RISK ASSESSMENT OF SYHT0H2 SOYBEAN

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

Country Taking Decision:	South Africa
Title:	Risk assessment of SYHT0H2 soybean in South Africa. This risk assessment is in support of the Syngenta South Africa and Bayer CropSciences LLC's Application for Commodity Clearance of SYHT0H2 soybean in South Africa.
Contact details:	**Name and Address and Contact details of the Importer**[2] Commodity imports are performed by various 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 South Africa Tel: +27 11 541 4000 Fax: +27 11 541 4072 Bayer (Pty) Ltd - 1968/011192/07 27 Wrench Road, Isando, South Africa Tel: +27 11 921 5168 **Name and Address and contact details of the Applicant**[3] Syngenta SA (Pty) Ltd. Building 10, Thornhill Office Park 94 Bekker Street Midrand, 1685 South Africa Tel: +27 11 541 4000 Fax: +27 11 541 4072 Bayer (Pty) Ltd 27 Wrench Road, Isando, South Africa Tel: +27 11 921 5168

[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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LMO information	
Name and identity of the living modified organism:	Soybean (*Glycine max* [L.] Merrill) SYHT0H2 has been genetically modified to express the genes *avhppd-03* derived from oat (*Avena sativa* L.) and *pat* from *Streptomyces viridochromogenes*.
Unique identification of the living modified organism:	The unique identifier assigned to this event is SYN-ØØØH2-5.
Transformation event:	Soybean has been genetically modified to express the genes *avhppd-03* derived from *A. sativa* L. and *pat* derived from *S. viridochromogenes*. The gene *avhppd-03* encodes a *p*-hydroxyphenylpyruvate dioxygenase (HPPD) enzyme, and the gene *pat* encodes the enzyme phosphinothricin acetyltransferase (PAT).
Introduced or Modified Traits:	Altered growth, development and product quality: herbicide tolerance.
Techniques used for modification:	The SYHT0H2 soybean was produced through *A. tumefaciens*-mediated transformation with the plasmid pSYN15954.
Description of gene modification:	Soybean has been genetically modified to express the genes *avhppd-03* derived from *A. sativa* L. and *pat* from *S. viridochromogenes*. The gene *avhppd-03* encodes an HPPD enzyme, designated AvHPPD-03, that catalyses the formation of homogentisic acid, the aromatic precursor in plastoquinone and vitamin E biosynthesis. In comparison with the native soybean HPPD, the AvHPPD-03 isozyme from oat has lower binding affinity for HPPD-inhibiting herbicides, such as mesotrione. Expression of *avhppd-03* in SYHT0H2 soybean plants confers an HPPD-inhibitor-tolerance phenotype. The gene *pat* encodes the enzyme PAT, which inactivates the herbicide glufosinate ammonium, which is an inhibitor of glutamine synthetase, an enzyme in the nitrogen assimilation pathway. Expression of *pat* confers a glufosinate-ammonium-tolerance phenotype.
Vector characteristics:	The SYHT0H2 soybean was produced through *A. tumefaciens*–mediated transformation with the plasmid pSYN15954.
Insert or inserts (Annex III.9(d)):	The region of insertion contains the *avhppd-03* gene, derived from oat. This gene is under the control of the synthetic minimal plant (SMP) promoter, the tobacco mosaic virus (TMV) enhancer, the figwort mosaic virus (FMV) enhancer, the cauliflower mosaic virus 35S enhancer region, and the terminator sequence from the nopaline synthase (NOS) gene of *A. tumefaciens*. The pSYN15954 plasmid consists of the following genetic elements: 1: an *avhppd-03* cassette, which contains the gene *avhppd-03* regulated by the FMV, 35S, and TMV enhancer sequences, the SMP promoter sequence, and the NOS terminator sequence. 2: a *pat-03-01* cassette, which contains the gene *pat-03-01* regulated by the 35S promoter sequence and the NOS terminator sequence. 3: a *pat-03-02* cassette, which contains the gene *pat-03-02* regulated by the cestrum yellow leaf curling virus promoter sequence, TMV enhancer sequence, and NOS terminator sequence 4: plasmid backbone, which contains the left and right border sequences

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	from the *A. tumefaciens* nopaline Ti-plasmid, the streptomycin adenylyltransferase *aadA* gene (*spec*) from *Escherichia coli,* the pVS1 replication protein gene (*repA*) from *Pseudomonas aeruginosa*, and the origin of replication sequences pVS1 ori from *P. aeruginosa* and ColE1 ori from *E. coli.*	
Recipient organism or parental organisms (Annex III.9(a):		
Taxonomic name/status of recipient organism or parental organisms:	**family name:**	Leguminosae
	genus:	*Glycine* Willd.
	species:	*Glycine max* (L.) Merr.
	subspecies:	Not applicable
	cultivar/breeding line:	'Jack' variety
	common name:	Soybean
Common name of recipient organism or parental organisms:	Soybean	
Point of collection or acquisition of recipient or parental organisms:	Soybean was domesticated around 1100 B.C. in northeast China (OECD, 2000). It might have been introduced to the east coast of Africa in the 19th century by Chinese traders. No species of the subgenus *soja* (to which *G. max* belongs) has ever been found in the wild in Africa (Hymowitz, 1970).	
Characteristics of recipient organism or parental organisms related to biosafety:	Several characteristics of soybean ensure biosafety: • Soybean is highly domesticated and cannot survive without human intervention. • Soybean does not display weedy characteristics. • Soybean seeds rarely display dormancy characteristics, and soybeans emerge as volunteers only under certain limited environmental conditions. • No sexually compatible wild relatives of soybean occur in South Africa; therefore, no cross-hybridisation or introgression is possible. <u>1. Sexual compatibility with other cultivated or wild plant species, including the distribution in South Africa of the compatible species.</u> The wild species *G. soja* and *G. gracilis* occur naturally in China, Taiwan, Korea, Japan, and along the border between China and the former Soviet Union (OECD, 2000). Because no wild relatives of *G. max* occur in South Africa, the potential for plant-to-plant genetic transfer and exchange is limited to other cultivated soybean plants. <u>2. Survivability</u> Cultivated soybean seeds rarely display dormancy characteristics, and soybeans emerge as volunteers only under certain limited environmental conditions. Volunteers do not compete well with perennial plants and are easily controlled by mechanical or chemical means. Therefore, survival of soybean plants outside of cultivation is limited by low competitiveness, lack of dormancy, susceptibility to plant pathogens, and	

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	adverse climatic conditions (OECD, 2000). 3. Dissemination: Soybean is a self-pollinating species. Soybean flowers are receptive to pollen for about 72 hours, and the anthers mature in the bud and pollinate the stigma of the same flower, resulting in a high percentage of self-pollination. For cross-fertilisation to occur, both flowering synchrony and pollen dispersal are required (OECD, 2000). Wind-mediated pollination in soybeans appears to be negligible (Yoshimura, 2011). Although cross-fertilisation between cultivated varieties is possible, natural cross-pollination rates are low (OECD, 2000). 4. Geographical distribution of the plant: Soybean is a commercially grown crop in more than 35 countries, with approximately 102 million hectares cultivated worldwide during 2010 (FAOSTAT, 2012). Over the last 50 years, the global production, use, and trade of soybeans have increased at least fivefold. Soybean production occupies close to 6% of the world's arable land, and it is expanding faster than production of any other major crop. The United States continues to be the leading soybean-producing country. 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. The traits expressed in SYHT0H2 soybean confer tolerance to HPPD-inhibiting herbicides, such as mesotrione, and herbicides containing glufosinate ammonium. In environments where these herbicides are used, the uptake of plant genes by micro-organisms, although a rare event, could hypothetically occur. It is important to note that horizontal gene transfer (HGT) is not in itself an adverse effect, but an occurrence that may or may not have adverse effects (Keese, 2008). Current scientific knowledge indicates that HGT of non-mobile DNA fragments between unrelated organisms (such as plants to micro-organisms) is extremely unlikely to occur under natural conditions. Barriers to HGT from plants to micro-organisms include the lack of efficient mechanisms for integration of unrelated chromosomal DNA and the limited potential for positive selection of the acquired recombinant-gene-encoded traits. Furthermore, genes similar to *avhppd-03* and *pat* are widespread in the environment. Therefore, no novel selective advantage that could not be conferred by natural micro-organisms is anticipated for hypothetical recipients expressing the *avhppd-03* and *pat* genes. In the unlikely event that HGT between SYHT0H2 soybean and soil micro-organisms occurred, it is unlikely that the transgenes would be expressed or become established in the bacterial genome, because *avhppd-03* and *pat* are under the control of plant promoters. In the very unlikely event that the genes became established in the genomes of micro-organisms, no adverse effects on human and animal health or the environment would be expected, as their expression would not confer a selective advantage. No antibiotic resistance genes were inserted into SYHT0H2 soybean.

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	A comparative safety assessment was conducted through a weight-of-evidence approach, considering molecular characterisation data as well as compositional and agronomic comparisons between SYHT0H2 soybean and its conventional soybean counterpart. The purpose of this assessment was to establish whether unintended changes in SYHT0H2 soybean had occurred as a result of the genetic modification. The comparative safety assessment identified no differences of biological relevance between SYHT0H2 soybean and its conventional counterpart, thus demonstrating that SYHT0H2 soybean can be considered equivalent to conventional soybean varieties. The probability that the introduced traits confer a selective advantage or disadvantage to the GM crop or to sexually compatible wild relatives was also assessed. The main limiting factors preventing the spread of soybean outside of agro-ecosystems are cultivation dependence and susceptibility to adverse climatic conditions; therefore, the herbicide-tolerance traits are unlikely to confer a selective advantage or disadvantage to soybean. 6. Wild plant species Hybridisation is possible between *G. max* and its wild relatives in the subgenus *soja, G. soja* and *G. gracilis*. *G. soja* and *G. gracilis* do not occur naturally in Africa. The genus *Glycine* also includes the subgenus *glycine*, which contains 12 wild perennial species; these species also do not occur naturally in Africa. Attempts to produce fertile hybrids between *G. max* and species of the subgenus *glycine* were unsuccessful; hybrids were created only with difficulty in a laboratory environment and were sterile (OECD, 2000).
Centre(s) of origin of recipient organism or parental organisms:	Soybean was domesticated in China between the 11th and 17th centuries B.C. and was introduced and cultivated from the 17th century A.D. in other Asian countries, including Indonesia, Japan, the Philippines, Vietnam, Thailand, Malaysia, Nepal, and northern India. It is now cultivated in over 35 countries worldwide and is the most prevalently grown oilseed. The major producers worldwide are the United States, Brazil, Argentina, and China.
Centres of genetic diversity, if known, of recipient organism or parental organisms:	Soybean was domesticated around 1100 B.C. in northeast China (OECD, 2000). No species of the subgenus *soja* (to which *G. max* belongs) has ever been found in the wild in Africa (Hymowitz, 1970).
Habitats where the recipient organism or parental organisms may persist or proliferate:	Cultivated soybean seeds rarely display dormancy characteristics, and soybeans emerge as volunteers only under certain limited environmental conditions. Volunteers do not compete well with perennial plants and are easily controlled by mechanical or chemical means. Therefore, survival of soybean plants outside of cultivation is limited by low competitiveness, lack of dormancy, susceptibility to plant pathogens, and adverse climatic conditions (OECD, 2000).

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Donor organism or organisms (Annex III.9(b)):	
Taxonomic name/status of donor organism(s)	*Avena sativa:* The source of the gene *avhppd-03* is common oat (*Avena sativa* L.), a member of the grass family Poaceae, which also includes maize and other cereals such as wheat, barley, and rye (Kellogg, 1998). *A. sativa* is commonly grown for food and animal feed (FAO, 2012) and is an important rotation crop for wheat and oil crops. *Streptomyces viridochromogenes:* The source of the gene *pat* is the aerobic bacterium *S. viridochromogenes* strain Tü494, of the order Actinomycetales. It is a Gram-positive, sporulating, soil-inhabiting bacterium that is widespread in the environment (OECD, 1999).
Common name of donor organism(s):	*A. sativa* L. is common oat. *S. viridochromogenes* is an aerobic bacterium.
Point of collection or acquisition of donor organism(s):	*A. sativa* and *S. viridochromogenes* are widely prevalent in the environment.
Characteristics of donor organism(s) related to biosafety:	*A. sativa* and *S. viridochromogenes* 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 SYHT0H2 soybean in South Africa.
Receiving environment (Annex III.9(h)):	The intended use of SYHT0H2 soybean 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 SYHT0H2 soybean genomic DNA, a real-time quantitative TaqMan polymerase chain reaction method has been developed by Syngenta and BCS. This detection method has been submitted for validation to the European Union Reference Laboratory of the Joint Research Centre of the European Commission as part of the European application to EFSA (EFSA-GMO-DE-2012-111).
Evaluation of the likelihood of adverse effects (Annex III.8(b)):	As no wild relatives of soybean occur in South Africa, any vertical gene transfer from SYHT0H2 soybean would be limited to other cultivated soybean plants. However, the scope of this application does not include the cultivation of SYHT0H2 soybean in South Africa; therefore, it is highly unlikely that SYHT0H2 soybean would grow in South Africa as a result of import or use in food and feed. In the rare event that small amounts of SYHT0H2 soybean seed were accidentally released into the environment, their survival would be very unlikely, and no significant interaction with other organisms would occur without successful establishment and spread of large numbers of SYHT0H2

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	soybean plants. Any plants germinating from this seed could easily be controlled by the agronomic measures currently used to control other commercially available soybean. Soybean is a self-pollinating plant species. Soybean flowers are receptive to pollen for about 72 hours, and the anthers mature in the bud and pollinate the stigma of the same flower, resulting in a high percentage of self-pollination. For cross-fertilisation to occur, both flowering synchrony and pollen dispersal are required (OECD, 2000). Wind-mediated pollination in soybeans appears to be negligible (Yoshimura, 2011). Although cross-fertilisation between cultivated varieties is possible, natural cross-pollination rates are low (OECD, 2000). Soybean has been genetically modified to express the genes *avhppd-03* derived from oat *(A. sativa)* and *pat* from *S. viridochromogenes*. The gene *avhppd-03* encodes an HPPD enzyme, designated AvHPPD-03, that catalyses the formation of homogentisic acid, the aromatic precursor in plastoquinone and vitamin E biosynthesis. In comparison with the native soybean HPPD, the AvHPPD-03 isozyme from oat has lower binding affinity for HPPD-inhibiting herbicides, such as mesotrione. Expression of *avhppd-03* in SYHT0H2 soybean plants confers an HPPD-inhibitor-tolerance phenotype. The gene *pat* encodes the enzyme PAT, which inactivates the herbicide glufosinate ammonium, which is an inhibitor of glutamine synthetase, an enzyme in the nitrogen assimilation pathway. Expression of *pat* confers a glufosinate ammonium-tolerance phenotype, which was also used as a selectable marker in the development of SYHT0H2 soybean. A weight-of-evidence assessment of potential allergenicity supported the conclusion that AvHPPD-03 and PAT are unlikely to be allergenic and that no further testing is required. In particular, no specific serum screening is considered to be necessary. In addition, based on the information available, Syngenta and BCS consider it unlikely that interactions could occur that would change the allergenicity of these proteins. The proteins AvHPPD-03 and PAT 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 during *in vitro* digestibility assays; and they show no acute oral toxicity in mammalian studies. Assessment of potential dietary exposure to the novel proteins in SYHT0H2 soybean was based on the maximum concentrations of AvHPPD-03 and PAT found in unprocessed soybean seed on a fresh-weight basis in a study conducted in Argentina. The assessment was based on the assumption that 100% of the soybean consumed in South Africa would be SYHT0H2 soybean, and it did not take into account that the levels of the AvHPPD-03 and PAT would decrease during processing of the soybean seed into food products. Furthermore, the assessment was based on the amount of soybean or soybean-derived food products ingested on a single day in cluster diets. The theoretical daily intake of protein produced by SYHT0H2 soybean was calculated based on a body weight of 60 kg and a daily soybean consumption

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	of 11.2 g per day (WHO, 2011), margins of exposure were calculated by comparing the no-observed-effect levels in acute oral toxicity studies in rodents with the expected daily intakes. The expected intakes of AvHPPD-03 and PAT through consumption of SYHT0H2 soybean in South Africa are very low. The margins of exposure exceed factors of at least 431332 for AvHPPD-03 and 409 for PAT, supporting the conclusion that the risk to consumers is negligible. Studies comparing the composition of SYHT0H2 soybean plants and conventional, non-transgenic soybean support the conclusion that SYHT0H2 soybean is substantially equivalent to conventional soybean. SYHT0H2 soybean is highly unlikely to have adverse effects on human or animal health, given that its effects are unlikely to be different from those of conventional, non-transgenic soybean. The import and food, feed, or processing use of SYHT0H2 soybean seed is highly unlikely to have environmental effects through interactions with other organisms.
Evaluation of the consequences (Annex III.8(c)):	Cultivation of SYHT0H2 soybean in South Africa is not within the scope of this Application for Commodity Clearance of SYHT0H2 soybean. The import and food, feed, or processing use of SYHT0H2 soybean seed and processed products is not expected to have any adverse consequences for human or animal health or the South African environment. As discussed above, detailed evaluation of the characteristics of SYHT0H2 soybean and analysis of the likelihood of any adverse effects support the conclusion that SYHT0H2 soybean is substantially equivalent to conventional soybean and is therefore highly unlikely to have any adverse consequences for on human or animal health or the South African environment.
Overall risk (Annex III.8(d)):	The overall risk of potential adverse effects on human and animal health or the environment from the import and food, feed, or processing use of SYHT0H2 soybean seed or processed products is negligible in the context of the intended uses of SYHT0H2 soybean.
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.

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Additional information	
Availability of detailed risk assessment information:	More information on the SYHT0H2 soybean and the assessment of risk can be obtained from the application.
Any other relevant information:	No
Attach document:	Not applicable
Notes:	Not applicable

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