

**CONSOLIDATED REPORT OF SOYBEAN DP305423  
FOR DIRECT USE AS FOOD AND FEED, OR FOR PROCESSING**

**EXECUTIVE SUMMARY**

On April 13, 2018, Pioneer Hi-Bred submitted soybean DP305423 for direct use as food and feed, or for processing to the Bureau of Plant Industry (BPI) under the DOST-DA-DENR-DOH-DILG Joint Department Circular (JDC) No. 1 Series of 2016. After reviewing the Risk Assessment Report and attachments submitted by the applicant, the assessors namely: Scientific and Technical Review Panel (STRP), BPI- Plant Products Safety Services Division (BPI-PPSSD) and Bureau of Animal Industry (BAI), concurred that DP305423 is as safe for human food and animal feed as its conventional counterpart.

The Department of Environment and Natural Resources – Biosafety Committee (DENR-BC), after a thorough scientific review and evaluation of the documents related to Environmental Risk along with the submitted sworn statement and accountability of the proponent, recommended the issuance of a biosafety permit for this regulated event provided that the conditions set by them are complied.

Also, the Department of Health – Biosafety Committee (DOH-BC), after a thorough scientific review and evaluation of documents related to Environmental Health Impact, concluded that soybean DP305423 will not pose any significant risk to health and environment and that any hazards could be managed by the measures set by the department. DOH-BC also recommended for the issuance of biosafety permit for soybean DP305423.

Furthermore, the Socio-economic, Ethical and Cultural (SEC) Considerations expert also recommended for the issuance of biosafety permit for this regulated article after assessing the socio-economic, social and ethical indicators for the adoption of Genetically Modified Organisms.

**BACKGROUND**

In accordance with Article VII. Section 20 of the JDC, no regulated article, whether imported or developed domestically, shall be permitted for direct use as food and feed, or for processing, unless: (1) the Biosafety Permit for Direct Use has been issued by the BPI; (2) in the case of imported regulated article, the regulated article has been authorized for commercial distribution as food and feed in the country of origin; and (3) regardless of the intended use, the regulated article does not pose greater risks to biodiversity, human and animal health than its conventional counterpart.

The BPI Biotech Office provided the assessors the complete dossier submitted by Pioneer Hi-Bred Inc.

Upon receipt of the individual reports from the assessors, the BPI Biotech Office prepared this consolidated risk assessment report for the information of the public.

## **STRP, PPSSD and BAI ASSESSMENT**

**Soybean DP 305423** was developed by Pioneer Hi-Bred Philippines, Inc. through the use of modern biotechnology. The said event was developed through microprojectile co-bombardment transformation of soybean cells with PHP19340A and PHP17752A plasmid vectors carrying the *gm-fad2-1* fragment which provides seed with increased levels of monosaturated (oleic) fatty acid and decreased level of polyunsaturated fatty acids and to a lesser extent, palmitic acid, via a mechanism of gene silencing. The plasmid vector also contains *gm-hra* gene that encodes GM-HRA protein which confers tolerance to Acetolactate Synthase (ALS)-inhibiting herbicides.

### *Host Organism (Glycine max L.)*

**Soybean (*Glycine max L.*)** has been grown world-wide as an important staple food for humans and feed ingredient for animals. Its major products are seeds, oil, and meal. Unprocessed soybeans are not suitable for food and their use for animal feed remains limited because they contain anti nutritional factors such as trypsin inhibitors and lectins which are inactivated by heat processing. Humans consume soybean mostly in processed form such as soy milk, milk curd/ tofu, whole cooked seed, edible soy oil, soy protein concentrate, isolated soy protein, hydrolyzed vegetable protein, textured soy protein and soy protein fibers. It is also being consumed by animals in the form of seed, forage/silage, hay, meal and hulls (OECD, 2012).

The STRPs and other agencies concurred that soybean is a source of key nutrients such as proteins, fat, ash, acid detergent fiber (ADF), neutral detergent fiber (NDF), total dietary fiber (TDF), crude fiber, carbohydrates, amino acids, fatty acids, minerals and vitamins (OECD, 2012). Soybean contains anti-nutritional factors such as oligosaccharides, trypsin inhibitors, lectins, phytic acid and other compounds such as isoflavones, phospholipids, sterols and saponins (OECD, 2012). Trypsin inhibitors and lectins are easily degraded upon heating. Soy saponins are not considered to be true anti-nutrients. High levels of soy saponins were found to have no adverse effect upon induction to laboratory animals. It has a weak effect on intestinal permeability and has little impact on active nutrient transport.

*History of safe use* was attributed to soybean. Based on OECD report, soybeans are commonly consumed in processed form and primary source of oil and protein. Heat processing eliminates the anti-nutritional factors in soybean. Toxicants are not commonly found in soybean.

### *Transgenic Plant*

DP305423 soybean has been reviewed and approved for food and/or feed use in many countries including Australia (Food, 2010), Brazil, (Food and Feed, 2018), Canada (Food, 2009), China (Food, 2011), Colombia (Food, 2016), European Union (Food, 2015), Japan (Food, 2010), Malaysia (Food, 2017), Mexico (Food, 2008), Philippines (Food, 2013), Singapore (Food, 2014), South Africa (Food, 2011), South Korea (Food, 2010), Taiwan (Food, 2010; Feed, 2017) and United States (Food, 2009).

Based on the documents provided by the developer, the assessors agreed that consumption patterns by population subgroups are not expected to be altered.

## Donor Organisms

*Glycine max* (L.) is the donor of *gm-fad2-1* and *gm-hra* genes. The donor organism is not known for human or animal pathogenicity and is not commonly allergenic (Pioneer, 2018). As previously indicated in the “Host Organism” part of this summary report, history of safe use was attributed to the donor organism.

## Transformation System

The event, DP 305423 was developed through microprojectile co-bombardment transformation of soybean cells with PHP19340A and PHP17752A plasmid vectors carrying the *gm-fad2-1* fragment which provides seed with increased levels of monosaturated (oleic) fatty acid and decreased level of polyunsaturated fatty acids and to a lesser extent, palmitic acid, via a mechanism of gene silencing. The plasmid vector also contains *gm-hra* gene that encodes GM-HRA protein which confers tolerance to ALS-inhibiting herbicides.

Further, the two (2) linear DNA fragments, PHP19340A and PHP17752A were derived from plasmids PHP19340 and PHP17752, respectively. Plasmid PHP19340 and fragment PHP19340A contain the *gm-fad2-1* cassette. Plasmid PHP17752 and fragment PHP17752A contain the *gm-hra* gene cassette. The developer provided sufficient information on the experimental protocol used (Pioneer, 2018, Part D. p. 14). The complete list of all genetic components used in the transformation of DP 305423 soybean was presented in Part D. Table 1 (p. 20 and 23) of the submitted dossier by the developer.

## Inserted DNA

Southern blot analysis was used for the molecular characterization of DP 305423 soybean to evaluate insert copy number, insert integrity and presence of plasmid backbone sequences. It was determined that multiple intact and truncated copies of PHP19340A have been inserted into 305423 soybean that contain, in total, eight copies of the KTi3 promoter, seven copies of the *gm-fad2-1* fragment, and five copies of the KTi3 terminator. A single, intact copy of the *gm-hra* gene cassette of fragment PHP17752A has been inserted into 305423 soybean that contains the SAMS regulatory region, the *gm-hra* gene and the als terminator. Based on the data provided, 305423 soybean has incorporated four insertions that are genetically linked and segregated as a single locus. (Pioneer, 2018, Part E. p. 24) Southern blot analysis was also used to investigate the integration pattern of the inserted DNA in 305423 soybean using selected restriction enzyme digestion and probes homologous to each genetic element of *gm-fad2-1* and *gm-hra*.

In addition, the STRPs, PPSSD and BAI noted that multiple copies, both intact and truncated, of PHP19340A have been inserted into the genome of DP 305423 soybean comprising, in total, eight copies of the KTi3 promoter, seven copies of the *gm-fad2-1* fragment, and five copies of the KTi3 terminator. One copy of the PHP19340A fragment is intact and contains a complete KTi3 promoter, *gm-fad2-1* fragment, and KTi3 terminator. Four copies of the PHP19340A fragment have some truncation but contain portions of all three cassette elements. Two copies of the PHP19340A fragment retain only the KTi3 promoter with the *gm-fad2-1* fragment. Finally, one copy of the KTi3 promoter is associated with a small non-functional fragment of backbone DNA. For the PHP17752A fragment, it was determined

that a single, intact *gm-hra* cassette has been inserted into the genome of DP 305423 soybean.

Bioinformatic analysis were used to evaluate DNA sequences spanning the junctions between the soybean genomic sequence and insert to determine whether the amino acid sequences of *in silico* translations have biologically relevant sequence similarity to known or putative proteins allergens and toxins should they be expressed *in planta*. The developer used COMPARE 2017 to analyze the nucleotide sequences in the MZIR098 element-to-element and genome-to-insert junctions. A total of 116 *in silico* translated ORFs meeting the minimum frame size were identified across the four insertions. None of the identified translated ORFs in 305423 soybean Insertion Regions 1 through 4 returned alignments from the search against the toxin database, indicating that there is no toxicity concern regarding the identified translated ORFs in 305423 soybean Insertion Regions 1 through 4.

A small non-functional fragment from the plasmid backbone was present in DP 305423 soybean. It was determined through Southern Blot Analysis with the probes covering the complete backbone DNA of plasmids PHP19340 and PHP17752 was used to examine the presence of regions outside the two transformation fragments, PHP19340A and PHP17752A, in 305423 soybean. This fragment was found to be associated with a truncated copy of the KTi3 promoter as a part of Insertion 3 in 305423 soybean.

### *Genetic Stability*

The multigenerational genetic stability of the insert and absence of plasmid backbone sequence in the DP 305423 Soybean genome was assessed by Southern blot analyses over three generations. Results of the analysis showed that the inserted T-DNA in DP 305423 soybean is stable from one generation to the other.

The assessors also added that a chi-square test of trait inheritance from three different generations was performed to determine the Mendelian heritability and stability of the genes. The results of this analysis are consistent with the finding of a single, genetically-linked locus of insertion that segregates in DP 305423 soybean progeny following Mendelian law.

### *Expressed Material*

GM-HRA protein encoded by the *gm-hra* gene confers plant tolerance to ALS-inhibiting herbicides. This feature was used as a selectable marker in the process of development of 305423 soybean.

The concentrations of *gm-hra* were quantified by enzyme-linked immunosorbent assay (ELISA) to establish an expression profile for these proteins as produced in DP 305423 soybean. The mean levels of GM-HRA in leaves, roots and grains were 4.0, 0.18 and 2.5 ng/mg, respectively.

### *Toxicological and Allergenicity Assessment*

The safety assessment of novel protein, GM-HRA, includes digestibility, heat inactivation, oral toxicity and amino acid sequence comparison studies to determine its potential to cause toxicity or allergenicity to humans (Pioneer, 2018).

Digestibility study demonstrated that GM-HRA was readily degraded upon incubation in simulated gastric fluid (SGF) with pepsin and simulated intestinal fluid (SIF) with pancreatin at less than 30 seconds and less than 1 minute, respectively (Comstock, 2006).

The temperature dependence of GM-HRA enzymatic activity was determined through acetolactate synthase/acetohydroxyacid synthase (ALS/AHAS) activity assay. The results of the assay showed that the GM-HRA enzyme activity was completely lost after incubation for 15 minutes at 50°C (Comstock, 2007).

Bioinformatics analysis against the UniProtKB/Swiss-Prot and public domain databases and FASTA a-type algorithm search showed that GM-HRA has no homology to any known toxins or allergens (Baxevanis, 2005; Pearson and Lipman, 1988; Pioneer, 2018).

Acute oral toxicity study of GM-HRA that administration of GM-HRA in mice have no treatment related adverse clinical observations and effect in body weight and food consumption (OECD, 2001). The no observed effect level (NOEL) is 436 mg GM-HRA/kg body weight.

*Escherichia coli* is used as source protein. Equivalency of the *E. coli*-produced GM-HRA and the plant produced GM-HRA was demonstrated with respect to identity, integrity, and insecticidal activity (Metcalf et al., 1996).

GM-HRA protein is 0.000607% of total protein in soybean seed. GM-HRA is not expected to be found in refined oil which is the major food use of soybean.

The immunoblot and ELISA inhibition data indicate that the IgE binding of the 305423 soybean was not altered and is similar to the non-transgenic control line. Therefore, the levels of endogenous allergens in 305423 soybean are comparable to those in nontransgenic control soybean.

Results of the toxicological and allergenicity assessment indicate that GM-HRA protein being expressed in DP 305423 soybean is not toxic or allergenic to humans (Pioneer, 2018).

### *Nutritional Data*

Compositional analysis provided by the developer indicating the nutritional data of DP 305423 in comparison with the non-transgenic soybean, range of commercial varieties and range of literature values (ILSI, 2004, Kim et al., 2005, Taylor et al., 1999, OECD, 2001). Results of the analysis indicated that there is no differences in the proximate, fiber, amino acid, fatty acid, vitamin, mineral, anti-nutrient and secondary metabolite levels of DP 305423 soybean and the non-transgenic soybean that can be considered biologically relevant with the exception of some of the fatty acids, which had altered profile as expected.

### *Conclusion*

For the transgenic **DP305423** soybean, weight of evidences approach indicates that the single event, soybean 305423, is substantially equivalent with the conventional counterpart in terms of nutritional composition and food safety other than the intended

increased levels of monounsaturated (oleic) fatty acid, decreased levels of polyunsaturated fatty acids (linoleic and linolenic) and to a lesser extent, palmitic acid, and tolerance to ALS-inhibiting herbicides. After reviewing the provided material of Pioneer Hi-Bred Philippines, Inc. and other literatures, the STRPs, PPSSD, and BAI, therefore concluded that DP305423 soybean is as safe as its conventional counterpart.

### **DENR RECOMMENDATION**

After a comprehensive review and evaluation of the documents including the scientific evidences from provided references and literature submitted by Pioneer Hi-Bred Philippines Inc., on its application for Direct Use as FFP of Soybean (DP305423), hereunder are the observations and appropriate actions:

1. The direct use of the regulated article whether for food, feed, or for processing will not cause any significant adverse effect on the environment (land and water) and biodiversity. The transgenic crop will not increase its weediness potential in case the seeds spill out into the environment because the GM-HRA enzyme produced by the transgenic crop will degrade upon exposure to the natural environment and general condition that is high temperature, 50°C and above, varying pH, enzyme digestion, etc. (Comstock, 2007). Also, there is no significant difference between the conventional and genetically modified soybean in terms of its percent germination rate, percent dead seed, and percent viable firm seed since there was no genetic modification performed in the reproductive and growth characteristics of soybeans under abiotic and biotic stresses (CFIA, 2009).
2. Acute oral mouse toxicity study (Mathesius et al., 2009) shows that GM-HRA protein does not cause oral toxicity.
3. The project description report (PDR) discusses the specified environmental management plan indicating the possible risk and harm to the environment and biodiversity as well as the mitigating measures and contingency plan. Furthermore, the chances of unintended release or planting of the regulated article is very minimal and will not cause any damaging and lasting effects because the receiving environment (areas near the port, roads, railways, etc.) is not conducive for plant growth. Also, soybeans generally are very highly domesticated and does not survive well without human intervention (FAO, 2014).
4. The Bureau of Plant Industry (BPI) shall ensure the proper and secure packaging of the regulated article for transport and the safety and durability of the transport vehicle, for prevention of any possible spillage or unintended release during transport/import based on BPI's inspection in the port area.

Based on the review and evaluation, the DENR-BC considered the regulated article safe to the environment and biodiversity, and hereby submits the technical report relative to the application of Pioneer Hi-Bred Philippines, Inc. Soybean DP305423 for Biosafety Permit for direct use as food, feed or for processing.

## **DOH RECOMMENDATION**

After a thorough review and evaluation of the documents provided by the proponent, Pioneer Hi-Bred Inc., through the Bureau of Plant Industry (BPI), in support of their application for approval for Direct Use as Food, Feed or for Processing (FFP) of Soybean DP305423, We,

Find that the regulated article applied for Direct Use as Food, Feed or for Processing (FFP) is safe as its conventional counterpart and shall not pose any significant risk to human and animal health and environment.

The following are the observations and recommendations:

1. Scientific pieces of evidence from Toxicity studies and references, find that the regulated article will not cause significant adverse health effects to human and animal health.
2. Dietary exposure to the regulated article is unlikely to result in allergic reaction.
3. The regulated article is as safe as food or feed derived from conventional soybean varieties.
4. The regulated article is not materially different in nutritional composition from that of the non-transgenic soybean or the conventional soybean.
5. It is suggested that the Bureau of Plant Industry (BPI) ensure that there shall be clear instructions that the product is only for the purpose of direct use for FFP and is not to be used as planting materials.
6. Based on the above considerations and with the submitted sworn statement and accountability of the proponent, we hereby submit our evaluation to BPI relative to the application of a Biosafety Permit for Direct Use as Food, Feed, or for Processing (FFP) of Soybean DP305423.

## **SEC EXPERT EVALUATION**

Based on SEC expert review of the SEC questionnaire answered by the applicant:

In terms of production, the proponent has successfully provided the supply and demand situation of the Philippine Soybean industry from 2014-2018. In addition, it has also provided data on major soybean producers of the world in 2000. It was also made clear by the proponent that the import of GM soybean event DP305423, will only be used for food and feed processing.

In addition, the data on the volume of total soybean imports in the Philippines, by product type were shown by Pioneer Hi-Breed, Philippines from 2013-2017. The data trend clearly indicates that the imports are strongly augmenting the meager domestic supply for food-feed processing in the Philippines soybean industry.

Thus, after a thorough and scientific review and evaluation of the documents provided by the technology developer (Pioneer Hi-Bred) relevant to DP305423, the SEC Expert recommend for the approval and issuance of biosafety permit of the said GM product.