13 COMMON FORMAT FOR RISK ASSESSMENT

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

	Risk assessment details	
1.	Country Taking Decision:	South Africa
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	LMO information	
4.	Name and identity of the living modified organism:	Soybean (*Glycine max* L. Merr.) CV127 that is tolerant to the imidazolinone class of agricultural herbicides
5.	Unique identification of the living modified organism:	BPS-CV127-9
6.	Transformation event:	Event 127 (CV127)
7.	Introduced or Modified Traits:	Choose the trait from the following list: Altered growth, development and product quality **Chemical tolerance** - Herbicide tolerance
8.	Techniques used for modification:	A PvuII DNA fragment containing the *csr1-2* gene from *Arabidopsis thaliana*, excised from plasmid pAC321 was used to transform soybean embryogenic axis tissue using particle bombardment transformation technique and tissue culture regeneration.

9. Description of gene modification:	A 6.2 kilobase pairs (kb) PvuII DNA fragment, containing the *csr1-2* gene cassette derived from *Arabidopsis thaliana*, was excised from the constructed plasmid pAC321 and was used to transform embryogenic axis tissue derived from the apical meristem of a single soybean seed of the commercial variety Conquista. This variety and tissue type was chosen for insertion of the *csr1-2* gene because it responds well to particle bombardment transformation and tissue culture regeneration. Biolistic transformation (microprojectile or particle bombardment) was used to produce soybean transformation events containing the *csr1-2* gene. These cells were transferred to a selective media containing the equivalent of 100 g active ingredient (ai)/ha imazapyr, an imidazolinone herbicide, and only those cells transformed with the *csr1-2* gene continued to grow. From this process a tolerant T0 plant was identified and named Soybean Event 127 (CV127).
	Characteristics of modification
10. Vector characteristics (Annex III.9(c)):	Vector pAC321 Plasmid pAC321 is approximately 8.7 kb and the DNA fragment derived from it that was used in the transformation of soybean tissues is an approximately 6.2 kb PvuII fragment. This fragment was cleaved from plasmid pAC321 by restriction with the restriction endonuclease PvuII and was purified prior to use in transformation. Plasmid pAC321 consists primarily of the *E. coli* cloning plasmid pBluescript SK(-) (Stratagene, La Jolla, CA) with a 5.7 kb XbaI fragment comprising the *csr1-2* gene cassette from the genome of *Arabidopsis thaliana* cloned into it. The *csr1-2* gene cassette consists of the *csr1-2* gene encoding the acetohydroxyacid synthase large subunit (AtAHASL) protein which is responsible for the imidazolinone herbicide tolerance trait in CV127 soybeans, with its native promoter and 3'untranslated region from *Arabidopsis thaliana*. In addition to the *csr1-2* native gene promoter, the 5' region upstream of the *csr1-2* coding sequence contains the complete coding sequence of the *A. thaliana* SEC61γ protein, which is a component of the DNA fragment used for transformation. The backbone section of plasmid pAC321 (the section outside of the borders of the transformation fragment) consists of genes and genetic elements derived from the *E. coli* cloning plasmid pBluescript SK(-).
11. Insert or inserts (Annex III.9(d)):	See 10 above.

Recipient organism or parental organisms (Annex III.9(a)):		
12. Taxonomic name/status of recipient organism or parental organisms:	Kingdom Subkingdom Division Class Subclass Order Family Genus Species	Plantae -- plants Tracheobionta -- vascular plants Magnoliophyta -- angiosperms, flowering plants Magnoliopsida -- dicots Rosidae Fabales Fabaceae *Glycine* Willd. -- soybean *Glycine max* (L.) Merr. -- soybean
13. Common name of recipient organism or parental organisms:	Soybean	
14. Point of collection or acquisition of recipient or parental organisms:	Brazil	
15. Characteristics of recipient organism or parental organisms related to biosafety:	Cultivated soybean is an erect, bushy herbaceous summer annual that can reach a height of 1.5 meters. None of the soybean varieties are frost tolerant, and they do not survive freezing winter conditions. The primary leaves are unifoliate, opposite and ovate, the secondary leaves are trifoliolate and alternate, and compound leaves with four or more leaflets are occasionally present. The nodulated root system consists of a taproot from which emerges a lateral root system. The plants of most cultivars are covered with fine trichomes, but glabrous types also exist. Its papilionaceous flowers consist of a tubular calyx of five sepals, a corolla of five petals, one pistil, and nine fused stamens with a single separate posterior stamen. The pod is straight or slightly curved, varies in length from two to seven centimetres, and consists of two halves of a single carpel which are joined by a dorsal and ventral suture. The shape of the seed, usually oval, can vary amongst cultivars from almost spherical to elongate and flattened (OECD, 2000).	

16. Centre(s) of origin of recipient organism or parental organisms:	The cultivated soybean, *Glycine max* (L.) Merr., a diploidized tetraploid (2n = 40) that belongs to the family Fabaceae and to the Genus *Glycine* Willd. Cultivated soybeans are not found in natural habitats in the wild outside of cultivation. The genus *Glycine* Willd. contains two subgenera, *Glycine* and *Soja* (Moench) F.J. Herm. The subgenus *Glycine* comprises 22 wild perennial species that are indigenous to Australia, islands in the west, central and southern Pacific Ocean, China, Russia, Japan, Indonesia, Korea, Papua New Guinea, the Philippines, and Taiwan (Hymowitz, 2004). The cultivated soybean, *G. max* (L.) Merr. and its wild annual relatives from Asia, *G. soja* Sieb. and Zucc. are classified in the subgenus *Soja*. *Glycine soja* is an annual that grows in the wild in fields, hedgerows, roadsides, and riverbanks in many countries of East Asia. This plant has a slender build with narrow trifoliate leaves. The flowers are purple, or on rare instances white, and are found on short, slender racemes. The pods are short and tawny with hirsute pubescence and contain oval-oblong seeds (Hermann, 1962). In addition to *G. max* and *G. soja*, the subgenus *Soja* also contains a form known as *G. gracilis*. This semi-cultivated or weedy plant is found only in Northeast China and is intermediate in morphology between *G. max* and *G. soja*. *G. gracilis* was first proposed as a new species of *Glycine* but more recent examinations have concluded it to be a variant of *G. max* (Hermann, 1962; Wang, 1976; Shoemaker et al., 1986). Within the tribe Phaseoleae, the genus *Glycine* is the only genus containing species that have diploid chromosome numbers of 40 and 80 and not 20 (Lackey, 1980). Based on taxonomic, cytological, and molecular systematics evidence, it has been proposed that the unique chromosome number of *Glycine* is most likely derived from an unknown progenitor species with a chromosome base number of 11. From this ancient progenitor, a putative ancestor of *Glycine* arose in Southeast Asia with 2n = 20 (Kumar and Hymowitz, 1989; Singh and Hymowitz, 1999; Lee and Hymowitz, 2001; Singh et al., 2001). Tetraploidization (2n = 2x = 40) through auto- or allopolyploidy of this ancestor species occurred at some time to produce a species in which 2n = 40. The sequence of events in the development of *G. max* from the ancient progenitor species is proposed by Singh et al. (2001) to be: wild perennial (2n = 4x = 40; unknown or extinct) to wild annual (2n = 4x = 40; *G. soja*) to soybean (2n = 4x = 40; *G. max*).
17. Centres of genetic diversity, if known, of recipient organism or parental organisms:	See 16 above.

18. Habitats where the recipient organism or parental organisms may persist or proliferate:	Soybean is commonly considered one of the oldest cultivated crops in the world. It is grown as a commercial crop in over 35 countries. The major producers of soybean are the U.S.A., China, Argentina, and Brazil. Soybean is a quantitative short day plant and hence flowers more quickly under short days. As a result, photoperiodism and temperature response are important in determining areas of cultivar adaptation. Soybean cultivars are identified based on bands of adaptation that run east-west, determined by latitude and day length. There are thirteen maturity groups (MG), from MG 000 in the north (45° latitude) to MG X near the equator. Within each maturity group, cultivars are described as early, medium, or late maturing. The seed will germinate when the soil temperature reaches 10°C and will emerge in a 5 - 7 day period under favourable conditions. In new areas of soybean production an inoculation with *Bradyrhizobium japonicum* is necessary, for optimum efficiency of the nodulated root system. Soybeans do not yield well on acid soils and the addition of limestone may be required. Soybeans are often rotated with such crops as corn, winter wheat, spring cereals, and dry beans.
Donor organism or organisms (Annex III.9(b)):	
19. Taxonomic name/status of donor organism(s)	The *csr1-2* gene that has been inserted into the genome of soybean CV127 is derived from *Arabidopsis thaliana* (L.) Heynh. (common name thale cress). *A. thaliana* is not known to be a toxicant, irritant, a human or animal pathogen and is not known to cause allergic reactions in humans. Arabidopsis is a member of the mustard (*Brassicaceae*) family, which includes cultivated species such as cabbage and radish. It is not known to be a source of allergens or toxins and it is widely used as a model organism in plant biology and genetics research.
20. Common name of donor organism(s):	Thale cress

21. Point of collection or acquisition of donor organism(s):	Arabidopsis is generally considered a weed and originates from Europe. It occurs naturally throughout temperate regions of the world including East Africa, Asia, and Japan. It is also found in North America and Australia, following introduction to these regions. Its natural habitat is open free draining ground, such as poor sandy or gravelly soil and can commonly be found growing on wasteland. *A. thaliana* is not sexually compatible with any other cultivated or wild plant species. Outcrossing in natural populations of *A. thaliana* is a very rare phenomenon and the average outcrossing in the wild does not exceed 0.3% (Abbott and Gomes, 1989). Accordingly, the natural transfer of the *SEC61γ* subunit gene from Arabidopsis to other individuals of the same species is highly unlikely. The same is true for the *csr1-2* allele of the *ahasl* gene if it was present in wild populations of Arabidopsis. Both the AHAS and the SEC61γ proteins are ubiquitous in plants and have a history of safe use as they are commonly present in food crops.
22. Characteristics of donor organism(s) related to biosafety:	See 21 above.
Intended use and receiving environment	
23. Intended use of the LMO (Annex III 9(g)):	Tolerance to applications of non selective herbicides belonging to the imidazolinone group. CV127 soybeans were produced by introduction of the imidazolinone-tolerant acetohydroxyacid synthase large subunit gene *csr1-2* from *Arabidopsis thaliana* into the soybean plant genome. The *csr1-2* gene encodes an AHASL protein that is tolerant to imidazolinone herbicides due to a single nucleotide mutation that results in a single amino acid substitution in which the serine residue at position 653 of the protein is replaced by asparagine (S653N). This amino acid change in plant AHAS proteins is known to prevent the binding of imidazolinone herbicides and thereby to result in tolerance to these herbicides (Tan et al., 2005).
24. Receiving environment (Annex III.9(h)):	The receiving environment of soybeans containing the CV127 soybeans will be the soybean lands of Argentina and Brazil where soybeans are currently planted. Regarding biological diversity and centres of origin refer to 16, 17, and 18 above.

	Risk assessment summary
25. Detection/Identification method of the LMO (Annex III.9(f)):	Molecular Methods: An event-specific real-time PCR method for the detection of CV127 soybean is available that can be applied to determine the relative content of CV127 soybean DNA in total soybean DNA. The protocol is capable of detecting one CV127 soybean grain in a mixture of 2500 conventional soybean grains (0.04%). The lowest amount of CV127 in a sample that can be reliably quantified with an acceptable level of precision and accuracy is 0.080%. Bio Assay. The herbicide tolerance in CV127 soybean will allow treatment of the soybean crop with imidazolinone herbicides for weed control without causing injury to the soybean plant at normal field application rates. Tolerance of soybeans to non-selective imidazolinone herbicides can be utilized to indirectly provide evidence for the presence of the *csr1-2* gene from Arabidopsis.
26. Evaluation of the likelihood of adverse effects (Annex III.8(b)):	Considering the following: a) the nature of the donor organism, b) the history of safety for the recipient soybean plant, c) the substantial equivalence of soybeans containing the CV127 event to conventional soybean, d) the absence of hazard potential associated with the introduced trait, and e) the history of safety for the AHAS protein, no adverse effects are anticipated.
27. Evaluation of the consequences (Annex III.8(c)):	See 26 above. Considering a) to e) above no adverse effects are anticipated. In the highly unlikely event of an adverse effect being recognized and recognizing the biology of the soybean, such effects could be controlled by stopping the sale and planting of seed. Any established planting or escapes could be controlled by either mechanical or chemical measures (excluding imidazolinone herbicides).
28. Overall risk (Annex III.8(d)):	Considering that no adverse effects have been identified in 26 above, no specific measures are anticipated in terms of risk management considering the substantial equivalence of CV127 soybeans to conventional soybeans. Soybeans have a very long history of safety both in terms of feed, food, and human safety also including impacts on the environment.
29. Recommendation (Annex III.8(e)):	No adverse environmental risks for CV127 soybeans have been identified that are any different from conventional soybeans. CV127 soybeans should therefore be treated as conventional soybeans that have a long history of safe use for consumption and environmental impacts.

30. Actions to address uncertainty regarding the level of risk (Annex III.8(f)):	No uncertainties have been identified regarding CV127 soybeans. Considering a long history of safe production of crops (since 2003 Clearfield® crops maize, wheat, rice and rape seed) that contain an imidazolinone herbicide-tolerant AHASL protein with the same S653N amino acid substitution as that in the AtAHAS encoded by the *csr1-2* gene in CV127 soybeans, the level of risk is the same as for conventional soybeans. No special actions are required.
	Additional information
31. Availability of detailed risk assessment information:	All the current information is contained in the application form. Further detail of risk assessments is available in the applications for general release of CV127 soybeans as presented in Brazil and Argentina petitions. This information probably accompanied these petitions.
32. Any other relevant information:	None
33. Attach document:	*Not applicable to applicant* <Specific types of entry: option to choose a file from the local source and 'upload' a copy to the BCH server>
34. Notes:	