RISK ASSESSMENT OF MIR162 MAIZE

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

Title:	Risk assessment of MIR162 maize in South Africa. This risk assessment is in support of the Syngenta SA Application for Commodity Clearance of MIR162 maize in South Africa.
Country Taking Decision:	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. Syngenta SA (Pty) Ltd. Building 10, Thornhill Office Park 94 Bekker Street Midrand, 1685 Tel: +27 11 541 4000 Fax: +27 11 541 4072 **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:	Event MIR162 maize, (hereafter referred to as 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.
Unique identification of the living modified organism:	SYN-IR162-4
Transformation event:	MIR162 maize contains the *vip3Aa20* and *pmi* genes.
Introduced or Modified Traits:	**Altered growth, development and product quality:** Insect resistance

[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.

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Techniques used for modification:	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).
Description of gene modification:	• a Vip3Aa protein (designated Vip3Aa20) for control of certain lepidopteran pests like *Heliothis zea* (corn earworm), *Agrotis ipsilon* (black cutworm), *Spodoptera frugiperda* (fall armyworm), 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.
Vector characteristics	The plasmid pNOV1300 was used for transformation of MIR162 maize.
Insert or inserts (Annex III.9(d)):	The region intended for insertion contains the *vip3Aa19* gene[4], 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 terminator from *A. tumefaciens*.
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 (CFIA, 1994).

[4] 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.

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Characteristics of recipient organism or parental organisms related to biosafety:	*Zea mays* is an allogamous plant that propagates through seed produced predominantly by wind-borne cross-pollination. Self-pollination of up to 5% may be observed (Messeguer *et al.*, 2006). Male and female flowers are separated on the plant by about 1 – 1.3m (Aylor, 2003). *Z. mays* has staminate flowers in the tassels and pistillate flowers on the ear shoots. *Z. mays* is a plant with protoandrous inflorescence; however, decades of conventional selection and breeding have also produced varieties of maize with protogyny (Angevin *et al.*, 2008). With the protoandrous maize varieties pollen shedding precedes silk emergence by up to five days. The key critical stages of maize reproduction are tasselling, silking, pollination and fertilization. Climatic and drought stress affect pollen viability and silk longevity, thus, potentially limiting the period of possible cross-pollination. Maize pollen is very sensitive to dehydration as it loses water rapidly. Other factors like rainfall or irrigation inhibit pollen emission, because the anther dehiscence is limited by the mechanical layer. In general, maize pollen is only viable for a few hours after emission (Aylor, 2004). As maize pollen is large and heavy it tends to be deposited close to the source plant (Raynor *et al.*, 1972; Pleasants *et al.*, 2001) and studies have indicated that most maize pollen falls within 5m of the field's edge (Sears and Stanley-Horn, 2000). In general, these studies have shown that over 98% of maize pollen remains within a radius of 25-50m of the source, although some grains can travel several hundred meters (EEA, 2002; Jarosz *et al.*, 2005). Climatic conditions also affect grain and seed production, especially under drought conditions during flowering, tasseling and silking. If severe drought occurs during these phenological stages, the grain yield is reduced (Wolf and Van Diepen, 1995). <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, 1994) and there are no wild relatives of maize in South Africa.

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	<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. (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 ensures that, other than deliberate cultivation, the only means by which it can persist in the environment is accidental dispersal of kernels into disturbed soil 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 soil outside agricultural areas can germinate to give rise to 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 can only 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. Maize is a cross-pollinated plant, relying on wind for the dispersal of its pollen. The

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	rate of cross-fertilization between fields depends on pollen viability, flowering synchrony and the relative concentration of pollen in the donor and receptor plots (Messeguer *et al.*, 2006). Effective pollen transport (gene flow) depends on viable pollen reaching and fertilizing the ovules on target plants (Aylor, 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. A meta-analysis of existing cross-fertilization studies concluded that most cross-pollination events occur within 50m of the pollen source (Riesgo *et al.*, 2010). (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). Compared to other wind-pollinated species, maize pollen grains are relatively large and therefore settle rapidly in the soil (Aylor, 2003) and usually have a short flight range (Jarosz *et al.*, 2005). 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. Although vertical wind movements or gusts during pollen shedding can lift pollen up high in the atmosphere and distribute it over significant distances, concentrations of viable pollen considerably decrease with height (Aylor *et al.*, 2006) and distance (Jarosz *et al.*, 2005) from the source. Hence, only low levels of cross-pollination could occur over longer distances under suitable climatic conditions (Bannert and Stamp, 2007). In addition, most maize varieties are protoandrous so pollen shedding precedes silk emergence by up to five days. 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 approximately 174 million hectares worldwide[5] with a yield estimation for

[5] http://www.fas.usda.gov/psdonline/circulars/production.pdf (accessed January 2013)

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	the 2011/12 season of 876 million tons[6]. It is distributed over a wide range of locations and 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. <u>5. Other potential interactions, relevant to the GMO, of the plant with organisms in the ecosystem where it is usually grown, or elsewhere, including information on toxic effects on humans, animals and other organisms</u>. 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. <u>6. Wild plant species</u> 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 exhibit 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, T. floridanum, T. 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 South Africa. *Tripsacum* species are geographically restricted to the Americas (CFIA, 1994). Only two species are known to be found

[6] http://www.igc.int/downloads/gmrsummary/gmrsumme.pdf (accessed January 2013)

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	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, 1994). *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 (CFIA, 1994).
Centres of genetic diversity, if known, of recipient organism or parental organisms:	Maize originates from the Mesoamerican region, i.e. Mexico and Central America (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 (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 the native *vip3Aa1* gene is *Bacillus 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). Several subspecies of *B. thuringiensis* have been described; many of them have been extensively studied and used in commercial insecticide preparations. Insecticidal products using *B. thuringiensis* 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

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	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. *B. thuringiensis* and *E. coli* are bacteria and therefore not related to the recipient organism maize.
Common name of donor organism(s):	Bacteria or Micro-organisms: *B. thuringiensis*, *E. coli*
Point of collection or acquisition of donor organism(s):	*B. thuringiensis* and *E. coli* 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* and *E. coli* are widespread in the environment.
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 MIR162 maize in South Africa.
Receiving environment (Annex III.9(h)):	The intended use of MIR162 maize does not currently include cultivation in South Africa.
Risk assessment summary	
Detection/Identification method of the LMO (Annex III.9(f)):	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

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Evaluation of the likelihood of adverse effects (Annex III.8(b)):	Cultivation of maize derived from MIR162 maize in South Africa is not within the scope of Syngenta's Application for Commodity Clearance of MIR162 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 in the soil 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. MIR162 maize is unlikely to become more persistent, weedy or invasive than maize varieties currently cultivated in South Africa, as the expression of the Vip3Aa20 and PMI 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 Vip3Aa20 and PMI, proteins expressed in MIR162 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.

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	The dietary exposure also takes a worst case assumption that 100% of the maize consumed in South Africa is MIR162 maize. Taking into consideration the level of expression of the Vip3Aa20 and PMI proteins, based on an average maize consumption of 248.1 g/person/day (WHO, 2012), the theoretical daily intake for each of the proteins produced by MIR162 maize (Vip3Aa20 and PMI) was calculated, based on a bodyweight of a 60kg person. In addition, margins of exposure have been calculated by comparing the no-observed-effect-level 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 Vip3Aa20 and PMI through consumption of MIR162 maize in South Africa will be very low. Margins of exposure exceed a factor of at least 2000, supporting the conclusion that the risk to consumers is negligible and confirming the results previously obtained. Studies comparing the agronomy, composition and whole food safety of MIR162 maize plants and non-transgenic maize lead to the conclusion that this maize is substantially equivalent to conventional maize. MIR162 maize is highly unlikely to have adverse effects on humans or animals; thus, the effects of MIR162 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 MIR162 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 MIR162 maize in South Africa is not within the scope of Syngenta's Application for Commodity Clearance of MIR162 maize. The importation and use as food, feed or for processing of grain from MIR162 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 MIR162 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 environment. Therefore, no adverse consequences will result from the importation and use as food, feed or for processing of grain from MIR162 maize in South Africa.

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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 MIR162 maize is negligible. None of the components introduced by MIR162 maize is considered to be dangerous to human health or the environment. None of the proteins expressed by MIR162 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 MIR162 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 MIR162 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