects on human or animal health, or the environment.
Evaluation of the consequences:	As discussed above, the potential risk for adverse consequences of Bt11 × MIR162 × MON 89034 × GA21 maize cultivation in SA is negligible.
Overall risk:	None of the components introduced into Bt11 × MIR162 × MON 89034 × GA21 maize are considered to be dangerous to human health or the environment. There is no indication that the combination of Bt11, MIR162, MON 89034 and GA21 maize by conventional breeding would result in any adverse effects or changes in maize toxicity to humans or animals. None of the proteins expressed by Bt11, MIR162, MON 89034 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 × MIR162 × MON 89034 × 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.
Recommendation:	No risks have been identified. Detailed stewardship practices will be implemented by Syngenta.
Actions to address uncertainty	Full compliance with IRM and WRM conditions imposed by the National Competent Authority.

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regarding the level of risk:	
Additional information	
Availability of detailed risk assessment information:	More information on Bt11 × MIR162 × MON 89034 × GA21 maize and the assessment of risk can be found in part I of this application.
Any other relevant information:	None
Attach document:	Not applicable
Notes:	Not applicable