Application for authorization to placing on the market in the European Union of products produced from genetically modified environmental stress-tolerant and glufosinate-tolerant soybean IND-ØØ41Ø-5, for food and feed uses, import and processing, under Regulation (EC) No 1829/2003 and Commission Implementing Regulation (EU) No 503/2013

EFSA-GMO-XXX-2020 / EFSA-Q-2020-XXX

ANNEX I

PART VII

SUMMARY OF APPLICATIONS

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1. GENERAL INFORMATION

1.1. Details of application

(a) Member State of application

Belgium

(b) Application number

Not available at time of application – to be determined.

(c) Name of the product (commercial and any other names)

IND-ØØ41Ø-5 soybean (“HB4 soybean”).

(d) Date of acknowledgement of valid application

Not available at time of application – to be provided.

1.2. Applicant

(a) Name of applicant

Verdeca LLC (a joint venture between Arcadia Biosciences, Inc. and Bioceres S.A.).

(b) Address of applicant

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Davis, CA 95618 United States

(c) Name and address of the representative of the applicant established in the Union (if the applicant is not established in the Union)

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1.3. Scope of the application

This application is for food, feed and processing uses of IND-ØØ41Ø-5 soybean. No cultivation of IND-ØØ41Ø-5 soybean is intended in the European Union.

(a) Genetically modified food

Yes Food containing or consisting of genetically modified plants.

Yes Food produced from genetically modified plants or containing ingredients produced from genetically modified plants.

(b) Genetically modified feed.

Yes Feed containing or consisting of genetically modified plants.

Yes Feed produced from genetically modified plants.

(c) Genetically modified plants for food or feed uses.

Yes Products other than food and feed containing or consisting of genetically modified plants with the exception of cultivation.

No Seeds and other plant propagating material for cultivation in the Union.

1.4. Is the product or the uses of the associated plant protection product(s) already authorised or subject to another authorisation procedure within the Union?

___ No

X Yes (in that case, specify)

IND-ØØ41Ø-5 soybean contains the *bar* gene, expressing the PAT protein, which confers tolerance to glufosinate-based herbicides. This plant protection product has been already authorised within the EU (see, for example, EFSA, 2016a, 2017b, d). There is also an EFSA peer reviewed risk assessment document referring to glufosinate (EFSA, 2012b).

1.5. Has the genetically modified plant been notified under Part B of Directive 2001/18/EC?

___ Yes

X No (in that case, provide risk analysis data on the basis of the elements of Part B of Directive 2001/18/EC)

Deliberate release of IND-ØØ41Ø-5 soybean into the environment in the European Union is not within the scope of this application. The ERA included in this application, which did not identify adverse effects to human, animal or the environment for the proposed uses of IND-ØØ41Ø-5, has the purpose of contemplating the unlikely possibility of unintended, accidental release.

1.6. Has the genetically modified plant or derived products been previously notified for marketing in the Union under Part C of Directive 2001/18/EC?

X No

___ Yes (in that case, specify)

1.7. Has the product been subject to an application and/or authorised in a third country either previously or simultaneously to this application?

_ No

X Yes (In that case, specify the third country, the date of application and, where available, a copy of the risk assessment conclusions, the date of the authorisation and the scope of the application).

Pre-authorisations

Field trials. Soybean event IND-ØØ41Ø-5 soybean have been field tested in four countries by Verdeca since 2009, under the corresponding permits of the relevant Competent Authorities for regulated release.

- Trials were conducted in Argentina in 2009, 2010, 2011, 2012, 2013 and 2014 under permit numbers 324663/2008, 21385/09, 283911/2009, 11555-1/10, 24817-1/2011, 95477/2012, 531006/2013 and 569139/2013 (extended permit) from CONABIA (Comisión Nacional Asesora de Biotecnología Agropecuaria), the Argentine agro-ecosystem biosafety regulatory authority. Additional trials for variety development have been conducted in Argentina after deregulation in 2015.

- Trials were conducted in the United States (US) in 2011, 2012, 2013, 2014, 2015, 2017, 2018 and 2019 under US Department of Agriculture (USDA) notification numbers 11-122-110n, 12-097-106n, 12-121-104n, 12-131-102n, 13-018-102n, 13-106-105n, 13-130-103n, 14-101-108n, 15-106-103n, 15-183-103n, 17-111-104n, 17-117-102n, 17-151-102n, 18-157-101n, 19-114-103n and 19-175-102n.

- Field trials were conducted in Paraguay in 2016-2017, under Resolución MAG (Ministry of Agriculture and Livestock) # 582.

- Trials were also conducted in Brazil during 2016-2017 and 2017-2018 (Processo n° 01200.001068/2016-98, Extrato Prévio: 5071/2016 and 5693/2017). Additional trials for variety development have been conducted in Brazil after approval.

- A couple of field trials were carried out in China for seed increase: Permits NongJiAnShenZi (2016) No.011, NongJiAnBanBaoGaoZi (2018) No.259

Submission under review.

- The IND-ØØ41Ø-5 soybean safety dossier was submitted in Uruguay Ministry of Livestock, Agriculture and Fisheries on February 9, 2015, requesting approval for environmental release for commercial production and for food and feed consumption and processing (Case no. 2015/7/1/1/378 - 02/09/15).

- IND-ØØ41Ø-5 soybean was presented to the regulatory authorities of China for food and feed consumption and processing (October 2016).

- Soybean event IND-ØØ41Ø-5 was submitted to Health Canada, Canadian Food Inspection Agency's Plant Biotechnology Office and Animal Feed divisions in October 1, 2018 under file U-18-VER1-0000-SOY-01 and is currently under review. Verdeca is requesting approval for environmental release, food and feed consumption.

- Dossiers submitted to the Regulatory Authorities of Bolivia (November 2019) and India (January 2019) for the purposes of approval of its use in food and feed are currently under review in these countries.

Argentina authorization status:

CONABIA and the Biotechnology Directorate of the Ministry of Agriculture, Livestock, & Fisheries completed their reviews and concluded that soybean event IND-ØØ41Ø-5 is as safe for the environment as current commercial soybeans varieties (on April 29, 2015). IND-ØØ41Ø-5 soybean has been also approved for the Argentina's National Service of Agricultural and Food Health and Quality (SENASA, in 2015) for food and feed use. On October 6, 2015, Argentina´s Ministry of Agriculture, Livestock and Fisheries (2015, Resolución 397/2015) provided approvals for food safety and environment but commercial planting for local cultivation was delayed for international trade issues, pending on China import approval. This delay is not related by any safety issues, but solely due to the strong dependence of soybean exports for the country trade income.

Post-authorisation

- In the US, Verdeca completed on August 7, 2015 an Early Food Safety Evaluation (EFSE) from the US Food and Drug Administration (FDA) Center for Food Safety and Applied Nutrition for the HAHB4 protein variant (HAHB4) produced by HB4 soybeans (FDA 2015; NPC 000016; HAHB4). The FDA concluded it had no further questions. Information on this approval can be found online at: https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=NPC

The soybean event IND-ØØ41Ø-5 safety assessment for this new plant variety was submitted for consultation to the FDA on May 12, 2016 (BNF 000155) and a full FDA consultation was completed on August 2, 2017. The FDA concluded it had no further questions. Information on this approval can be found online at: https://www.accessdata.fda.gov/scripts/fdcc/?set=Biocon These two FDA approvals are for food and feed consumption of IND-ØØ41Ø-5 soybean within the US.

For environmental release in the US, a Petition for Determination of Nonregulated Status (17-223-01P) was submitted to the USDA August 7, 2017 and was deregulated on August 7, 2019. Within the USDA's Plant Pest Risk Assessment process, it was concluded HB4 soybeans (event IND-ØØ41Ø-5) "was unlikely to pose a greater plant pest risk than unmodified organism from which it was derived". Complete information on the USDA environmental release deregulation of IND-ØØ41Ø-5 can be found online at: https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/permits-notifications-petitions/petitions/petition-status .

The USDA plant pest risk assessment and environmental risk assessment portions of the deregulation are attached among the references's pdf files (USDA, 2019a, b).

- The IND-ØØ41Ø-5 soybean safety dossier was submitted in Brazil by Tropical Melhoramento e Genética S/A (TMG) in June 2018 for environmental release, food and feed consumption and was approved in May 2019 (Extrato De Parecer Técnico Nº 6.450/2019. Information on the deregulation of IND-ØØ41Ø-5 in Brazil can be found online at http://ctnbio.mctic.gov.br/liberacao-comercial/-/document_library_display/SqhWdohU4BvU/view/614405#/liberacao-comercial/consultar-processo).

- In November 2018, Soybean event IND-ØØ41Ø-5 was presented to the regulatory authorities of Paraguay and successfully completed the regulatory review process on November 2019 and received approval for its IND-ØØ41Ø-5 soybean drought and herbicide tolerant soybeans from the Paraguayan Minister of Agriculture, through the National Commission for

Agricultural and Forestry Biosafety (Resolución 269/2019, https://conbio.mag.gov.py/index.php/resoluciones).

1.8. General description of the product

(a) Name of the recipient or parental plant and the intended function of the genetic modification:

The recipient plant is soybean.

Soybean event IND-ØØ41Ø-5 was developed through the introduction of two genes: the *HaHB4* (*Helianthus annuus homeobox 4)* gene from sunflower (*Helianthus annuus*) and the *bar* (bialaphos-resistance) gene from *Streptomyces hygroscopicus*. The *HaHB4* gene encodes the HAHB4 protein, a transcription factor that has been demonstrated to be upregulated by various environmental stresses (Dezar et al., 2005; Manavella et al., 2006; Cabello et al., 2007; Chan, 2009). The *HaHB4* gene confers improved yield under a range of soybean growing conditions. IND-ØØ41Ø-5 soybean also contains the *bar* gene, expressing the protein L-phosphinothricin N-acetyltransferase (PAT) which provides field tolerance to the glufosinate herbicides (trade names BASTA, Buster, Liberty).

The IND-ØØ41Ø-5 soybean traits, maintenance of yield opportunity under environmental stress and glufosinate tolerance, were engineered in Argentina into Williams 82, a soybean variety developed by the USDA and the Illinois Agriculture Experiment Station in 1981.

(b) Types of products planned to be placed on the market according to the authorisation applied for and any specific form in which the product must not be placed on the market (such as seeds, cut-flowers, vegetative parts) as a proposed condition of the authorisation applied for:

According to this application, authorisation for placing on the market is sought for products that will consist of IND-ØØ41Ø-5 soybean grain, meal, protein-derived products, such as oil, and other processed food and feed components commonly derived from soybean grain. Soybean seed is not intended for placing on the EU market and therefore seed or cultivation are beyond the scope of this application.

(c) Intended use of the product and types of users:

IND-ØØ41Ø-5 soybean grain, meal, protein-derived products, oil and other food and feed components of or derived from soybean grain are intended to be used in the same way as other commodity soybean grain products and components already on the EU market.

(d) Any specific instructions and recommendations for use, storage and handling, including mandatory restrictions proposed as a condition of the authorisation applied for:

IND-ØØ41Ø-5 soybean and its derived products will be used, stored, handled, transported, packaged and labeled in the same way as other GM soybean varieties authorized in the EU. There are no specific instructions or recommendations other than those required as conditions of authorisation under Regulation (EC) No 1829/2003. There are not mandatory restrictions which might be proposed as a condition of the authorization applied for. However, the same as conventional soybean derived products, proper allergenic awareness labelling will be mandatory.

(e) If applicable, geographical areas within the Union to which the product is intended to be confined under the terms of the authorisation applied for:

Not applicable.

(f) Any type of environment to which the product is unsuited:

Not applicable within the European Union.

(g) Any proposed packaging requirements:

None. IND-ØØ41Ø-5 soybean grain and derivatives will be packaged similarly as other commodity soybean products.

(h) Any proposed labelling requirements in addition to those required by other applicable EU legislation than Regulation (EC) No 1829/2003 and when necessary a proposal for specific labelling in accordance with Article 13(2) and (3), Article 25(2)(c) and (d) and Article 25(3) of Regulation (EC) No 1829/2003.

In the case of products other than food and feed containing or consisting of genetically modified plants, a proposal for labelling which complies with the requirements of point A (8) of Annex IV to Directive 2001/18/EC must be included.

There are not specific labelling requirements for IND-ØØ41Ø-5 soybean. IND-ØØ41Ø-5 soybean will be labelled as conventional soybean, which includes an allergenicity warning advice. In accordance with Articles 12-14 and 24-26 of Regulation (EC) No 1829/2003, Article 13(2)f and Annex IV of Directive 2001/18/EC, and with Article 4 of Regulation (EC) No 1830/2003, IND-ØØ41Ø-5 soybean grain and derivatives will be labelled as “genetically modified soybeans” or “produced from genetically modified soybeans.” in accordance with Articles 12-14 and 24-26 of Regulation (EC) No 1829/2003 and the requirements of Article 5 of Regulation (EC) No 1830/2003.

Verdeca does not intend handling of commodity soybeans in the EU. Grain operators handling or using IND-ØØ41Ø-5 soybean grain, meal, protein-derived products, oil and other food and feed components of, or derived from soybean grain will be required to comply with the same legal obligations regarding traceability and labelling of these products as with conventional soybean. These requirements for the traceability and labelling of GMOs and derived foods and feeds are laid down in Regulations (EC) No 1829/2003 and 1830/2003.

(i) Estimated potential demand

(i) In the EU

The potential demand of the products derived from IND-ØØ41Ø-5 soybean will not impact on the current demand of the same products derived from other sources in the EU. IND-ØØ41Ø-5 soybean will be a commodity variety and will be grown and harvested outside the EU and placed on the market into the EU as whole grain, meal, oil or other soybean grain derived products. These products will enter the EU soybean commodity market streams along with other soybean products derived from other soybean commodity grains and since the nutrition, composition, quality and use-related features are indistinguishable in food or feed, IND-ØØ41Ø-5 soybean will not drive any associated specific demand in the food and feed markets.

(ii) In EU export markets

Should the EU to re-export IND-ØØ41Ø-5 soybean products, there will be no difference between IND-ØØ41Ø-5 soybean and other commodity soybean products where approvals are in place. Demand of soybean-derived products by prospective trade partners should not be affected by the placing IND-ØØ41Ø-5 soybean on the EU market.

(j) Unique identifier in accordance with Regulation (EC) No 65/2004.

IND-ØØ41Ø-5 soybean.

1.9. Measures suggested by the applicant to take in the case of unintended release or misuse of the product as well as measures for its disposal and treatment.

Unintended release or misuse of IND-ØØ41Ø-5 soybean can be treated in the EU with the same measures as any other conventional soybean product. If unintended release reached the status of a grown (volunteer) plant, IND-ØØ41Ø-5 soybean will remain susceptible to all usual soybean management practices, including promoting germination and growth and control with herbicides other than products containing glufosinate. Disposal by landfill, burial, burning and processing for consumption can all be applied to IND-ØØ41Ø-5 soybean in the same way as to conventional soybeans.

2. INFORMATION RELATING TO THE RECIPIENT OR (WHERE APPROPRIATE) PARENTAL PLANTS.

The biology of soybean is fully described in the Consensus Document on the Biology of *Glycine max* (L.) Merr. (Soybean) (OECD, 2000). Language from the OECD document is incorporated in this dossier to provide the following details of soybean biology.

2.1. Complete name:

IND-ØØ41Ø-5 (HB4 soybean).

(a) family name; Fabaceae – legumes.

(b) genus; *Glycine* Willd.

(c) species; Glycine max (L.) Merr.

(d) subspecies; not applicable.

(e) cultivar, breeding line; Williams 82.

(f) common name; soybean, soya bean, soy, soya.

2.2. Geographical distribution and cultivation of the plant, including the distribution within the Union.

Soybean is grown as a commercial crop in over 35 countries. Total world production in 2019/2020 was 342 million metric tons (USDA, 2020). The six major soybean producers in the 2019/2020 season were Argentina, Brazil, China, India, Paraguay and the US, accounting for

92% of the total world production. In the 2019/2020 season, production in the four major producers were (in million metric tons) 126 (Brazil), the US (97), Argentina (54) and China (18).

In 2019/2020, the EU imported more than 15 million metric tons of soybean (grain equivalent).

The production of soybean in the EU continent is increasing; the harvest production was 2.9 million tons in 2018, up from 2.7 million tons in 2017 and from the 0.8 million tons harvested in 2008 (https://ec.europa.eu/eurostat/statistics-explained/index.php/Agricultural_production_-_crops#Oilseeds). Soybean is grown in fifteen Member States (FAO, 2019). The four major soybean producing countries in the EU (Ukraine excluded) are Italy (1,139), Serbia (646), Romania (466) and France (400) (numbers correspond to 2018 and are expressed in thousand tons) (FAO, 2019).

Soybean is grown to produce seeds and the derived products including oil and meal as food and animal feed, respectively. Soybean has a multitude of uses in the food and industrial sectors and represents one of the major sources of edible vegetable oil and of proteins for livestock feed use.

2.3. Information concerning reproduction (for environmental safety aspects).

IND-ØØ41Ø-5 soybean does not differ from the conventional recipient plant or from other soybean varieties in terms of habitat, reproduction, generation time, dissemination or survivability. Soybean is a quantitative, short day plant and hence flowers more quickly under short days. As a result, photoperiodism and temperature response is important in determining areas of variety adaptation (OECD, 2000).

From the environmental safety perspective, reproductive physiology and habitat of IND-ØØ41Ø-5 soybean are the same as conventional soybean.

(a) Mode(s) of reproduction.

Soybean is considered a self-pollinated species, propagated commercially by seed. Artificial hybridization is used for variety breeding (OECD, 2000). The stress tolerance and glufosinate tolerance traits do not affect the reproductive behavior, as confirmed in field trial results in Argentina and the US.

(b) Specific factors affecting reproduction.

There are no specific factors of IND-ØØ41Ø-5 soybean that would affect its reproductive physiology as compared to conventional soybean varieties. The stress tolerance and glufosinate tolerance traits did not introduce any specific factor that may have affected reproduction, as was confirmed in field trial results in Argentina and the US field trials.

(c) Generation time.

IND-ØØ41Ø-5 soybean, the same as conventional soybeans, has a generation time of 3-5 months. The stress tolerance and glufosinate tolerance traits have not affected the generation time, as determined from field trial results in Argentina and the US.

2.4. Sexual compatibility with other cultivated or wild plant species (for environmental safety aspects)

There are no wild plant species in the EU sexually compatible with *Glycine max.* Also, there are no sexually compatible relatives in North and South America; consequently, pollen-mediated gene flow can only occur between cultivated varieties. However, such gene flow would be minimal because of the biology of soybean, an essentially self-pollinated species.

Glycine max (L.) Merr., the cultivated soybean, is a summer annual herb that has never been found in the wild (Hymowitz, 1970). The subgenus *Soja* contains, in addition to *G. max* and *G. soja*, the form known as *G. gracilis*, a form morphologically intermediate between the two. This is a semi-cultivated or weedy form and is known only from Northeast China (OECD, 2000).

Glycine soja is distributed throughout China, the adjacent areas of the former USSR, Korea, Japan and Taiwan. It grows in fields and hedgerows, along roadsides and riverbanks" (OECD, 2000).

Soybean is a self-pollinating crop that can only cross with other members of genus *Glycine*, that is subgenus *Soja*. The potential for gene flow in soybean is limited by 1) very low natural cross-pollination (less than 1%) with nearby soybean plants and 2) geographic isolation. Wild soybean species are endemic in China, Korea, Japan, Taiwan and the former USSR. These species are not naturalized in North and South America, and although they could occasionally be grown in research plots, there are no reports of their escape from such plots to unmanaged habitats (OECD, 2000). Natural outcrossing in soybeans is so low that US Certified Seed Regulations (CFR, Part 201) allows for seed production to be adjacent with only a separation distance adequate to prevent mechanical mixture. Consequently, from the perspective of environmental safety, the probability of gene transfer from IND-ØØ41Ø-5 soybean to other soybean plants via crossing with sexually compatible plants is very low and are not different than that of conventional soybean.

Harlan and de Wet (1971) developed the concept of three gene pools—primary (GP-1), secondary (GP-2), and tertiary (GP-3) based on the success rate of hybridization among/between species. Soybean GP-1 consists of biological species that can be crossed to produce hybrids that possess seed fertility. It contains the accessions of *G. max* and *G. soja* (i.e., they have sexual compatibility). GP-3 is the third outer limit of potential genetic sexual compatibility. Hybrids between GP-1 and GP-3 are lethal, or completely sterile, and gene transfer is not possible or requires radical techniques (Harlan and de Wet, 1971). GP-3 includes the 26 wild perennial species of the subgenus *Glycine.* Therefore, besides the obvious geographic isolation of cultivated areas, gene flow is not a risk to the biodiversity for domesticated soybean.

2.5. Survivability (for environmental safety aspects).

(a) Ability to form structures for survival or dormancy.

Cultivated soybean seed rarely displays any dormancy characteristics and only under certain environmental conditions grows as a volunteer in the year following cultivation. If this should occur, volunteers do not compete well with the succeeding crop and can easily be controlled mechanically or chemically. The soybean plant has not weedy lifestyle. In North and South America, *Glycine max* is not found outside of cultivation (OECD, 2000).

Soybean is not frost tolerant and does not survive freezing winter environments and is susceptible to depredation, rotation or winter germination which results in death of the plant (CFIA, 2012). These properties of conventional soybean did not change in IND-ØØ41Ø-5 soybean

(b) Specific factors affecting survivability.

Soybean is not a weedy species. It is non-invasive and does not grow in wild habitats. These properties have not been altered in IND-ØØ41Ø-5 soybean.

Soybean does not exhibit survival ability (voluntary plants, persistence in the seed bank). It is an annual non-latent crop, whose seeds generally do not survive from one growth cycle to another. The natural propagation is by seed, which are the only survival structures. Wild soybean species have not been found outside its center of origin. In the agro-ecosystems, soybeans generally do not survive due to predation, rotation, germination during winter resulting in the death of the seeds, or management practices used prior to planting the next crop (CFIA, 2012).

2.6. Dissemination (for environmental safety aspects).

(a) Ways and extent of dissemination.

All *Glycine max,* including Williams 82 and IND-ØØ41Ø-5 soybean chiefly disseminate through seed only. A small percentage, usually lower than 1%, of cross pollination has been measured (Weber and Hanson, 1961; Caviness, 1966; Lu, 2005). Soybean seed grows successfully only by human cultivation. Other methods of seed dissemination, including biotic and abiotic tolerance in unmanaged fields are low, as seeds do not possess adaptive abilities. Seed may disperse during transportation and handling, e.g. at sowing or during harvest. However, soybean is not an invasive crop and volunteer plants will usually not establish (ferality) due to unfavorable environmental conditions. Commercial soybean does not demonstrate weedy feature have not been found moving into non-agricultural ecosystems, and are not listed as a noxious weed species in the US (CFR, Part 360). Furthermore, soybean does not possess any of the attributes generally associated with weeds (Baker, 1974), such as seed dispersal and establishment as a dominant species in ecosystems. Nor do soybeans have ability to compete well with native vegetation in North and South America. Because soybean seed lacks dormancy and germinates quickly under adequate temperature and moisture conditions, it is easily controlled in cultivated fields (OECD, 2000).

(b) Specific factors affecting dissemination.

The introduced traits in IND-ØØ41Ø-5 soybean provide: 1) an increased yield opportunity under typical environmental conditions of soybean production (which include hydric stress), and 2) tolerance to glufosinate herbicides, compared with the Williams 82 control. These characteristics are not considered as conferring weediness potential and do not promote dissemination. Results from disease and insect damage, arthropods abundance, volunteer monitoring, symbiont interactions, dormancy and germination and pollen fertility demonstrated nearly no significant biological differences between IND-ØØ41Ø-5 soybean and the Williams 82 control that would indicate an overall selective advantage which would promote dissemination. Disease susceptibility of soybean IND-ØØ41Ø-5 is the same as displayed by the range found in conventional soybean varieties. In addition, any IND-ØØ41Ø-5 soybean volunteers would be controlled similarly to conventional soybeans, using herbicides (other than glufosinate) and cultivation to remove unwanted plants.

Therefore, there are no specific factors affecting dissemination in IND-ØØ41Ø-5 soybean which would differ from conventional soybean.

2.7. Geographical distribution within the Union of the sexually compatible species (for environmental safety aspects).

There are no wild plant species in the EU that are sexually compatible with *Glycine max*. Soybean is sexually compatible only with other species of the genus *Glycine* subgenus *Soja*. Therefore, the only sexually compatible species within the Union are other soybean cultivated (domesticated) varieties. However, it is unlikely IND-ØØ41Ø-5 soybean grain intended for use in the EU only for food and feed (as per the scope of this application) would be present at soybean-growing areas. Soybeans do not persist under non-agricultural conditions

Soybean is grown in 15 Member States (EC, 2017). The major soybean producing countries are Italy, France, Romania, Croatia, Austria and Hungary (USDA, 2017). Other soybean-growing Member States are Bulgaria, Czech Republic, Germany, Greece, Lithuania, Poland, Slovenia, Slovakia and Spain (Eurostat. 2018).

2.8. In the case of plant species not normally grown in the Union description of the natural habitat of the plant, including information on natural predators, parasites, competitors and symbionts (for environmental safety aspects).

Not applicable. Soybean is grown in the European Union.

2.9. Other potential interactions, relevant to the genetically modified plant, of the plant with organisms in the ecosystem where it is usually grown, or used elsewhere, including information on toxic effects on humans, animals and other organisms (for environmental safety aspects).

IND-ØØ41Ø-5 soybean does not have any traits or characteristics different from conventional soybean varieties that would display potential interactions with other organisms in the ecosystem or additional toxic effects on humans and other life forms.

Soybean contains compounds, such as protein allergens, trypsin inhibitors and lectins that may have a negative effect on human and animal nutrition. Although processing removes most of the deleterious compounds, labelling is used to alert allergen-sensitive consumers of foods containing soy proteins, as it is with conventional soybean-derived products.

Verdeca is not aware of any data or observations regarding IND-ØØ41Ø-5 soybean that would result in adverse environmental consequences from its introduction or any kind of interactions different from those exhibited by conventional soybean. As determined through field and laboratory studies, the only biologically relevant phenotypic differences between IND-ØØ41Ø-5 soybean and conventional varieties are 1) the very low-level of expression of the transcription factor encoded by the *HaHB4* gene and the resultant phenotype that provides an increased yield opportunity under a broad array of environmental conditions, and 2) the expression of field-effective levels of the PAT protein, conferring glufosinate herbicide tolerance. Multiple lines of evidence support the conclusion that IND-ØØ41Ø-5 soybeans will not have adverse consequences: 1) molecular analysis, 2) protein expression analysis, 3) HAHB4 protein history of consumption, 4) compositional analysis, and 5) characterisation of the plant agro-phenotypic characteristics.

3. MOLECULAR CHARACTERISATION.

3.1. Information relating to the genetic modification.

(a) Description of the methods used for the genetic modification.

The soybean transgenic event IND-ØØ41Ø-5 was generated through *Agrobacterium*-mediated transformation of cotyledonary nodes of soybean seedlings variety Williams 82 with the binary plasmid pIND2-HB4.

(b) Nature and source of the vector used.

The plasmid vector used for transformation, pIND2-HB4 (Figure 3.1), derives from the pPZP family of *Agrobacterium* binary vectors (Hajdukiewicz y col, 1994). These vectors use the T-DNA borders of *Agrobacterium* plasmid pTiT37, the pBR322bom (basis of mobility) site for mobilisation from *Escherichia coli* to *Agrobacterium*, and the replication origins of pBR322 (*ColE1)* and pVS1 plasmids for replication in *E. coli* and *A. tumefaciens*, respectively. The transformation vector carries an *aadA* marker gene for selection conferring resistance to amino-glucoside antibiotics streptomycin/spectinomycin. This antibiotic resistance gene is only used as selection marker during selection of transformed cells and is outside the T-DNA region inserted in soybean IND-ØØ41Ø-5.

Agrobacterium tumefaciens strain EHA101 was used for the transformation process (Hood et al., 1986). The T-DNA includes a 2x35S-*bar*-Tvsp cassette and an LPF-*HaHB4*-nos cassette, where LPF is one of the allelic forms of the sunflower natural promoter of the *HaHB4* gene.

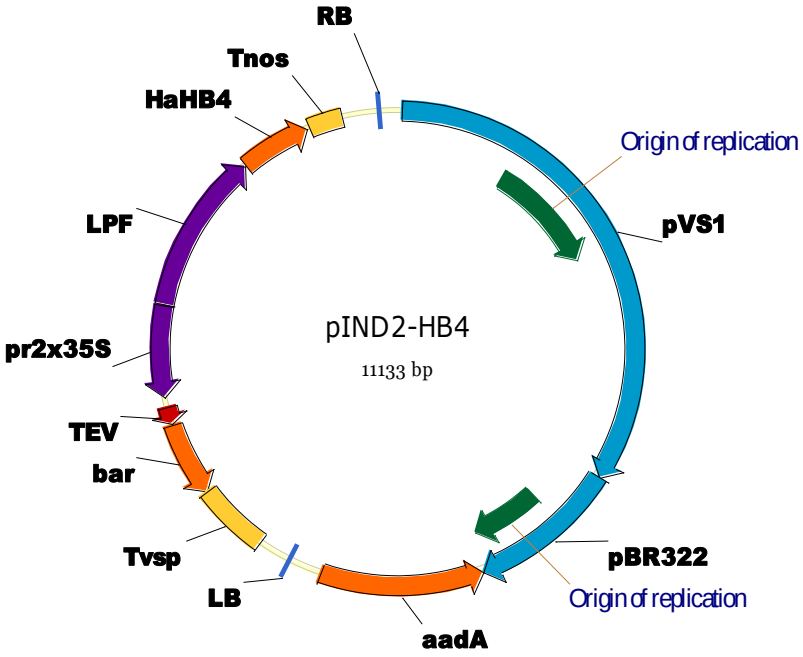

Figure 3.1. pIND2-HB4 Vector Map. Binary vector used to transform soybean cultivar Williams 82. All the genetic elements including coding (*HaHB4* and *bar*) and regulatory sequences are indicated.

(c) Source of donor nucleic acid(s) used for transformation, size and intended function of each constituent fragment of the region intended for insertion.

A summary of the IND-ØØ41Ø-5 genetic elements and their position on the source vector are provided in Table 3.1. The table shows the constituents of the vector and what was transformed into IND-ØØ41Ø-5 in column 1.

Table 3.1 Genetic Elements in the pIND2-HB4 vector and its presence in IND-ØØ41Ø-5 insertion

Genetic element	Size (nt)	Description - Function	Donor	References
Intervening sequence	8	Sequence introduced for/during cloning	Sequence used in DNA cloning	
pVS1	3771	Sequence derived from plasmid pVS1. The sequence between nt 948 and 1948 corresponds to the origin of replication pVS1ori, for replication and maintenance in *A. tumefaciens*.	*Pseudomonas aeruginosa*	Itoh et al., 1984; Itoh and Haas, 1985; Hajdukiewicz et al., 1994
pBR322	1131	Sequence derived from plasmid pBR322. The sequence between nt 4233 and 4852 corresponds to the origin of replication Col E1 for replication and maintenance in *E. coli*	*Escherichia coli*	Yanisch-Perron et al., 1985
aadA	1243	Aminoglycoside 3'-(O) adenylyltransferase gene confers resistance to spectinomycin/streptomycin. For selection in *E. coli* and *A. tumefaciens* (Complementary sequence).	*Shigella flexneri* Type 2a	Fling et al.,1985; Chinault et al., 1986
Intervening sequence	165	Sequence introduced for/during cloning	Sequence used in DNA cloning	
Intervening sequence	78	Sequence introduced for/during cloning	Sequence used in DNA cloning	
Left Border (LB)	25	The T-DNA left border sequence from the nopaline type pTi plasmid from *A. tumefaciens*.	*Agrobacterium tumefaciens*	Zambryski et al., 1983; Yadav et al., 1982
Intervening sequence	239	Sequence introduced for/during cloning.	Sequence used in DNA cloning	
Tvsp	549	Sequence of the 3' terminator from a soybean vegetative storage protein gene. (Complementary sequence)	*Glycine max*	Rapp et al., 1990
bar	561	L-Phosphinothricin (L-PPT) acetyltransferase gene that confers tolerance to glufosinate herbicides by N-acetylation of L-PPT. (Complementary sequence).	*Streptomyces hygroscopicus*	Thompson et al., 1987; White et al., 1990; Becker et al., 1992
Intervening sequence	11	Sequence introduced for/during cloning	Sequence used in DNA cloning	

Table 3.1. Genetic Elements in the pIND2-HB4 vector and its presence in IND-ØØ41Ø-5 insertion (Continued)

Genetic element	Size (nt)	Description - Function	Donor	References
TEV	130	Viral 5′ leader sequence, acting as translational enhancer (Complementary sequence).	Tobacco Etch Virus	Carrington and Freed, 1990; Gallie et al., 1995
Intervening sequence	72	Sequence introduced for/during cloning.	Sequence used in DNA cloning	
pr2x35S	687	2 x CaMV 35S promoter (duplicated CaMV 35S) (Complementary sequence).	Cauliflower Mosaic Virus	Odell et al., 1985, Haq et al., 1995
Intervening sequence	12	Sequence introduced for/during cloning.	Sequence used in DNA cloning	
LPF (large promoter fragment)	1209	One of the allelic forms of the natural promoter of the *HaHB4* gene (direct orientation)	*Helianthus annuus*	Dezar et al., 2005b
Intervening sequence	11	Sequence introduced for/during cloning.	Sequence used in DNA cloning	
HaHB4	531	Gene coding for the transcription factor HAHB4, involved to improve yield under varying environmental conditions (direct orientation). Translates to generate the HAHB4 protein.	*Helianthus annuus*	Dezar et al., 2005a, b; Manavella et al., 2008a; Gago et al*.,* 2002
Intervening sequence	19	Sequence introduced for/during cloning	Sequence used in DNA cloning	
Tnos	253	A 3´ nontranslated region of the nopaline synthase gene from *A. tumefaciens*, which functions to terminate transcription. (Direct orientation).	*Agrobacterium tumefaciens*	Depicker et al., 1982
Intervening sequence	288	Sequence introduced for/during cloning.	Sequence used in DNA cloning	
Right Border (RB)	25	The T-DNA right border sequence from the nopaline type pTi plasmid from *A. tumefaciens*	*Agrobacterium tumefaciens*	Zambryski et al., 1983; Yadav et al., 1982
Intervening sequence	16	Sequence introduced for/during cloning	Sequence used in DNA cloning	
Intervening sequence	98	Sequence introduced for/during cloning	Sequence used in DNA cloning	

Boxes highlighted in grey indicate genetic elements intended to be inserted

3.2. Information relating to the genetically modified plant

3.2.1. Description of the trait(s) and characteristics which have been introduced or modified

IND-ØØ41Ø-5 soybean contains the following introduced genes: 1) the *HaHB4* gene, expressing the transcription factor HAHB4 (*Helianthus annuus* homeobox-leucine zipper protein),

that provides the opportunity to protect yield under hydric stress, and 2) the *bar* gene from *Streptomyces hygroscopicus*, that confers resistance to glufosinate herbicides.

3.2.2. Information on the nucleic acid(s) sequences actually inserted or deleted

The *bar* and *HaHB4* genes are the only coding nucleic acids inserted in IND-ØØ41Ø-5 soybean. The *bar* gene was incorporated into IND-ØØ41Ø-5 to provide field-efficient tolerance to glufosinate-based herbicides. The *HaHB4* gene provides the potential for increased yield under environmental stresses found in the natural range of soybean production areas.

The entire insert region in IND-ØØ41Ø-5 was independently sequenced by Eurofins and the sequence along with certified reference materials under Regulation (EC) No 1829/2003 were submitted to the EURL-GMFF (European Union Reference Laboratory for GM Food and Feed) in July 2019. These genes are described in Table 3.1.

(a) The copy number of all detectable inserts, both complete and partial.

One complete insert at one single location has been inserted into soybean to generate the IND-ØØ41Ø-5 event. No partial sequences from the T-DNA, except those associated to the Left and Right border. No elements of the vector backbone have been inserted.

(b) In the case of deletion(s), size and function of the deleted region(s).

A deletion of 142 base pair and a single nucleotide change in the left flanking junction of the T-DNA insert of IND-ØØ41Ø-5 were detected in the soybean genome, which were in a non-coding, intergenic region region and therefore without any biological significance. The insertion does not interrupt any soybean coding sequence or any other known annotated feature in the soybean genome.

(c) Subcellular location(s) of insert(s) (nucleus, chloroplasts, mitochondria, or maintained in a non-integrated form) and methods for its/their determination.

The insert is located in chromosome 9 and is stably maintained in the *Glycine max* nuclear genome. The single insertion has been confirmed by sequencing of the T-DNA and 1kb of flanking soybean genomic DNA on both ends of the insert region. The sequenced genomic DNA flanking the T-DNA insertion was compared with published soybean genome sequence and the location was confirmed as indicated.

(d) The organisation of the inserted genetic material at the insertion site

The genetic elements of the inserted T-DNA (as defined by the right and left border sequences) are in the same order as in the transformation vector. There were no rearrangements or modifications in the T-DNA when integrated into the soybean genome.

(e) In the case of modifications other than insertion or deletion, describe function of the modified genetic material before and after the modification, as well as direct changes in expression of genes as a result of the modification.

There were no modifications other than insertion of the T-DNA a deletion of 142 base pair and a single nucleotide change in the left flanking region of the T-DNA insert of IND-ØØ41Ø-5 in the soybean genome, were in a non-coding, intergenic region region and therefore without any biological significance. Sequencing of the flanking regions in the insertion locus has shown that

this 142 bp deletion does not interrupt any soybean coding sequence or any other known annotated feature in the soybean genome and therefore has no biological significance.

3.2.3. Information on the expression of the insert

(a) Information on developmental expression of the insert during the life cycle of the plant

The expression levels of the HAHB4, driven by the native sunflower Large Promoter Fragment (LPF) of the native *HaHB4* gene and the *bar* gene driven by the constitutive double 35S were measured. During development of IND-ØØ41Ø-5 it was determined that very low levels of HAHB4 protein are expressed in soybean IND-ØØ41Ø-5 tissues. These levels were below the lower limit of quantification (LLOQ) by the highly sensitive LC-MS/MS assay in any seed samples (LOD of 0.007 µg/g DW seed), and could only be detected in two leaf samples at 0.004 and 0.005 µg/g DW. Expression of the PAT protein was measured in IND-ØØ41Ø-5 tissues using a commercially available, validated ELISA and found to be expressed constitutively throughout the plant during development.

(b) Parts of the plant where the insert is expressed

IND-ØØ41Ø-5 soybean plants has two newly expressed proteins: HAHB4 and PAT. The levels of HAHB4 protein were determined in soybean seed and leaf tissue harvested from field trials in Argentina and in the US. A specific and sensitive LC-MS/MS method was developed and validated to detect the expected low levels of this transcription factor. HAHB4 protein expression levels in the event IND-ØØ41Ø-5 were found below the LLOQ, and even below the Limit of Detection, in most of the samples. Samples were collected from field trials grown across varying regions from plants that were subjected to various types of environments. Even under possible environmental stress conditions, the expression of HAHB4 driven by the native LPF promoter did not elicit expression at mensurable levels at the time of harvest, as might be predicted for a transcription factor. Only two leaf samples showed an HAHB4 detectable level, but even then, below the LLOQ, of 0.004 and 0,005 µg/g DW.

In conclusion, the HAHB4 protein was barely detectable in IND-ØØ41Ø-5 tissues-.

The *bar* gene is driven by a double 35S promoter and the PAT protein is present throughout the growing phases and throughout the plant. PAT protein was detected in IND-ØØ41Ø-5 soybean forage and seeds, but not in any of the Williams 82 samples, as expected. Using a standard assay, the measured values of PAT were generally equal to or below previous reports of other crops expressing the transgenic protein. Results of measurements of PAT levels determined by ELISA were similar in all tissues. The highest value measured in the IND-ØØ41Ø-5 seed samples in an individual field trial was 69 µg/g FW and 12.68 µg/g FW in leaves.

3.2.4 Genetic stability of the insert and phenotypic stability of the genetically modified plant

***Genetic* Stability**

The stability of the IND-ØØ41Ø-5 insertion across several generations was monitored by qPCR of junction sequences. Leaf samples of IND-ØØ41Ø-5 (generations 8 and 13) and Williams 82 plants cultivated in a growth chamber were collected for DNA extraction. A set of primers amplifying the regions located at both ends of the insertion were used (Figure 3.2.4a).

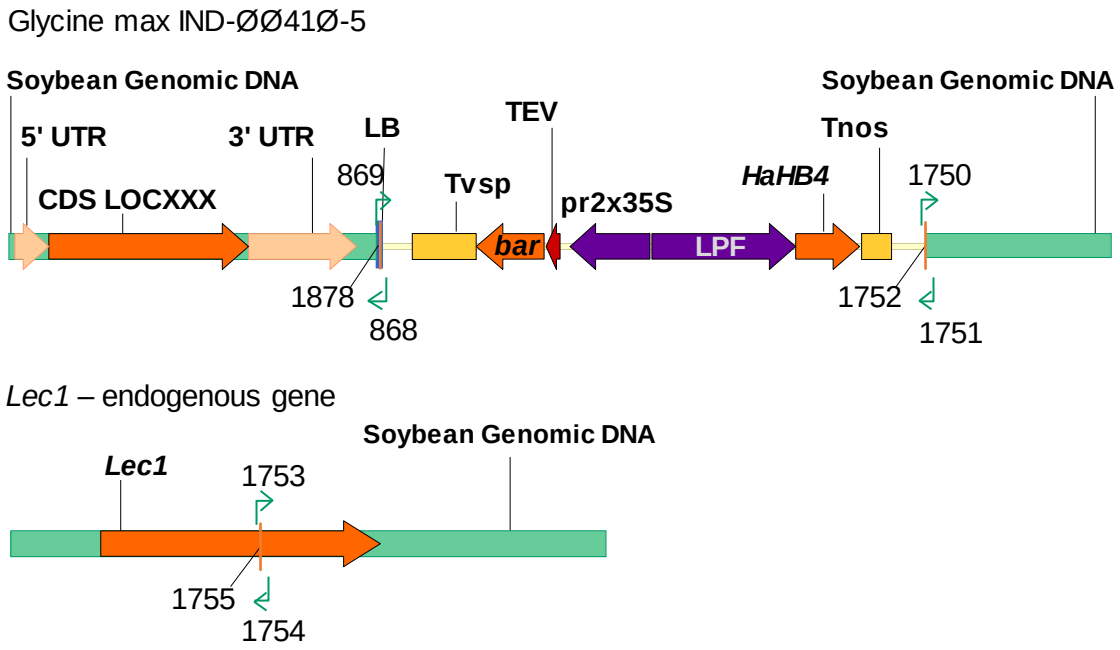

Figure 3.2.4a. Junctions sequences amplifications and reference sequence used to test the stability of the insertion in IND-ØØ41Ø-5. Upper panel: Schematic representation of elements presents within the T-DNA in soybean event IND-ØØ41Ø-5. The primers 868 - 869 and 1750 - 1751 (indicated as green broken arrows) and probes 1878 and 1752 (indicated as light orange vertical lines) were used to test for the presence of the Left and Right Border Junction, respectively. Lower panel: Schematic representation of *Lec1* reference gene. The primers 1753 and 1754 and the probe 1755 were used as an endogenous indicator of soybean genome.

The left junction was amplified using oligonucleotides 868 and 869 that produced a 81 bp fragment comprising the left end portion of the T-DNA inserted in IND-ØØ41Ø-5 soybean and a contiguous soybean genome flanking sequence. For the right border, oligonucleotides 1750 and 1751 were used to amplify a 138 bp fragment which include a short sequence of the inserted T-DNA and the right flanking sequence (Figure 3.2.4a). Probes 1878 and 1752 were used for the detection of the left and right border junction's amplification respectively.

As an endogenous indicator of the native soybean genome, oligonucleotides 1753 and 1754 for the lectin gene (*Lec1*, Accession number, GeneBank: K00821 and M30884) were used. These oligonucleotides generate a 68 bp amplicon sequence detected with probe 1755.

The results of this analysis are displayed in Table 3.2.4 a and confirm that the event is stable across six generations.

Table 3.2.4a. Results of junction's amplification to assess IND-ØØ41Ø-5 stability

Sample	Generation	Left junction	Right junction	*Lec1*
IND-ØØ41Ø-5	F8	+	+	+
		+	+	+
	F13	+	+	+
		+	+	+
Williams 82	-	-	-	+

Signs indicate the presence (+) or absence (-) of the amplicon

Details of genetic stability studies are presented in Appendix 4.

Segregation

Similar methodology to that use to analyse insert stability, junction sequences amplification and verification of the reference identification, was also used to study the inheritance pattern of IND-ØØ41Ø-5 insertion. Segregation of the T-DNA inserted into the IND-ØØ41Ø-5 soybean was assessed in an F2 population of plants obtained after crossing this transgenic event with a commercial soybean cultivar (Bio 6.5). A homozygous IND-ØØ41Ø-5 plant was crossed with Bio 6.5 to produce the F1 progeny. Four F1 plants were self-pollinated to produce the F2 seeds that were used for the segregation analysis.

This study was run using DNA from 73 individuals of the F2 generation as template. The zigosity of F2 plants was determined based on the presence of amplicons for the Left Border Junction and for the native allele (see Table 3.2.4b).

Table 3.2.4b. Classification based on the result obtained by the segregation analysis

Designation	Left Border Junction	WT soybean allele
I: Homozygous for the IND-ØØ41Ø-5 insertion	+	-
H: Hemizygous	+	+
W: Homozygous for the native Williams 82 cultivar allele	-	+

Figure 3.2.4b shows the different sets of primers amplifying either the DNA junction at the Left Border or the native (WT) soybean allele. For the native allele, oligonucleotides 934 and 935 were used to amplify a 205 bp fragment formed by flanking sequences of the IND-ØØ41Ø-5 insertion site. This amplicon includes part the 142 bp sequence which was deleted during transformation (absent in the IND-ØØ41Ø-5 event but detectable in the untransformed line) (Figure 3.2.4b). Probe 936 labelled was used for detection. For the soybean event IND-ØØ41Ø-5. oligonucleotides 868 and 752 were used to amplify a chimeric fragment of 356bp (Figure 3.2.4b) formed by left flanking sequence and part of the IND-ØØ41Ø-5 insert.

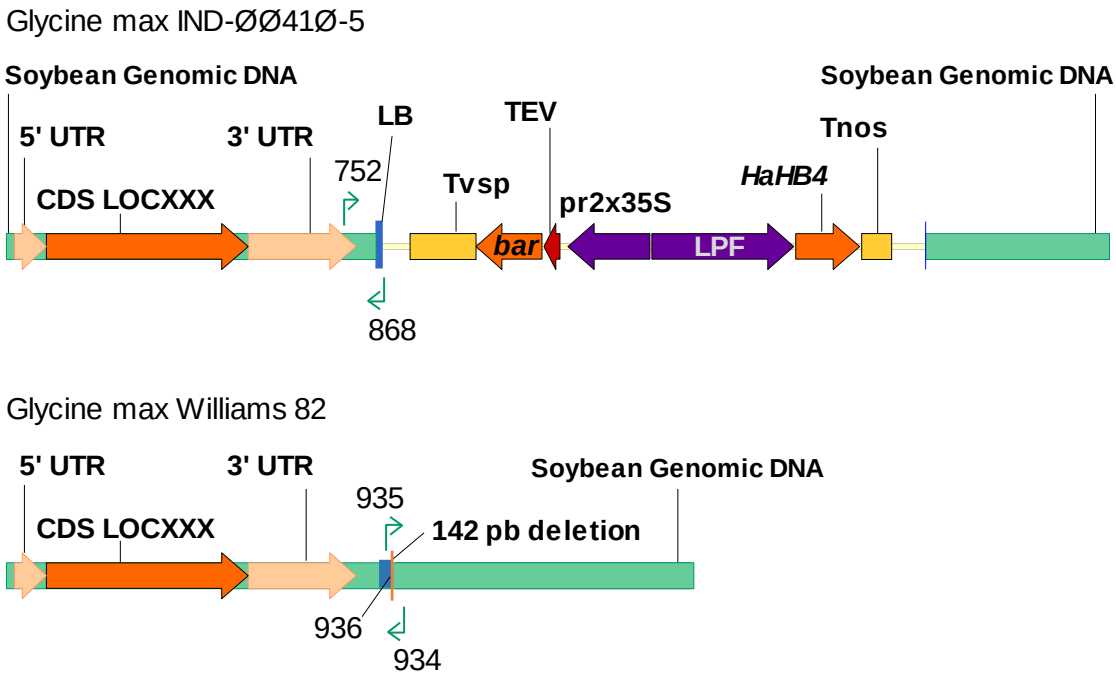

Figure 3.2.4b. Junctions amplification to test segregation of the insertion in IND-ØØ41Ø-5. Upper panel: scheme of the insertion in soybean event IND-ØØ41Ø-5 showing the elements present within the T-DNA, and the primers (indicated as green broken arrows) used for segregation analysis. The primers 868 and 752 were used to test for the presence of the Left Border Junction. Lower panel: scheme of native allele showing the elements present in insertion region (without the T-DNA). Primers 934 and 935 and probe 936 (indicated as a light orange vertical line) were used to test for the presence of the native allele. UTR: untranslated region, CDS LOCXXX: coding sequence of the closest gene.

The results of the segregation analysis are shown in Table 3.2.2.4c

Table 3.2.4c. Analysis of the segregation of IND-ØØ41Ø-5 T-DNA in F2 plants

Expected Genotypes (Number of Plants)			Observed Genotypes (Number of Plants)			χ2	p-value
I	H	W	I	H	W		
18.25	36.5	18.25	17	39	17	0.34246	0.8426

I: IND-ØØ41Ø-5 homozygous; H: hemizygous; W: Williams 82 homozygous.

The χ2 value (df=2, α=0.05) indicate no statistically significant difference between the observed data and the expected 1:2:1 (I:H:W) genotypic segregation. These results support the conclusion that the IND-ØØ41Ø-5 insertion resides at a single locus within the soybean genome and is inherited according to Mendelian inheritance principles.

As a summary of the stability and segregation studies, it is concluded that the insertion was proved to be stable after several generations of self-pollinated soybean event IND-ØØ41Ø-5 and that the T-DNA insertion was shown to segregate in a Mendelian fashion following outcrossing.

Phenotypic Stability

The intended phenotypes of IND-ØØ41Ø-5 were the potential for increased yield compared to the parental variety Williams 82 across the current range of environments in which soybean are grown commercially and glufosinate herbicides tolerance. The stability of the phenotype of

soybean event IND-ØØ41Ø-5 is demonstrated by the results included in this submission. Field trials included in Appendix 9 were conducted in soybean production locations during the 2012-2013 growing season in Argentina and the US 2013 growing season, using a T6 generation. Results of these field studies are presented in Section 1.3 of Part II, Scientific Information, and in detail in Appendix 9. In these studies, values of the grain yield of IND-ØØ41Ø-5 soybean were greater than yield of parental control Williams 82 at sites having environmentally based low potential. Concerning the glufosinate tolerance, besides the original phenotype used for event selection at the initial phase of the project, this feature was confirmed in field trials run in 2017 (Appendix 10). Results of these studies confirm the stability of the IND-ØØ41Ø-5 phenotype across widely different environments and after several generations.

3.2.5. Information (for environmental safety aspects) on how the genetically modified plant differs from the recipient plant in:

(a) Mode(s) and/or rate of reproduction

IND-ØØ41Ø-5 soybean does not differ from the recipient plant in terms of reproduction, dissemination or survivability. Data generated on IND-ØØ41Ø-5 soybeans over six years of field trials in Argentina and the US support a conclusion that the *HaHB4* gene confers increased tolerance to environmental stresses that reduce crop yields. This increase yield tolerance phenotype is related to a decreased sensitivity to ethylene-induced senescence. IND-ØØ41Ø-5 soybean also expresses the glufosinate herbicide tolerance protein PAT at useful levels field conditions. The mode of action of PAT is the inactivation of L-phosphinothricin (the active molecule in glufosinate herbicides) through N-acetylation. These introduced traits and mechanisms of action do not modify the mode or rate of reproduction on the recipient plant in a manner that these characteristics would be different from those of the conventional counterpart.

(b) Dissemination

There are no specific factors of IND-ØØ41Ø-5 soybean that would affect reproduction or dissemination as compared to conventional soybean varieties. The stress tolerance trait has not affected reproductive physiology as determined from field trial results in Argentina and the US.

(c) Survivability

There are no specific factors in IND-ØØ41Ø-5 soybean that would affect survivability of the plant other than tolerance to hydric stress and to glufosinate herbicides: IND-ØØ41Ø-5 soybean will survive a treatment by up to 8x field level of any herbicide containing glufosinate. However, IND-ØØ41Ø-5 soybean remains sensitive to other herbicides towards which conventional soybean is susceptible. Under conditions of environmental stress, the HAHB4 trait will provide increased grain yield compared to a soybean not containing the *HaHB4* gene, but this characteristic is not related to survivability but with the normal physiological response of the plant to environmental stress. This increased yield opportunity would not contribute to increase of, or otherwise modify survivability of soybean, when compared with conventional varieties.

(d) Other differences

None.

3.2.6. Any change to the ability of the genetically modified plant to transfer genetic material to other organisms (for environmental safety aspects)

(a) Plant to bacteria gene transfer

The risk from any plant-to-bacteria horizontal gene transfer from IND-ØØ41Ø-5 soybean is negligible. The risk of horizontal gene transfer (HGT) from plants to other organisms under natural conditions is extremely low and is expected to be lower than background rates (Schlüter et al, 1995). Therefore, HGT from GM plants poses negligible risks to human health or the environment (Mohr and Tebbe, 2007; Keese, 2008; Rizzi et al., 2012). Animal feeding studies have demonstrated that a minor amount of fragmented dietary DNA few nucleotides long may resist the digestive process, but stable integration and expression of internalized DNA has not been demonstrated and it is considered very unlikely. Over the past years, there have been reports concerning and homology-facilitated-illegitimate-recombination from GM plants to genetically conditioned bacteria (with artificially introduced plant-recombinogenic sequences, see for example, de Vries and Wackernagel, 2002, de Vries et al., 2004) under microcosmos conditions, but no evidence of HGT under natural conditions was found to occur involving genetically modified plants (see for example, Schlüter et al., 1995). The Entransfood network of the European Commission concluded that the probability of occurrence of such HGT is extremely low (van den Eede et al., 2004).

The possibility of HGT from IND-ØØ41Ø-5 soybean to environmentally occurring bacteria was also investigated. Bioinformatic analyses were performed to assess the potential for HGT of sequences within the insertion and its flanking regions. The results of this search showed that the insertion in IND-ØØ41Ø-5 soybean has no sequence homologies able to participate in a recombination-mediated HGT.

(b) Plant to plant gene transfer

The genetic modification in IND-ØØ41Ø-5 soybean would not alter plant-to-plant gene transfer potential in soybeans. Over multiple generations, the insert was found to be stably integrated in the genome of IND-ØØ41Ø-5 soybean and therefore any mobilization event can be excluded. There is no mechanism where the soybean genes could be transferred plant-to-plant other than a cross with a sexually compatible species. Since there are no wild compatible species in the EU, the risk of plant-to-plant transfer is limited to cultivated soybean. If IND-ØØ41Ø-5 soybean was mixed with traditional soybean a small amount of outcrossing may occur, which would be similar to outcrossing between two conventional soybean varieties and result in no difference, other than an expected pollen-mediated breeding.

4. COMPARATIVE ANALYSIS

4.1. Choice of the conventional counterpart and additional comparators

The parent variety, Williams 82, was used as the conventional soybean comparator (control) in the safety assessment of IND-ØØ41Ø-5 soybean. Williams 82 was developed by the USDA-ARS and the Illinois Agriculture Experiment Station and released in 1981. It is a late group III indeterminate variety (relative maturity 3.8). This follows the EFSA guidance (EFSA, 2011d) to select an isogenic line as a comparator. Williams 82 shares the exact same genome as IND-ØØ41Ø-5 soybean, with the exception of the transgene insert.

In addition to the use of the above comparator, locally adapted modern commercial varieties of soybean representative of the soybean growing conditions were also grown in randomized plots at each field test to establish the ranges of natural variations of typical measurements for soybean at each trial site. These local reference varieties were chosen specifically for each site in both Argentina and the US and are described in Appendix 11. The prime criterion for selecting the local reference varieties was that they are adapted for optimal yield and general agronomic performance for each growing region, which was actually the primary objective of the comparative analysis. Thus, selection of such varieties provided the appropriate comparison for IND-ØØ41Ø-5 soybean *in real agricultural settings*, which is the ultimate reason of this part of the comparative analysis. These commercial comparators contain the *CP4 epsps gene* of the CP4 strain of *Agrobacterium tumefaciens* from MON-Ø4Ø32-6 (for glyphosate tolerance). As more than 99% of soybeans grown in Argentina contains this transgene, including all modern commercial varieties, we submit that these are therefore appropriate comparators for the objective of this analysis. Therefore, these soybean varieties can be considered as commercial standards (Appendix 12). These commercial comparators were derived from lines that were risk assessed on the basis of experimental data collected according to the principle of the EFSA MC and FF risk assessment and were commercially available in the country of cultivation. Soybean products from these varieties freely enter worldwide trade and use with no restrictions.

4.2. Experimental design and statistical analysis of data from field trials for comparative analysis

Compliance statement

Field trials for agronomic, phenotypic, compositional and environmental studies were conducted in 2012 and 2013, before the publication of EFSA's GLP-related guidance (EFSA, 2015c). Therefore, the Argentina and US trials and data analysis were started prior going into effect the Implementing Regulation (EU) No 503/2013. However, field trials were completed under rigorous standard scientific principles including testing (organization, management supervision), process (planning, job assignments), performance (chain of custody, standard operation procedures, control, integrity), validation (methodology and regulatory compliance), certification (overall quality assessment), reporting and archive, all supporting data quality and quality assurance principles.

Also, two randomized block trials were conducted in 2017 to check glufosinate tolerance. These trials demonstrated that the PAT protein expressed in soybean IND-ØØ41Ø-5 provides field tolerance to glufosinate-based herbicides.

Randomized block field trials were conducted at fifteen locations in Argentina during the 2012-2013 growing season and at ten locations in the US during the 2013 growing season. Six locations in Argentina representing the environmental variation over the range of the 15 locations were selected for compositional analysis (Appendix 13). Five of the ten locations in the US were similarly selected for compositional analysis. Field trials were located within the major soybean production areas of both Argentina and the US thereby covering the diverse environmental conditions for soybean in these two countries. Four replicated plots of each seed line (IND-ØØ41Ø-5, Williams 82 and the commercial reference varieties) were planted using a randomized complete block field design. At each test site and planting date, harvested seed and forage materials were collected from each of the seed lines.

Seed (at BBCH 89) and forage (at BBCH 71-75) material from transgenic event IND-ØØ41Ø-5, its conventional comparator Williams 82 and the commercial reference varieties were collected from eleven locations in Argentina and the US (Appendix 13). Compositional analyses were conducted according to the OECD consensus document for soybean (OECD 2012). Plants were tested at T6 generation. Nutrients measured included proximates (moisture, protein, fat, ash, and carbohydrates), fiber (acid detergent fiber (ADF), neutral detergent fiber (NDF) and crude fiber), minerals (Calcium and Phosphorus), fatty acids, vitamin E, and amino acids in seed and proximates and fiber (ADF and NDF) in forage. Anti-nutrients measured in seed included stachyose, raffinose, phytic acid, lectin, trypsin inhibitors and isoflavones (daidzein, genistein, and glycitein). The levels of 11 endogenous soybean allergens were also measured (Appendix 14). All nutrients and anti-nutrients (except for lectin) for Argentine locations were measured at Melacrom Laboratories (Mercedes, Buenos Aires, Argentina). Lectin for these sites was measured by Covance Laboratories (Madison, WI, USA). All nutrients and anti-nutrients for locations in the US were measured at Covance Laboratories (Madison, WI, USA). Allergen levels were also measured by Convance Laboratories (Madison, WI, USA).

No tests materials from field trials in which the GM plant was exposed to glufosinate herbicide have been collected. We submit that such tests would no add biologically relevant information to the comparative compositional analysis of IND-ØØ41Ø-5 soybean as reported in this application when using the appropriate comparator and the commercial reference varieties. We base this statement on the detailed report given in Appendix 15 and 16. In short, based on the experience gained in previous EFSA Scientific Opinions, plus extensive scientific literature, it has been demonstrated that comparativel analyses do not differ between sprayed and unsprayed materials (Apendice 15 and 16).

The statistical methodology used to analyse the data obtained in the present study was the one described in the EFSA guideline (EFSA, 2010a). The differences between IND-ØØ41Ø-5 (the GMO) and its conventional comparator William 82, as well as, the equivalence with the commercial varieties cultivated at the same locations were assessed. The null hypothesis and alternative hypothesis when testing for differences were:

$$\boldsymbol{H_0}: \boldsymbol{\Delta_{GC}} = \mathbf{0} \quad \boldsymbol{H_1}: \boldsymbol{\Delta_{GC}} \neq \mathbf{0}$$

where $\boldsymbol{\Delta_{GC}}$ is the true difference on an appropriate scale between the GMO and the conventional counterpart. When testing for equivalence of the GMO and a reference, the null and alternative hypotheses were:

$$\boldsymbol{H_0}: \boldsymbol{\Delta_{GR}} \geq \boldsymbol{EL} \quad \boldsymbol{H_1}: \boldsymbol{\Delta_{GR}} < \boldsymbol{EL}$$

where $\boldsymbol{\Delta_{GR}}$ is the true difference between the GMO and the reference, and $\boldsymbol{EL}$ is the equivalence limit for this difference. A linear mixed model was fitted to study the average difference and the average equivalence over sites. Random site effects were assumed, and a logarithmic transformation of the response variable was used.

$$\boldsymbol{y_{ijkl}} = \boldsymbol{Mean} + \boldsymbol{Site_i} + \boldsymbol{Block_{ij}} + \boldsymbol{GenotypeGroup_k} + \boldsymbol{IndRef_l} \times \boldsymbol{Genotype_l} + \boldsymbol{\epsilon_{ijkl}}$$

where GenotypeGroup is a 3-level fixed factor (GMO, conventional counterpart, reference varieties) and Genotype is a random factor for the variety (GMO, conventional counterpart, and each of the reference varieties). In this model, IndRef is an indicator variable with value 1 for the reference varieties, and 0 otherwise, which allows the contrast for difference and equivalence to be assessed against the proper residual variation, excluding the variance between genotypes. The indices $\boldsymbol{i}$, $\boldsymbol{j}$, $\boldsymbol{k}$ and $\boldsymbol{l}$ in the model are for site, block within site, treatment group (counterpart,

GMO or reference) and reference varieties, respectively. The tests of difference and equivalence were carried out by calculating the 90% confidence intervals for the specific contrasts of interest between GMO and its counterpart, and between GMO and the group of references, respectively. This implies that each difference test has a 90% confidence level, and each equivalence test a 95% confidence level.

To establish equivalence limits, the following model was fitted:

$$y_{ijkl} = Mean + Site_i + Block_{ij} + GenotypeGroup_k + Genotype_l + \epsilon_{ijkl}$$

which includes the variance between genotypes as it does not include the IndRef indicator.

Mixed models were fitted using the residual maximum likelihood algorithm and the degrees of freedom were calculated by the Kenward-Roger method. The approximate two-sided $\mathbf{100}(\mathbf{1}-\boldsymbol{\alpha})\%$ confidence intervals were constructed using the standard errors of difference and $\mathbf{100}(\mathbf{1}-\boldsymbol{\alpha}/\mathbf{2})\%$ point of the appropriate Student's t distribution. To facilitate visual interpretation, an additional adjustment to the equivalence limits was performed so that only the confidence interval for the difference test is needed to assess both equivalence and difference (EFSA, 2010a; van der Voet et al., 2011). The confidence intervals for difference and the equivalence limits are displayed in a multiplicative scale to the counterpart. In this scale, a confidence interval covering the value of 1 implies no difference. The assessment of equivalence was performed by inspecting the position of the confidence interval respective to the equivalence limits. A one-way ANOVA was used to perform a by-site statistical analysis taking a p value below 0.05 as a significant difference.

4.3. Selection of material and compounds for analysis

To conduct the compositional analyses, both forage and seed samples were collected from the 11 field trials. Four plots each of IND-ØØ41Ø-5 soybean and Williams 82 were harvested for sampling, in addition to pooled samples of each commercial reference variety. This led to a total of 143 samples each of forage and seed. To maintain sample identity, each sample was identified with an ID or a barcode (US samples) and tracked at multiple points during the process: at harvest, upon receipt to the holding facility if used (US Cold Storage for US samples), prior to shipment to Covance and Melacrom and upon receipt at both analytical laboratories. Additionally, Covance used the barcode numbers to track the sample data and provide them in the final report. The forage samples were maintained at or below -20 °F (US samples) or dried and held at room temperature as seeds.

4.4. Comparative analysis of agronomic and phenotypic characteristics

The agronomic performance of soybean transgenic event IND-ØØ41Ø-5 was evaluated in comparison with the conventional variety Williams 82 and with the commercial comparators. The results of these studies support the conclusion that the soybean IND-ØØ41Ø-5 event is equivalent to commercial soybeans and does not pose a specific plant pest or environment damaging risks.

The trials were conducted in several locations in Argentina (AR) in the 2012-2013 soybean growing season and the US in the 2013 growing season. Locations as planted at both countries have similar temperate growing seasons, environmental conditions and cultivation practices. In addition to the IND-ØØ41Ø-5 event and Williams 82 soybean entries, a set of commercial check

varieties locally adapted in each country were included as references, providing a range of natural variability values for assessment of phenotypic, agronomic and environmental interactions.

Biological parameters were evaluated to compare the characteristics of germination, growth, pest and disease susceptibility and environmental stress responses between the mentioned entries. Results of these agronomic, phenotypic and ecological evaluations under field conditions (or laboratory, when appropriate) showed no statistically significant differences between IND-ØØ41Ø-5 soybean and control Williams 82 soybean for most of the agronomic and phenotypic parameters in the combined across all field-sites analysis. Statistically significant differences were observed between the two treatments in the combined sites analysis for yield, emergence, initial stand, final stand and maturity. However, except for maturity and yield, values for IND-ØØ41Ø-5 soybean for all these parameters were within the range of the commercial soybean varieties in the field studies. Regarding Yield and Maturity both test materials, IND-ØØ41Ø-5 and conventional counterpart, are outside the limits of equivalence of the commercial varieties, but values of IND-ØØ41Ø-5 are statistically higher than those of the conventional counterpart, laying between the values of conventional counterpart and the reference varieties. The references are current varieties available in the market, and the differences between the conventional counterpart and IND-ØØ41Ø-5 may be produced by an expected improvement of the genetic background of the conventional counterpart, due to the favorable environmental effect of the genetic modification.

Therefore, it was concluded that vegetative and reproductive growth and development of IND-ØØ41Ø-5 soybean is equivalent to that of control Williams 82 and to other commercial soybean varieties (see Appendix 9 for a complete description of these studies).

Results of the seed germination and dormancy studies showed that germination of IND-ØØ41Ø-5 soybean seed is equivalent to that of Williams 82 seed as well as to seed of the commercial soybean comparators under all of the temperature regimes used in the study (Appendix 20).

Also, no statistically significant differences in any of the measured pollen parameters between IND-ØØ41Ø-5 soybean and parental control Williams 82 soybean (Appendix 21). Therefore, regarding to pollen characteristics, IND-ØØ41Ø-5 soybean is expected to have the same very low outcrossing rate to other cultivated soybeans as Williams 82 and other commercial soybean varieties.

Results of the growth chamber studies showed no statistically significant differences for interactions with nitrogen-fixing *Bradyrhizobium japonicum* bacteria between IND-ØØ41Ø-5 soybean, the control Williams 82 and other commercial soybean varieties. Therefore, no meaningful hazards were identified with respect to interactions of IND-ØØ41Ø-5 soybean with non-target organisms or with *Bradyrhizobium japonicum* in the EU. Therefore, it is concluded that there is no risk that any adverse effects on biodiversity through interactions between IND-ØØ41Ø-5 soybeans with NTOs or with *Bradyrhizobium japonicum* bacteria will develop in the EU as a result of import of IND-ØØ41Ø-5 soybeans.

Gene transfer ability, either plant-to-pant or plant-to-bacteria, were also shown negligible as the same properties of conventional soybeans. Bioinformatic analyses were performed to assess the potential for HGT of any sequences within the insertion and in the flanking regions. The results of these searches showed that the insertion in IND-ØØ41Ø-5 soybean does not have sequence homologies able to participate in a recombination-mediated HGT.

The studies outlined above showed the lack of biologically significant differences in agronomic, phenotypic and ecological characteristics between IND-ØØ41Ø-5 soybean and Williams 82 soybean, as well as to other commercial soybean varieties, except for the intended traits of increased yield opportunity under conditions of environmental stress and tolerance to the herbicide glufosinate. Overall, the results demonstrate that IND-ØØ41Ø-5 soybean poses no greater potential for persistence and invasiveness in different environments or have any greater ecological or environmental impact than do the parental control comparator Williams 82 and other commercial soybean varieties.

4.5. Effect of processing

From the food processing technology perspective, IND-ØØ41Ø-5 soybeans are unchanged compared with the non-transgenic parental control line Williams 82 and with conventional soybeans. Compositional analyses of IND-ØØ41Ø-5 soybeans (a critical characteristic for food processing) showed no significant changes compared to the control Williams 82 soybean. Consequently, processing effects would be the same as those of currently undergone by conventional soybeans.

5. TOXICOLOGY

(a) Toxicological testing of newly expressed proteins

Comprehensive risk/safety assessment of soybean IND-ØØ41Ø-5 including molecular, compositional, agronomic and phenotypic analyses, shows no substantial modifications in the composition, no indication of possible unintended effects and no indication of differences in the interactions which may be relevant for food and feed safety. Demonstration of the compositional equivalence between the GM crop to the conventional comparators has been a keystone for the assessment of the safety and nutritional characteristics of the food and feed derived from the GM crop (Kuiper et al., 2001; EFSA 2008; Privalle et al., 2013). Moreover, from the food safety perspective, the newly expressed HAHB4 protein in soybean IND-ØØ41Ø-5 results in several relevant favorable safety characteristics. First, the source of the native protein HAHB4 (sunflower - *Helianthus annuus* L.) has been in the food and feed chain for several thousand years. Therefore, it has a history of safe food and feed use. Also, HAHB4 is a transcriptional factor of normal endogenous plant pathways, i.e., the expressed phenotype relies on the natural physiology and metabolic pathways of the plant. Therefore, no proteins or metabolites other than the natural plant ones are expressed in the transgenic event. Finally, being a transcription factor, HAHB4 is expressed at extremely low levels which, added to the safety of the source, makes its presence in foods of no safety concern. The PAT protein from the *bar* gene, also expressed in soybean IND-ØØ41Ø-5, can be also considered as having a history of safe food use as it has been introduced in many crops (233 edible crops, as of February 28, 2018; ISAAA, 2018a) and consumed since the very beginning of crop genetic modification technology.

Bioinformatic analysis searching for sequence homologies confirmed that HAHB4 protein does not have biologically significant homologies with known toxic proteins.

(b) Testing of new constituents other than proteins

Not applicable. No constituents other than the proteins and metabolites resulting from the expression of the genetic elements introduced (HAHB4 and PAT proteins) are generated in soybean IND-ØØ41Ø-5 as the result of the transformation.

(c) Information on natural food or feed constituents

The two introduced traits did not affect the compositional equivalence of IND-ØØ41Ø-5 soybeans to conventional soybeans regarding natural food or feed constituents. This includes the natural endogenous allergenic proteins and anti-nutrients.

The results of the compositional analysis (Appendix 13) and the quantitative measurement of soybean allergens (Appendix 14), indicated that constituents of the IND-ØØ41Ø-5 event are similar to those found in conventional soybean.

(d) Testing of the whole genetically modified food and feed

Absence of toxicity associated with IND-ØØ41Ø-5 soybean grain was confirmed by a 90-day rodent feeding study in which the GM-derived meal was compared with the meal from soybeans of the parental control Williams 82. Meal of each treatment was incorporated at a level of 30% (w/w) in the diets. The results of this study showed that the growth and health of the rodents fed IND-ØØ41Ø-5 soybean meal were equivalent to the growth and health of the rodents fed Williams 82 meal. There were no deleterious effects identified in either rat population at the end of the test. For more detail see Appendix 18.

6. ALLERGENICITY

(a) Assessment of allergenicity of the newly expressed protein

The HAHB4 protein

The HAHB4 protein expressed in IND-ØØ41Ø-5 soybean does not present an allergenic risk since it is not derived from source generally recognised as allergenic, is present in very low amounts in soybean grain (allergens are usually major food protein components), it has amino acid sequence homology to similar proteins with a history of safe use in food and feed products (the plants HD-Zip family of transcription factors), it does not possess amino acid sequence or structural similarities to known food allergens and is rapidly digested in simulated gastric fluid (SGF) environments. Therefore, the HAHB4 protein expressed in IND-ØØ41Ø-5 soybean tissues does not possess any attributes of known protein food allergens, does not have any characteristics of known toxins and therefore presents no risks for human or animal consumption and is as safe as other dietary proteins present in conventional crops with a history of safe use in food and feed products.

The PAT Protein

Genetically modified foods and feed containing the *bar* gene (or the similar *pat* gene) expressing the PAT enzyme have been approved by regulatory agencies throughout the world and are currently widely commercialized and consumed thereby establishing a history of safe use. The lack of allergenic properties of the PAT enzyme expressed in IND-ØØ41Ø-5 soybean has been thoroughly confirmed by regulatory agencies worldwide. The PAT enzyme has shown to possess no allergenic sequences by bioinformatic searches, and it is quickly digested in SGF and SIF (Herouet et al, 2005; CERA, 2011).

(b) Assessment of allergenicity of the whole genetically modified plant

In compliance with Implementing Regulation (EU) No 503/2013, this application includes the quantitative measurement of soybean allergens (Appendix 14). The risk hypothesis behind this

investigation would be the possibility of changes in the levels of endogenous allergens in a manner that would adversely impact on human and animal health (EFSA, 2017e). According to the above regulation and taking into account EFSA Guidance Document (EFSA, 2017e), the levels of 11 endogenous soybean allergens were measured using an isotopic dilution LC-MS/MS method in soybean event IND-ØØ41Ø-5 and compared to those in its conventional counterpart Williams 82 and with those found in several commercial varieties to provide a reference range of natural variability. Results showed that the levels of the relevant protein allergens in soybean event IND-ØØ41Ø-5 were not different to those measured in the conventional comparator Williams 82. When analysed in the context of the weight-of-evidence approach (Metcalfe et al., 1996, Thomas et al., 2008), these results indicate that there was no effect on the levels of endogenous allergens associated to the genetic modification in soybean event IND-ØØ41Ø-5.

7. NUTRITIONAL ASSESSMENT

(a) Nutritional assessment of the genetically modified food

The nutrient composition of grain and forage obtained from the soybean event IND-ØØ41Ø-5 was similar to that found in the non-transgenic counterpart and within the range of the commercial reference varieties, as supported by the compositional equivalence of the transgenic event with Williams 82 and conventional reference varieties. Therefore, the nutritional composition of seed, forage and other feed products derived from IND-ØØ41Ø-5 is equivalent to those derived from current commercially available soybean varieties.

(b) Nutritional assessment of the genetically modified feed

The composition of grain and forage obtained from the soybean event IND-ØØ41Ø-5 was similar to that found in the non-transgenic counterpart and within the range of the commercial reference varieties, supporting the compositional equivalence of the transgenic event with Williams 82 and conventional reference varieties. Nutritional equivalence was also strongly suggested by the results of the 90-day rodent feeding study.

Therefore, the nutritional composition of seed, forage and other feed products derived from IND-ØØ41Ø-5 soybeans, as well as the levels of endogenous allergens is equivalent to current commercially available soybean varieties (Appendixes 13 and 14). These data support the conclusion that soybean event IND-ØØ41Ø-5 is equivalent to conventional soybean varieties which are currently safely grown and consumed. In fact, Verdeca does not identify toxicological and allergenicity concerns regarding the HAHB4 and PAT proteins expressed in IND-ØØ41Ø-5 soybean and finds no evidence that the genetic modification would change the overall nutritional features of the GM plant. The nutritional impact of food and feed from IND-ØØ41Ø-5 soybean is expected to be the same as that of food and feed from the conventional counterpart and the tested commercial non-GM soybean reference varieties. Verdeca then concludes that IND-ØØ41Ø-5 soybean is as safe as and nutritionally equivalent to the conventional counterpart and the tested non-GM soybean reference varieties.

8. EXPOSURE ASSESSMENT—ANTICIPATED INTAKE OR EXTENT OF USE.

Commercialization of IND-ØØ41Ø-5 soybeans is not expected to significantly increase the use of soybean products in food or feed as it does not represent any consumer-oriented characteristic. The IND-ØØ41Ø-5 soybean and all derived food, feed and processed products are expected to join the stream of similar products from commercial soybean production already on

the market. Therefore, the exposure and the anticipated intake or extent of use or consumption of soybean products derived from IND-ØØ41Ø-5 soybean will not change the exposure and intake levels of soybean products already on the EU market.

Estimated dietary exposure of the HAHB4 and PAT proteins from IND-ØØ41Ø-5 soybean has been detailed in Annex I, Part II, Section 2 Exposure. Considering the levels of the new expressed protein in soybean event IND-ØØ41Ø-5, estimated exposure levels for HAHB4 range from 0.0000 to 0.3582µg/kg BW and 0.004 to 693.63 µg/kg BW for PAT. Lower values correspond to chronic exposure in average population and the high level is associated to acute exposure in high consumers (Appendix 19).

9. RISK CHARACTERISATION.

Foods and feeds derived from soybean have a long history of safe use. Information detailed in this application show that IND-ØØ41Ø-5 soybean is in all respects equivalent to conventional soybean with the exception of the transgenic insert. Risks of IND-ØØ41Ø-5 soybean (e.g., allergenicity, anti-nutrients) is the same as the risk of all conventional and authorised transgenic soybean products.

A detailed account of Risk Characterisation could be found in Annex I. Part II, Section 3.

10. POST-MARKET MONITORING OF THE GENETICALLY MODIFIED FOOD OR FEED.

The risk assessment confirmed IND-ØØ41Ø-5 soybeans are equivalent to commercially available soybean and no safety concerns were identified. Therefore, post-market monitoring of soybean food and feed products containing, consisting of or derived from IND-ØØ41Ø-5 soybeans is not deemed necessary. However, for the sake of completeness, a detailed description of the Post-Market Monitoring of the Genetically Modified Food or Feed derived from IND-ØØ41Ø-5 soybeans is shown in Section 4, Part II of this application

11. ENVIRONMENTAL ASSESSMENT

11.1. Mechanism of interaction between GM and target organisms

11.2. There are no target organisms for IND-ØØ41Ø-5 soybeans.

Potential changes in the interactions of the GM plant with the biotic environment resulting from the genetic modification

IND-ØØ41Ø-5 soybeans are similar to conventional commodity soybeans in all respects related to the environment, other than changes resulting from the expression of the transgenes: opportunity to yield maintenance under hydric stress and glufosinate herbicide tolerance. There are no other specific changes identified in this dossier or anticipated between IND-ØØ41Ø-5 soybeans in the interactions of the GM plant with the biotic environment resulting from the genetic modification (including the relevant symbiotic interactions) that wouldn’t be the same as with any conventional soybean variety.

(a) Persistence and invasiveness

Releases of soybeans in South and North America did not show altered persistence or invasiveness characteristics compared with conventional soybean. Soybean, including the IND-ØØ41Ø-5 event, did not show weed characteristics or any persistence or invasiveness behavior.

(b) Selective advantage or disadvantage

IND-ØØ41Ø-5 soybeans have a distinct advantage only when herbicides containing L-phosphinothricin are used in the field. Up to a certain agronomically useful level, this soybean tolerates the herbicide effects due the expression of the *bar* gene. There is no advantage to the plant when other or no herbicides are utilised. In times of environmental stress including drought, because it contains the *HaHB4* gene, IND-ØØ41Ø-5 soybeans generally yielded more grain than soybeans without the gene. This is comparable to better yielding and adapted varieties produced by traditional breeding methods, so the small selective advantage to the higher yield phenotype under stress does not result in an evolutionary pressure. In repeated releases and in all comparative analyses as submitted in this application, there was no disadvantage observed from the expression of the *bar* and *HaHB4* genes in IND-ØØ41Ø-5 soybeans, when compared with the parent, not modified variety Williams 82.

(c) Potential for gene transfer

A detailed report of this section is shown in Section 3.2.6.(a) in this Part VII. A bioinformatic analysis of this putative risk is shown in Appendix Bioinformatic

The possibility of HGT from IND-ØØ41Ø-5 soybean to environmentally occurring bacteria was also investigated. Bioinformatic analyses were performed to assess the HGT potential of the insertion and its flanking regions. The results of this search showed that the insertion in IND-ØØ41Ø-5 soybean does not have sequence homologies able to participate in a recombination-mediated HGT.

(d) Interactions between the GM plant and target organisms

The genetic modification in IND-ØØ41Ø-5 soybeans is not addressed at pest or disease control, i.e. does not express traits to control insect pests, pathogens or herbivores of the soybean plant. Therefore, there are no target organisms for IND-ØØ41Ø-5 soybeans. A detailed description of this characteristic is shown in Section 11.4 (e) in this Part VII.

(e) Interactions of the GM plant with non-target organism

IND-ØØ41Ø-5 soybean does not express traits to control insect pests, pathogens or herbivores of the soybean plant and therefore it was not expected to have any different interactions with NTOs compared to the interactions that Williams 82 or the commercial comparator soybean varieties have with NTOs. An ERA was completed showing that NTO interactions were similar as those of conventional soybeans. No unintended interactions between IND-ØØ41Ø-5 soybeans and NTOs have been observed or predicted with the use of this technology. Regarding to interactions with other organisms in the environment, IND-ØØ41Ø-5 soybeans are substantially equivalent to commodity soybeans. A detailed description of this characteristic is shown in Section 11.4 (e) in this Part VII.

(f) Effects on human health

An ERA was completed showing that there were no anticipated effects on human health as IND-ØØ41Ø-5 soybeans are substantially equivalent to commodity soybeans. Detailed information to support this statement is shown in Part II, Sections 1.4, 1.5, 1.6 and 7, including the subsections texts.

(g) Effects on animal health

An ERA was completed showing that there were no anticipated effects on animal health as IND-ØØ41Ø-5 soybeans are substantially equivalent to commodity soybeans. Detailed information to support this statement is shown in Part II, Sections 1.4, 1.6 and 7, including the subsections texts.

(h) Effects on biogeochemical processes

An ERA was completed showing that no biogeochemical processes will be affected as IND-ØØ41Ø-5 soybeans are substantially equivalent to commodity soybeans. Detailed information to support this statement is shown in Part II, Section 5. Environmental Assessment, Subsection 5.6, as is required by the Commission Implementing Regulation (EU) No 503/2013.

(i) Impacts of the specific cultivation, management and harvesting techniques

As per this Application, IND-ØØ41Ø-5 soybeans will not be cultivated in the European Union.

11.3. Potential interactions with the abiotic environment

IND-ØØ41Ø-5 soybeans are similar to conventional commodity soybeans in all respects related to the environment, other than changes resulting from the expression of the transgenes. In particular, expression of the *HaHB4* gene, conferring an increased potential opportunity to yield maintenance under hydric stress, may be considered as an intended new interaction with the abiotic environment with agronomic relevance, but only under specific environmental conditions. Under unrestricted water availability, the yield opportunity trait does not express nor produce a yield penalty to the crop. Other than that, there are no specific changes identified in this dossier or anticipated between IND-ØØ41Ø-5 soybeans and the abiotic environment that wouldn’t be the same as with another soybean variety.

11.4. Risk characterisation

Field trials carried out in several locations under different environmental conditions showed the lack of biologically significant differences in agronomic, phenotypic and ecological characteristics between IND-ØØ41Ø-5 soybean and the conventional counterpart Williams 82, as well as to other commercial reference varieties, except for the intended traits of increased yield opportunity under conditions of environmental stress and tolerance to the herbicide glufosinate

Vegetative growth, development and reproductive physiology of IND-ØØ41Ø-5 soybean are equivalent to these characteristics of the parent control Williams 82 and to other commercial reference soybean varieties.

Results of seed germination and dormancy studies showed that these properties of IND-ØØ41Ø-5 soybean seed are equivalent to those of Williams 82 seed as well as to those of

commercial reference varieties, under the wide range of temperature regimes used in these studies.

Also, no statistically significant differences in any of the measured pollen parameters between IND-ØØ41Ø-5 soybean and parental control Williams 82 soybean were found. Therefore, regarding to pollen characteristics, IND-ØØ41Ø-5 soybean is expected to have a very low outcrossing rate, the same that other cultivated soybeans as Williams 82 and other commercial soybean varieties.

Gene transfer ability, either plant-to-pant or plant-to-bacteria, were also shown to be highly unlikely. First, cultivated soybean is a self-pollinating plant, having a very low outcrossing potential. Second, bioinformatic analyses performed to assess the HGT potential of sequences in the insertion and in its flanking regions showed that the insertion in IND-ØØ41Ø-5 soybean does not have sequence homologies able to participate in a recombination-mediated HGT.

Field trials carried out in Argentina and in the US, IND-ØØ41Ø-5 soybean showed no differences in disease susceptibility and counts of both pest and beneficial arthropods at different plant developmental stages, compared to measurements of the same characteristics in plots of Williams 82 or the commercial reference plots.

Also, results of growth chamber studies showed no statistically significant differences for interactions with symbiotic nitrogen-fixing *Bradyrhizobium japonicum* bacteria between IND-ØØ41Ø-5 soybean, the control Williams 82 and other commercial soybean varieties. Therefore, no meaningful hazards were identified with respect to symbiotic interactions of IND-ØØ41Ø-5 soybean with *Bradyrhizobium japonicum* in the EU. Also, no meaningful hazards were identified with respect to interactions between IND-ØØ41Ø-5 soybean with non-target organisms in the environment.

Overall, the above results demonstrate that IND-ØØ41Ø-5 soybean poses no greater risk potential for persistence, invasiveness or adverse effects on biodiversity than do the parental control comparator Williams 82 and other commercial soybean varieties. Therefore, it is concluded that there are no risks associated with the interactions of IND-ØØ41Ø-5 soybean with the environment which are different from those of its conventional counterpart Williams 82 and of other commercial soybean varieties

12. ENVIRONMENTAL MONITORING PLAN

(a) General (risk assessment, background information)

IND-ØØ41Ø-5 soybean will not be cultivated in the EU, therefore any effects on the environment will be limited to accidental spills during handling, transport or processing. Accordingly, under Part II, Section 6. PMEM plan, a detailed account of IND-ØØ41Ø-5 soybeans Safety Features is shown, determining that Case-Specific Monitoring (CSM) is not required (EFSA, 2011f) as per this application. However, it is also shown there one each exercise of the (theoretical) application of GS and CSM to IND-ØØ41Ø-5 soybean as it might be eventually needed in case of unintended spills into the environment. IND-ØØ41Ø-5 soybeans will be treated as a conventional commodity soybean product. Verdeca's plan will be based on the current harmonized monitoring plan by EuropaBio and European trade associations.

(b) Interplay between environmental risk assessment and monitoring

IND-ØØ41Ø-5 soybean will not be cultivated in the EU, therefore any effects on the environment will be limited to accidental spills during handling, transport or processing. Since the introduced genes do not confer a selective advantage to soybeans, it is unlikely that monitoring will show an advantage effect of this soybean's use. Comprehensive environmental risk assessment has been completed with the review of the function of the genes within the IND-ØØ41Ø-5 soybean plant. Monitoring is in place for the rare event that there is a measurable effect on the environment after the introduction and use of IND-ØØ41Ø-5 soybean. A detailed description of the Interplay between environmental risk assessment and monitoring is developed in the Sections along this application, namely: Part II, Section 1. Hazard Identification and Characterisation, which included Molecular Characterisation (1.2), Comparative analysis (1.3), which included Comparative analysis of agronomic and phenotypic characteristics (1.3.5),

Section 3. Risk Characterisation, Section 4. Post-Market Monitoring on the Genetically Modified Food or Feed, Section 5. Environmental Assessment and Section 6. PMEM plan. Developed in all these Sections, an appropriate description is given on the safety features of IND-ØØ41Ø-5 soybeans and of the interplay between ERA and monitoring, with the conclusion that this GM plant does not constitute an agent of new environmental effects or interactions different from conventional soybeans. Moreover, no new applications are anticipated, and no new were identified by Verdeca with its use.

(c) Case-specific GM plant monitoring (approach, strategy, method and analysis)

IND-ØØ41Ø-5 soybean will not be cultivated in the EU, therefore any effects on the environment will be limited to accidental spills during handling, transport or processing. Accordingly, under Part II, Section 6. Post PMEM plan, a detailed account of IND-ØØ41Ø-5 soybeans Safety Features is shown, determining that CSM is not required (EFSA, 2011f) as per this application. Shown there is an exercise of the (theoretical) application of CSM to IND-ØØ41Ø-5 soybean as it might be eventually needed in case of unintended spills into the environment. Verdeca's ERA did not establish significant uncertainty or risks within IND-ØØ41Ø-5 soybean. CSM is required for cultivation of a GMO and where the ERA has established an uncertainty, or a risk hypothesis can be formulated that requires dedicated monitoring. Given that IND-ØØ41Ø-5 soybean will not be cultivated in the EU and that it has no toxicity or effects on TO or NTO, the need for CSM is deemed not necessary. If a putative adverse effect is identified within the scope of this or any IND-ØØ41Ø-5 authorisation, a GS program and a CSM protocol in the PMEM context will be commenced to study the newly identified risk and to explore the nature and level of the underlying uncertainty. The CSM will specifically identify the target population(s) or environment(s) where the risk is present. Then measure the exposure levels in the target populations and environments through measurements of the relevant endpoints and monitor the impacts of IND-ØØ41Ø-5 soybean on these groups or environments. The measurements and analysis will be recorded and reported as needed. Timely reports will be also submitted if the risk requires management by Verdeca or other parties.

(d) General surveillance of the impact of the GM plant (approach, strategy, method and analysis)

In the Subsection above, a comprehensive account of the opportunity and characteristics of a PMEM plant, which includes the GS issue is given. Verdeca's PMEM plan GS is based on the following:

- Analysis of data collected by EuropaBio and European trade associations on the import, distribution and use of IND-ØØ41Ø-5 soybean.
- Issued alerts or reports by authorities, existing networks and/or the press that discuss any potential effects of IND-ØØ41Ø-5 soybean.
- Scientific literature data concerning GMO, GMO Soybean, soybean or animal feeding that may have an impact on IND-ØØ41Ø-5 soybean use and dissemination in the environment.
- Verdeca stewardship practices and open communication with the various stakeholders that will create the pathway where any questions or issues concerning IND-ØØ41Ø-5 can be communicated to Verdeca directly.

(e) Reporting of the results monitoring

The annual report of this PMEM plan's GS activities will be published for general dissemination and use by any stakeholder. Raw data used to compile the annual report will be made available for scientific exchange. Periodically (e.g. every third year) the annual report will contain longer-term observations, summarizing the data collected over the lifecycle of the products and any effects noted during the products lifecycle along with the annual observations. Verdeca's stewardship of IND-ØØ41Ø-5 soybean will remain the highest priority of the company to protect the wholeness of IND-ØØ41Ø-5 soybean and maintain its safe use.

13. DETECTION AND IDENTIFICATION TECHNIQUES FOR THE GM PLANT

Verdeca has developed a method for detection of IND-ØØ41Ø-5 soybean and has forwarded the method, reference samples, reagents and raw data to the European Reference Laboratory as required in 2019.

14. INFORMATION RELATING TO THE PREVIOUS RELEASES OF THE GM PLANTS (FOR ERA ASCPECTS).

History of previous releases of the GM plant notified under Part B of the Directive 2001/18/EC and other Part B of Directive 90/2220/EEC by the same notifier.

There have been no releases of IND-ØØ41Ø-5 soybean under Part B of the Directive 2001/18/EC and other Part B of Directive 90/2220/EEC in the past and there not planned for the future.

14.2. History of previous releases of the GM plant carried outside of the Community by the same notifier

There is a history of regulated releases of IND-ØØ41Ø-5 Soybean in South and North America. The data required as per this Subsection is displayed in Tables 14.2.1 and 14.2.2.

14.2.1.

(a) Release Country:

Argentina

(b) Authority: CONABIA (Comisión Nacional Asesora de Biotecnología Agropecuaria), the Argentine agro-ecosystem biosafety regulatory authority.

(c) Release site	(d) Aim of Release	(e) Duration*
Zavallla, Santa Fe	Observation Breeding Seed increase	2008 - 2010
Rosario, Santa Fe	Observation Crossings Seed increase	2008 - 2015
Villa Mercedes, San Luis	Observation Seed increase	2009 - 2010
Girardet; Santiago del Estero	Observation Seed increase	2009 - 2010
Charata, Chaco	Observation Seed increase	2010 - 2011
Monte Buey. Cordoba	Observation Seed increase Regulatory data	2010 - 2013
Chilibroste, Cordoba	Regulatory and Composition data	2012 – 2014
Corral de Bustos, Cordoba	Regulatory data	2012 - 2013
Alto Alegre, Cordoba	Observation Seed Increase Regulatory data	2010 - 2012
Villa Saboya	Regulatory data	2012 – 2014
Carmen de Areco, Buenos Aires	Regulatory data	2012 – 2014
General Capdevilla, Chaco	Observation Seed Increase	2010 - 2011
Daireaux, Buenos Aires	Regulatory data	2012 - 2013
San Agustín	Regulatory data	2012 – 2014
Colonia Raquel, Santa Fe	Observation Seed Increase	2012 - 2012
Landeta	Regulatory data	2013 - 2014
Hughes, Santa Fe	Observation Seed increase Regulatory data	2010 - 2013

(c) Release site	(d) Aim of Release	(e) Duration*
Acevedo, Buenos Aires	Observation Seed Increase	2012 - 2012
Aranguren, Entre Rios	Observation Seed increase Regulatory data	2010 - 2013
San Enrique, Buenos Aires	Observation Seed Increase	2011 - 2012
Junin, Buenos Aires	Seed Increase Crossings	2013 - 2014
Bella Vista, Corrientes	Seed Increase	2014 - 2015

*: The range includes several releases involving seasons during the indicated years (approximately 5.5 months each). Regulated releases require a 1-year post harvest monitoring for volunteers or other persistence. In Argentina, a field trial finalisation report is mandatory

14.2.2.

(a) Release Country:

Unites States of North America

(b) Authority: USDA = United States Department of Agriculture, Animal and Plant Health Inspection Service, Biotechnology Regulatory Services

(c) Release site	(d) Aim of Release	(e) Duration*
American Falls, ID	Breeding Observation	2011 - 2012
American Falls, ID	Regulatory data Observations	2012 – 2013
Troy, OH	Regulatory data	2012 - 2013
Richland, IA	Observations	2012 - 2013
Kiowa, KS	Regulatory data	2012 - 2013
Ponce, PR	Seed Increase Crossing	2013
Ponce, PR	Seed Increase Crossing	2013 - 2014
Richland, IA	Regulatory data	2013 - 2014

(c) Release site	(d) Aim of Release	(e) Duration*
Highland, IL	Regulatory data	2013 - 2014
Carlyle, IL	Regulatory data	2013 - 2014
Effingham, IL	Regulatory data	2013 - 2014
Ladoga, IN	Regulatory data	2013 - 2014
Troy, OH	Regulatory data	2013 - 2014
York, NE	Regulatory data	2013 - 2014
Troy, KS	Regulatory data	2013 - 2014
Pemberton, OH	Regulatory data	2013 - 2014
Hinton, OK	Regulatory data	2013 - 2014
Ponce, PR	Seed Increase Crossing	2013 - 2014
Ponce, PR	Seed Increase Crossing	2013 - 2015
Dewitt, AR	Breeding	2016 - 2017
Ponce, PR	Crossing Seed Increase	2017
Pemberton, OH	Regulatory Data	2017 - 2018
Troy, OH	Regulatory Data	2017 - 2018
Newport, AR	Seed Increase	2018 – 2019
Newport, AR	Seed Increase	2019

*Duration of release is 1 season (approx. 5.5 months). Regulated releases require a 1-year post harvest monitoring for volunteers or other persistence.

14.2.3

(a) Release Country:

Brasil

(b) Authority:

CTNBio (Comissão Técnica Nacional de Biossegurança)

(c) Release site	(d) Aim of Release	(e) Duration*
Cambé, Paraná, (CB)	Regulatory Data	2016-2018
Costa Rica, Mato Grosso do Sul, (CR)	Regulatory Data	2016-2018
Rondonópolis, Mato Grosso (RL)	Regulatory Data	2016-2018

*: The range includes several releases involving seasons during the indicated years (approximately 5.5 months each)

14.2.4

(a) Release Country:

Paraguay

(b) Authority:

MAG (Ministry of Agriculture and Livestock)

(c) Release site	(d) Aim of Release	(e) Duration*
Capitán Miranda, Itapúa	Regulatory Data	2016-2017
Yhovy, Canindeyú	Regulatory Data	2016-2017
Tomás Romero Pereira, Itapúa	Regulatory Data	2016-2017

*: The range includes one season (approximately 5.5 months).

14.2.5

(a) Release Country:

China

(b) Authority:

MOA (Ministry of Agriculture)

(c) Release site	(d) Aim of Release	(e) Duration*
Beijing	Seed Increase	2016-2017
Tangshan, Hebei	Seed Increase	2018
Tongliao, Inner Mongolia	Seed Increase	2018

*: The range includes one season (approximately 5.5 months).

(f) Aim of post release monitoring:

f1. Argentina: post-harvest monitoring for volunteers or other forms of persistence, including accidental animal intrusion or feeding, compliance with isolation distances according to the crop, and reports from both, government inspectors and applicant professionals is mandatory. A finalization report by the applicant is mandatory, and failure of its submission determines that the applicant will not be allowed subsequent releases. Also, if applicable, appropriate use of seed processing equipment is also subject to inspection.

f2. North America

Post-release monitoring is performed to measure any effects due the release including observable effects on the site and surrounding the site. These include biotic and abiotic responses to the transgenic event. Survival, dormancy and degree of volunteerism is also measured.

(g) Duration of post-release monitoring

Post-release monitoring occurred for one season (typically one calendar year) post-harvest with the exception of Puerto Rico, that had a 6-month monitoring period due to the semi-tropical growth conditions that promoted seed germination. In Argentina, one-year monitoring and a post-harvest report is mandatory.

(h) Conclusion of post-release monitoring

In repeated releases within Argentina and the USA, no effects were observed during the post-harvest monitoring period that were unusual or not typical of commodity soybean cultivation. Volunteerism was infrequent and where soybean did eventually survive to germinate the following season (a very unlikely event), it was easily removed through standard agricultural practices. No persistence was observed. No effect on any non-target organism or abiotic system were observed. In Argentina, breach of isolation distances or animal intrusion and feeding determines either destruction of the crop in place or other forms of removal from field or human or animal exposure. Animals which eventually fed after accidental intrusion into the regulated field are subject to veterinary health control for a stipulated time period.

(i) Results of the release in respect to any risk on human health and environment.

There are no defined risks in the cultivation, handling and consumption of IND-ØØ41Ø-5 soybean. No risks to the environment and human health have been detected. This was confirmed through the extensive release history and observations of IND-ØØ41Ø-5 soybean. In Argentina, any breach of experimental release regulations determines either destruction of the crop in place or other forms of removal from field or human or animal exposure.

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Appendix 10. Glufosinate Tolerance of IND-ØØ41Ø-5 Soybean. Verdeca Technical Report, 1010315, 1-9.

Appendix 11. Description of Commercial Soybean Varieties Used as Comparators for IND-ØØ41Ø-5. Verdeca Technical Report, 1010313, 1-6.

Appendix 12. A Proposal Regarding the Selection of Appropriate Comparators for Comparative Assessment as per EFSA Guidelines. ""Guidance on Selection of Comparators for the Risk Assessment of Genetically Modified Plants and Derived Food and Feed. Verdeca Technical Report, 1010311, 1-7.

Appendix 13. Compositional Assessment of IND-ØØ41Ø-5 Soybean. Verdeca Technical Report, 1010305, 1-84.

Appendix 14. Endogenous Soybean Allergens Levels. Verdeca Technical Report, 1010304, 1-25.

Appendix 15. On the EFSA Requirement of Agronomic Performance and Phenotypic Characteristics in the Application for Import, Food and Feed Uses, and Processing, of Soybean Event IND-ØØ41Ø-5. Verdeca Technical Report, 1010315, 1-10.

Appendix 16. On the EFSA Requirement of Compositional Analysis in the Application for Import, Food and Feed Uses, and Processing, of Soybean Event IND-ØØ41Ø-5. Verdeca Technical Report, 1010314, 1-10.

Appendix 18. 90-Days Oral Toxicity Study of IND-ØØ41Ø-5 Soybean. Verdeca Technical Report, 1010307, 1-6.

Appendix 19. Human and Animal Dietary Exposure Assessment to Genetically Modified Soybean IND-ØØ41Ø-5. Verdeca Technical Report, 1010303, 1-28.

Appendix 20. IND-ØØ41Ø-5 Soybean Germination and Dormancy Evaluation. Verdeca Technical Report, 1010309, 1-6.

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