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Risk Assessment for the import of insect tolerant maize Event MIR604 into South Africa

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

Risk assessment details

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| 1. Country Taking Decision: | South Africa |
| 2. Title: | Risk Assessment for the import of insect tolerant maize Event MIR604 into South Africa |
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| 3. Contact details: | <p>Name and Address and Contact details of the Exporter/Importer
Grain from Event MIR604 will enter international commodity trade and be exported and imported by various traders. In order to allow importation of commodity grain containing Event MIR604, the technology developer, Syngenta, is applying for commodity clearance in South Africa.</p> <p>Name and Address and contact details of Syngenta
Syngenta SeedCo (Pty) Ltd.
Building 9 & 10, Thornhill Office Park
94 Bekker Street
Midrand, 1685</p> |
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LMO information

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| 4. Name and identity of the living modified organism: | Maize Event MIR604. |
| 5. Unique identification of the living modified organism: | SYN-IR604-5. |
| 6. Transformation event: | Event MIR604 |
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7. Introduced or Modified Traits:

Event MIR604 is a genetically modified (GM) maize expressing a modified Cry3A (*mCry3A*) gene, encoding for a protein that confers protection against the Western Corn rootworm (WCRW) (*Diabrotica virgifera virgifera*, Le Conte) and related coleopteran species. It also expresses a marker gene, *pmi*, that allows the plants to utilise mannose as a carbon source, acting as a selectable marker.

8. Techniques used for modification:

Agrobacterium-mediated transformation.

9. Description of gene modification:

Please refer to 7.

10. Vector characteristics

Plasmid pZM26, a binary vector used for *Agrobacterium* mediated plant transformation, was used to generate event MIR604.

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11. Insert or inserts (Annex III.9(d)):

Table1: Description of the elements of the foreign gene cassettes

Vector backbone components:

Component	Function and origin of the sequence
Spec	Streptomycin adenylyltransferase, <i>aadA</i> gene from <i>E. coli</i> Tn7 (GenBank Accession Number X03043). Confers resistance to erythromycin, streptomycin, and spectinomycin; used as a bacterial selectable marker (Fling <i>et al.</i> , 1985).
VS1ori	Consensus sequence for the origin of replication and partitioning region from plasmid pVS1 of <i>Pseudomonas</i> (similar to GenBank Accession Number U10487). Serves as origin of replication in <i>Agrobacterium tumefaciens</i> host (Ioth <i>et al.</i> , 1984).
ColE1ori	Origin of replication that permits replication of plasmid in <i>E. coli</i> . (similar to GenBank Accession Number V00268) (Itoh and Tomizawa, 1978).
LB	Left border region of T-DNA from <i>Agrobacterium tumefaciens</i> nopaline ti-plasmid (GenBank Accession number J01825). Short direct repeat that flanks the T-DNA and is required for the transfer of the T-DNA into the plant cell (Zambryski <i>et al.</i> , 1982).
RB	Right border region of T-DNA from <i>Agrobacterium tumefaciens</i> nopaline ti-plasmid (GenBank Accession number J01826). Short direct repeat that flanks the T-DNA and is required for the transfer of the T-DNA into the plant cell (Wang <i>et al.</i> , 1984).
virG	VirGN54D from pAD1289 (similar to GenBank Accession Number AF242881). The N54D substitution results in a constitutive <i>virG</i> phenotype. VirG is part of the two-component regulatory system for the <i>vir</i> regulon in <i>Agrobacterium</i> (Hansen <i>et al.</i> , 1994).
repA	pVS1 replication protein from <i>Pseudomonas</i> , which is a part of the minimal pVS1 replicon that is functional in gram-negative plant associated bacteria (GenBank Accession Number AF133831) (Heeb <i>et al.</i> , 2000).

MCry3A vector components:

Component	Function and origin of the sequence
MTL	Promoter derived from maize metallothionein-like gene, provides root preferential expression (US patent number 5466785).
mCry3A	A gene encoding a modified Cry3A protein which is related to a Cry3A protein from <i>Bacillus thuringiensis</i> .
NOS	Termination sequence of the nopaline synthase gene, isolated from <i>Agrobacterium tumefaciens</i> (Depicker <i>et al</i> 1982, Bevan <i>et al</i> 1983).

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Selectable marker:

Component	Function and origin of the sequence
ZmUbilIntron	Promoter of a maize ubiquitin gene together with the 1st intron of the gene (Christensen <i>et al.</i> , 1992).
PMI	Coding sequence of the phosphomannose isomerase gene isolated from <i>Escherichia coli</i> (Miles and Guest, 1984). This gene is used as a marker for the transformation, and allows positive selection on mannose (Bojsen <i>et al.</i> , 1994; Joersbo <i>et al.</i> , 1998; Reed 1999; Negrotto <i>et al.</i> , 2000).

11. Inserts

Refer to 11 above.

Recipient organism or parental organisms (Annex III.9(a)):

12. Taxonomic name/status of recipient organism or parental organisms:

Family name: Graminea
Genus: *Zea*
Species: *Zea mays* L.
Subspecies: *mays*

13. Common name of recipient organism or parental organisms:

Maize/corn.

14. Point of collection or acquisition of recipient or parental organisms:

Maize originates from the Mesoamerican region, i.e. Mexico and Central America region (CFIA, 2003).

15. Characteristics of recipient organism or parental organisms related to biosafety

Zea mays 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). 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.

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).

Survivability

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.

Survival of maize is dependent upon temperature, seed moisture, genotype, husk protection and stage of development. Maize cannot persist as a weed. Maize seed can only survive under a narrow range of climatic conditions. Volunteers are killed by frost or easily controlled by current agronomic practices including cultivation and the use of selective herbicides (Niebur, 1993). Maize is incapable of sustained reproduction outside of domestic cultivation and is non-invasive of natural habitats (OECD, 2003).

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16. Centre(s) of origin of recipient organism or parental organisms:	Refer to 14.
17. Centres of genetic diversity, if known, of recipient organism or parental organisms:	Refer to 14.
18. Habitats where the recipient organism or parental organisms may persist or proliferate:	Refer to 15.
Donor organism or organisms (Annex III.9(b)):	
19. Taxonomic name/status of donor organism(s)	<i>mCry3A</i> related to a <i>Cry3A</i> protein from <i>Bacillus thuringiensis</i> .sbsp. <i>tenebrionis</i> <i>pmi</i> from <i>Escherichia coli</i>
20. Common name of donor organism(s):	Refer to 19
21. Point of collection or acquisition of donor organism(s):	Not applicable.
22. Characteristics of donor organism(s) related to biosafety:	Event MIR604 plants have been modified to contain a <i>mCry3A</i> gene conferring tolerance to WCRW, along with a marker gene, <i>pmi</i> , that allows the plants to utilise mannose as a carbon source. None of these donors are known to be pathogenic and no pathogenic sequences have been introduced.
Intended use and receiving environment	
23. Intended use of the LMO (Annex III 9(g)):	As conventional maize grain, therefore for food, feed and industrial use.
24. Receiving environment (Annex III.9(h)):	MIR604 products will not be released into the environment intentionally. The products will used in the food and feed chains, or for industrial purposes.

Risk assessment summary

25. Detection/Identification method of the LMO (Annex III.9(f)):	PCR techniques.
26. Evaluation of the likelihood of adverse effects (Annex III.8(b)):	<p>No adverse effects on human or animal health or adverse consequences for the food/feed chain are anticipated following consumption of food or feed consisting or containing Event MIR604 maize under the scope of this application.</p> <p>Cultivation of maize derived from Event MIR604 in South Africa is not within the scope of this application. In the rare event that small amounts of maize kernels of event MIR604 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), especially under normal South African climatic conditions. The expression of the mCryA and PMI proteins do not affect the agronomic characteristics or weediness potential of Event MIR604, as demonstrated in field trials conducted to evaluate the agronomic performance of this event in comparison with the isogenic control. 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.</p>
27. Evaluation of the consequences (Annex III.8(c)):	Refer to 26.
28. Overall risk (Annex III.8(d)):	The risk of potential adverse effects from food and feed use can be considered negligible.
29. Recommendation (Annex III.8(e)):	Not applicable, as no risk was identified.
30. Actions to address uncertainty regarding the level of risk (Annex III.8(f)):	Not applicable, as no risk was identified.
Additional information	
31. Availability of detailed risk assessment information:	More details can be obtained from the applicant.
32. Any other relevant information:	Not applicable.

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33. Attach document: Not applicable to applicant.

34. Notes: Not applicable.

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COMMON FORMAT FOR Risk Assessment
(In accordance with Annex III of the Cartagena Protocol on Biosafety)

Risk assessment details

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| 1. Country Taking Decision: | Republic of South Africa. |
| 2. Title: | Application for Commodity Clearance of DAS-40278-9 in the Republic of South Africa. |
| 3. Contact details: | Dow AgroSciences South Africa, P.O. Box 76129 Lynnwoodridge, Pretoria 0040, South Africa. Telephone: (+27) 12 361 8120; Fax (+27) 12 361 8126. |
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LMO information

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| 4. Name and identity of the living modified organism: | The LMO is DAS-40278-9. DAS 40278-9 was generated from the publicly available maize line, Hi-II. Hi-II is a derivative of the A188 and B73 inbred maize lines, which are publicly available lines developed by the University of Minnesota and Iowa State University, respectively. |
| 5. Unique identification of the living modified organism: | DAS-40278-9 |
| 6. Transformation event: | DAS-40278-9 |
| 7. Introduced or Modified Traits: | Chemical tolerance
- Herbicide tolerance. |
| 8. Techniques used for modification: | Whiskers-mediated transformation. |
| 9. Description of gene modification: | DAS-40278-9 maize was developed by inserting a linear <i>Fsp</i> I fragment from plasmid pDAS1740 using direct Whiskers-mediated transformation to stably incorporate the <i>aad-1</i> gene from the soil bacterium, <i>Sphingobium herbicidovorans</i> , into maize. DAS-40278-9 produces the AAD-1 protein which degrades the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) into herbicidally-inactive 2,4-dichlorophenol (DCP). Additionally, AAD-1 converts certain aryloxyphenoxypropionate (AOPP) herbicides (such as quizalofop) into their corresponding inactive phenols. |
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Characteristics of modification

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10. Vector characteristics (Annex III.9(c)):	<p>The recipient maize line Hi-II was transformed using direct insertion of the DNA <i>Fsp</i> I fragment, carrying the <i>aad-1</i> expression cassette for insertion into the plant genome, from plasmid pDAS1740.</p> <p>The <i>aad-1</i> expression cassette contained in the pDAS1740/<i>Fsp</i> I fragment was designed to express the plant-optimized aryloxyalkanoate dioxygenase (<i>aad-1</i>) gene that encodes the AAD-1 protein. The <i>aad-1</i> gene was isolated from <i>Sphingobium herbicidovorans</i> and the synthetic version of the gene had the G+C codon optimized, to a level more typical for plant expression. The insertion of the <i>aad-1</i> gene into maize plants confers tolerance to 2,4-D and AOPP (“fop”) herbicides. The <i>aad-1</i> gene encodes a protein of 296 amino acids that has a molecular weight of approximately 33 kDa.</p>
11. Insert or inserts (Annex III.9(d)):	<p>The transgene insert in maize event DAS-40278-9 is a single, intact copy of the <i>aad-1</i> expression cassette from plasmid pDAS1740. The event is stably integrated and inherited across and within breeding generations, and no plasmid backbone sequences are present in DAS-40278-9 maize.</p>
<p>Recipient organism or parental organisms (Annex III.9(a)):</p>	
12. Taxonomic name/status of recipient organism or parental organisms:	<p>Common name: Maize, Family: Graminae, Genus: <i>Zea</i>, Species: <i>mays</i> (2n=20)</p>
13. Common name of recipient organism or parental organisms:	<p>Maize</p>
14. Point of collection or acquisition of recipient or parental organisms:	<p>Privately owned germplasm.</p>
15. Characteristics of recipient organism or parental organisms related to biosafety:	<p>Maize is a well-known crop plant worldwide. Maize is extensively cultivated in South Africa and has a history of safe use. Maize grain and forage, or derived products of maize, are not considered to have toxic effects on humans, animals and other organisms.</p>
16. Centre(s) of origin of recipient organism or parental organisms:	<p>It is believed that maize originated in south central Mexico, specifically in the Pacific slope of the modern Mexican states of Oaxaca, Tehuacán, and the Valley of Mexico. Specific geographic coordinates are unknown.</p>
17. Centres of genetic diversity, if known, of recipient organism or parental organisms:	<p>Centers of genetic diversity of maize are the same as its centre of origin.</p>

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18. Habitats where the recipient organism or parental organisms may persist or proliferate:	Maize does not persist or proliferate outside of agriculture in South Africa. There are no known populations in any natural habitat in the country.
Donor organism or organisms (Annex III.9(b)):	
19. Taxonomic name/status of donor organism(s)	<i>Sphingobium herbicidovorans</i> (Bacteria; Proteobacteria; Alphaproteobacteria; Sphingomonadales; Sphingomonadaceae; <i>Sphingobium</i>)
20. Common name of donor organism(s):	Bacteria
21. Point of collection or acquisition of donor organism(s):	Bacteria are ubiquitous in nature.
22. Characteristics of donor organism(s) related to biosafety:	There are no reports of <i>S. herbicidovorans</i> being implicated as a human pathogen, producing any allergens or adversely affecting livestock or the environment. Out of the ~20 recognized species of <i>Sphingobium</i> , only one, <i>S. yanoikuyae</i> has been isolated from a clinical environment. Other related genera however, are known to cause infrequent infections which are generally limited in virulence. Because of their ubiquity and adaptability, sphingomonads are often found in clinical settings, but usually not associated with infection. There are reports of sphingomonads producing antigenic glycolipids that may have use as therapeutics. Due to their biodegradative and biosynthetic capabilities, other related sphingomonads have been used for a wide range of biotechnological applications, including bioremediation of environmental contaminants and production of extracellular polymers such as sphingans which are used extensively in the food.
Intended use and receiving environment	
23. Intended use of the LMO (Annex III 9(g)):	For all uses as for any other maize, excluding cultivation.
24. Receiving environment (Annex III.9(h)):	This is not an application for release of DAS-40278-9 into the environment of South Africa.
Risk assessment summary	
25. Detection/Identification method of the LMO (Annex III.9(f)):	Detection methods to confirm the molecular identity of DAS-40278-9 maize as well as certified reference materials have been developed (see http://www.sumobrain.com/patents/wipo/Detection-aad-1-event-das/WO2011022471A1.pdf).

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26. Evaluation of the likelihood of adverse effects (Annex III.8(b)):	The scope of this application does not include cultivation of of DAS-40278-9 maize in South Africa. Any exposure to the environment will be limited to any unintended release of DAS-40278-9 maize, which could occur via accidental spillage during loading/unloading of the vessels, trains and trucks carrying the load of commodity grain, including DAS-40278-9 maize, destined for processing into animal feed or human food products. However, this limited exposure is highly unlikely to give rise to any adverse effect and, if necessary, any spillage could be easily controlled by the application of current agricultural practices used for the control of volunteer maize plants. Furthermore, maize is a poor competitor and cannot persist as a weed. Environmental conditions at the sites of handling are unlikely to be conducive to germination, growth and reproduction of maize grain that is incidentally released.
27. Evaluation of the consequences (Annex III.8(c)):	In the unlikely event that some grain containing DAS-40278-9 (from grain imports) may end up in the environment of South Africa before being milled, the impact would be negligible as data supported a conclusion that DAS-40278-9 is substantially equivalent to conventional maize; maize plants cannot survive without human intervention. Furthermore, South Africa is not the centre of origin for <i>Zea mays</i> and there are no wild relatives in South Africa with which maize can outcross and if the grain was to be used as seed for planting, it would represent a F2 generation of which the growth, development and yield is extremely variable and predominantly weak.
28. Overall risk (Annex III.8(d)):	The overall risk posed by this GMO is negligible.
29. Recommendation (Annex III.8(e)):	The overall risk is negligible. No recommendations other than procedures that may apply to conventional maize are applicable.
30. Actions to address uncertainty regarding the level of risk (Annex III.8(f)):	There is no uncertainty regarding the risk profile.
Additional information	
31. Availability of detailed risk assessment information:	All data relating to this risk assessment have been presented in this dossier.
32. Any other relevant information:	To the best of our knowledge, all relevant information has been supplied in this dossier.
33. Attach document:	<i>Not applicable to applicant</i> <Specific types of entry: option to choose a file from the local source and 'upload' a copy to the BCH server>

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34.Notes:

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35.Notes:

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