PART II

14. 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
2. Title:	Application for Commodity Clearance of Genetically Modified Organisms (GMO) – HB4 wheat
3. Contact details:	Trigall Genetics S.A of Ocampo 210 Bis, Predio CCT, Rosario, Argentina **Represented by** Biolegal (Pty) Ltd of 45 Rutland Road Parkwood Johannesburg South Africa

LMO information

4. Name and identity of the living modified organism:	Wheat *(Triticum aestivum L.)* transformed with the *HaHB4* gene from the sunflower and the *bar* marker gene from *Streptomyces hygroscopicus* to exhibit increased yield opportunity under environmental stress and tolerance to glufosinate herbicides.
5. Unique identification of the living modified organism:	Unique identifier IND-ØØ412-7
6. Transformation event:	*HaHB4* gene *bar* gene
7. Introduced or Modified Traits:	Choose the trait from the following list: A. Abiotic environmental tolerance - Drought or water tolerance - Other abiotic environmental tolerance B. Altered growth, development and product quality **Chemical tolerance** - Herbicide tolerance
8. Techniques used for modification:	Particle bombardment co-transformation with two plasmids each carrying the relevant genes: 1) *HaHB4* codifying for a sunflower transcription factor (TF) that is involved in the plant response to environmental stresses, and 2) the *bar* gene from *Streptomyces hygroscopicus*, expressing the glufosinate-inactivating enzyme phosphinothricin N-acetyl transferase (PAT).

9. Description of gene modification:	*HaHB4* encodes for a sunflower transcription factor which provides the plant with increased yield potential under environmental stresses. The *bar* gene from *Streptomyces hygroscopicus* encodes for the glufosinate-inactivating enzyme, phosphinothricin N-acetyl transferase (PAT) which provides the plant with increased tolerance to glufosinate-based herbicides.
Characteristics of modification	
10. Vector characteristics (Annex III.9(c)):	Two plasmids, *pIND4-HB4* and *pIND4-Bar*, carrying the *HaHB4* and *bar* genes, respectively, were used to generate wheat event IND-ØØ412-7 . These vectors are based on a set of plasmids that have been constructed on *pUC8* or *pUC19* plasmid sequences by fusing the same 1992 bp *PstI* fragment from the ubiquitin maize *Ubi-1* gene sequence to the relevant genes. This *Ubi-1 PstI* fragment contains 899 bp of promoter sequence, 83 bp of 5' untranslated exon, and 1010 bp of first intron sequence upstream of restriction sites used in the construction of the chimeric genes (the promoter "construct" is therefore *prUbi-1/Ubi-1Exon/Ubi-1Intron*). This *Ubi-1* promoter construct has been shown to be highly active in monocotyledonous plants, to facilitate efficient transformation and to drive gene expression. The original intron present in the 5'-untranslated region of the *Ubi-1* gene was retained in all the constructs of this series because previous studies have shown that introns frequently exert a strong enhancing effect on transgene expression in cereals.

11. Insert or inserts (Annex III.9(d)):	*HaHB4* encodes for a sunflower transcription factor which provides the plant with increased yield potential under environmental stresses. The *bar* gene from *Streptomyces hygroscopicus* encodes for the glufosinate-inactivating enzyme, phosphinothricin N-acetyl transferase (PAT) which provides the plant with increased tolerance to glufosinate-based herbicides.
Recipient organism or parental organisms (Annex III.9(a)):	
12. Taxonomic name/status of recipient organism or parental organisms:	*Triticum aestivum*
13. Common name of recipient organism or parental organisms:	Wheat (bread wheat)
14. Point of collection or acquisition of recipient or parental organisms:	No element used in the development of this product required collection from a natural source. All genetic elements used in the constructions were synthetic and the conventional variety used for the transformation is commercially available.
15. Characteristics of recipient organism or parental organisms related to biosafety:	None. IND-ØØ412-7 wheat has been shown to be substantially equivalent to conventional wheat. No import of propagating material (seed) or planting of any GM seed of IND-ØØ412-7 wheat is envisaged in terms of this commodity approval.

16. Centre(s) of origin of recipient organism or parental organisms:	Hexaploid wheat is believed to have originated in the "Fertile Crescent" of southwest Asia and to have resulted from the wild form of einkorn (*Triticum monococcum* L. sensulato, AA genome cluster) through two hybridization and spontaneous chromosome doubling events (Willenborg and Van Acker, 2008). Most of the wild and cultivated wheat are one of three species, *T. compactum* Host. (club wheat varieties), *T. turgidum* L. ssp. *durum* (Desf.) Husn. (durum wheat varieties), or *T. aestivum* (hexaploid wheat varieties), the latter being the most commonly cultivated species worldwide.
17. Centres of genetic diversity, if known, of recipient organism or parental organisms:	The exact location of the centre of origin of wheat is unknown, but currently believed to be in the "Fertile Crescent" - a hilly and mountainous region extending from the foothills of the Zagros mountains in south-western Iran, through the Tigris and Euphrates basins in northern Iraq and south eastern Turkey, continuing southwestward over Syria to the Mediterranean, and extending to central Israel and Jordan. Wild taxa belonging to 4 species of wheat and 17 species of the closely-related genus *Aegilops* are endogenous to this region, which most likely was the area of wheat domestication. This assumption is supported by ample archaeological evidence. The approximate centre of this area is given by: Longitude: 32.357; Latitude: 45.312

18. Habitats where the recipient organism or parental organisms may persist or proliferate:	Wheat (*T. aestivum*) exists only in cultivated fields. In South Africa this would be the winter and summer rainfall fall regions of the western Cape and Free State where conventional wheat is cultivated, as well as irrigation areas in the Northern Cape.
Donor organism or organisms (Annex III.9(b)):	
19. Taxonomic name/status of donor organism(s)	*Helianthus annuus* *Streptomyces hygroscopicus*
20. Common name of donor organism(s):	Common sunflower N/A
21. Point of collection or acquisition of donor organism(s):	No element used in the development of this product required collection from a natural source. All genetic elements used in the constructions were synthetic and the conventional variety used for the transformation is commercially available.

22. Characteristics of donor organism(s) related to biosafety:	Wheat is a predominantly self-pollinating crop. The frequency of pollen mediated gene flow (PMGF) in wheat is low and decreases rapidly with distance from the pollen source (Beckie & Hall, 2008; Martin 1990). Seeds are the only wheat reproductive structures. Early reproductive development is highly vulnerable to water deficit stress (Saini & Westgate, 1999; Feng *et al.*, 2017). Wheat is generally considered to have lost its seed dispersal mechanisms. Domesticated wheat is not able to compete well and lacks characteristic weedy lifestyle traits, such as prolonged seed dormancy, extended persistence in the soil, germination under a broad range of environmental conditions, rapid vegetative growth, a short life cycle, very high seed output, a high rate of seed dispersal (with high shattering), long distance seed dispersal and development of ecotypes (Keeler, 1985; Keeler, 1989). Additionally, wheat does not naturally grow outside of cultivation areas. Consequently, domesticated wheat should not be considered as having weediness or invasiveness potential.
Intended use and receiving environment	
23. Intended use of the LMO (Annex III 9(g)):	The intended use is for the Commodity Clearance of HB4 wheat to enable trade with countries that cultivate the LMO.
24. Receiving environment (Annex III.9(h)):	The application is not for cultivation in South Africa
Risk assessment summary	

25. Detection/Identification method of the LMO (Annex III.9(f)):	The detection method for the wheat event IND-ØØ412-7 using real time PCR has been provided to the Registrar along with this application.
26. Evaluation of the likelihood of adverse effects (Annex III.8(b)):	As determined through extensive field and laboratory studies, the only biologically relevant differences between wheat event IND-ØØ412-7 and conventional varieties are: 1) the expression of the HAHB4 protein and the resultant phenotype that provides an increased yield opportunity, and 2) the expression of the PAT enzyme, providing tolerance to glufosinate-ammonium-based herbicides. Multiple lines of evidence support the conclusion that wheat event IND-ØØ412-7 will not have adverse consequences: 1) molecular characterization, 2) protein analysis, 3) HAHB4 protein history of consumption and its food safety profile, 4) compositional analysis, and 5) characterization of the plant agro-phenotypic features. From a food and feed safety perspective, wheat event IND-ØØ412-7 variety will provide a reliable and safe source of wheat food products.

27. Evaluation of the consequences (Annex III.8(c)):	Studies confirmed that this product is agronomically and compositionally equivalent to its conventional counterpart and that there is no increased tendency towards weediness or an increased susceptibility to insect pests normally associated with wheat. The consequences of any risk materialising is minimal. No potentially adverse effects were detected based on the extensive characterization studies performed including molecular characterisation; expression analysis, protein assessments, compositional analysis and phenotypic evaluation. HB4 wheat showed no increased potential to persist in the environment without human intervention or to become more invasive when compared to its conventional counterpart.
28. Overall risk (Annex III.8(d)):	The overall risk, to human and animal health and the environment, associated with the commodity clearance of HB4 wheat is minimal.
29. Recommendation (Annex III.8(e)):	No risks have been identified and therefore no risk mitigation recommendations are made.
30. Actions to address uncertainty regarding the level of risk (Annex III.8(f)):	No risks have been identified and therefore no actions are recommended to address uncertainty. Any permit conditions issued will be complied with.
Additional information	
31. Availability of detailed risk assessment information:	Further detail is available in the full dossier provided in Part I.

32. Any other relevant information:	None
33. Attach document:	*N/A*
34. Notes:	None