Consolidated Risk Assessment Report of Monsanto's Cotton MON531 Application for Direct Use as Food, Feed or for Processing (FFP)

EXECUTIVE SUMMARY

On May 31, 2018, Monsanto Philippines Inc., submitted cotton MON531 application 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 cotton MON531 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, considered the regulated article safe to the environment and biodiversity.

The Department of Health – Biosafety Committee (DOH-BC), after a thorough scientific review and evaluation of documents related to Environmental Health Impact, found evidence that cotton MON531 will not pose any significant risk to health and environment and that any hazards could be managed by the measures set by the department.

Furthermore, the Socio-economic, Ethical and Cultural (SEC) expert recommended for the issuance of biosafety permit for this regulated article after reviewing and assessing the information provided by the applicant in the SEC questionnaire.

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 Monsanto Philippines, Inc.

Below is the summary of the evaluation conducted by the STRP, SEC Expert and regulatory agencies.

A. STRP, PPSSD, BAI ASSESSMENT

After thorough review of the technical documents submitted by the applicant, the assessors' findings are as follows:

A. Host Organism

OECD Consensus Document indicated that cotton is a source of key nutrients (OECD, 2009). It is a source of oil that is derived from cotton seed that can be consumed by humans and proteins which is present in cottonseed cake and meal making it appropriate as livestock feed.

Based on proximate analysis, cottonseed contains Neutral Detergent Fiber (NDF) with a range of 40-54.8 %, Acid Detergent Fiber (ADF) with 29-40.1% and Total Dietary Fiber of 5.77%.

Essential vitamins (B1, B2, B6, C E, Folate and Niacin) and minerals (Na, K, Ca, P, Mg, Fe and others) were also present in the cottonseed.

Cotton contains anti-nutrient components such as gossypol and cyclopropenoid fatty acids.

Gossypol is an oil-soluble complex polyphenolic compound which is highly toxic to swine and poultry. Gossypol is a terpenoid that is present in the secretory structure of most cotton plant tissues including seeds. The level of gossypol and related terpenoids in cotton seed vary due to different species, variety, fertilizer application, and environmental conditions. Gossypol can render amino acid lysine metabolically unavailable which affects the normal functioning of mitochondria.

The cyclopropenoid fatty acids, include dihydrosterculic acid, sterculic acid, and malvalic acid that account for 0.5 to 1.0 percent of the total fat content of cottonseeds. Cyclopropenoid fatty acids are anti-nutritional compounds, which interfere with the metabolism of saturated fats and reportedly have adverse effects on egg yolk discoloration and reduce hatchability in chickens.

Due to the presence of these anti-nutrients in cottonseed, only highly refined products (refine, bleached and deodorized oil and linters) are suitable for human consumption; the levels of gossypol and cyclopropenoid fatty acids are drastically reduced during processing.

OECD Consensus Document indicated that cotton is not a source of allergens (OECD, 2009).

B. Transgenic Plant

Cotton MON 531 has been reviewed and approved for food use in many countries including Argentina (1998), Australia (2000), Brazil (2005), Canada (1996), China (2008), Colombia (2003), European Union (2002), Japan (2001), Mexico (1996), New Zealand (2000), Paraguay (2007), Philippines (2004), Singapore (2014), South Africa (1997), South Korea (2003), Taiwan (2015), USA (1995).

The Philippines first approved MON 531 for direct use as Food and Feed, or for processing on February 5, 2004.

C. Donor Organism

The donor organisms, *Bacillus thuringiensis* variety *kurstaki* (*cry1Ac*) and *Escherichia coli* (*nptII* and *aad*), are not known for human or animal pathogenicity, and are not commonly allergenic. These organisms are not commonly used directly as a food or feed source, however are ubiquitous in nature and are likely present as contaminates on the food and feed consumed. In addition, *Escherichia coli*, is present in the digestive systems of humans and animals.

Bacillus thuringiensis variety *kurstaki*, which produces the B.t. insect control protein, is the basis of microbial formulations commercially available for Lepidopteran insect control for over 30 years. Based on the available scientific data, EPA and other regulatory agencies, worldwide, have determined that use of registered B.t. products pose no significant risks to human health, non-target organisms, or the environment (U.S. EPA, 1988). The protein produced by MON 531 is nearly identical (>99.8%) to that found in nature and in commercial B.t. formulations.

The *nptII* gene encodes a selectable marker enzyme, neomycin phosphotransferase II (NPTII), which was isolated from kanamycin resistant bacteria that contained the Tn5 transposon and has been used as a selectable marker in *Escherichia coli*, animal and human cells and plants. The NPTII protein, which has no insecticidal effect, is ubiquitous in the environment and found in microbes present on food and within the human digestive system. The safety of the NPTII protein has been reviewed and discussed broadly because of its wide use as a selectable marker for plant transformation.

The *aad* gene encodes the bacterial selectable marker enzyme 3'(9)-O-aminoglycoside adenylyltransferase (AAD), which allowed for the selection of the *Agrobacteria* on media containing spectinomycin or streptomycin. The *aad* gene is under the control of a bacterial promoter, and therefore the encoded protein is not expressed in plants derived from Bollgard® cotton event MON 531.

D. Transformation System

Cotton MON531 was generated via *Agrobacterium*-mediated transformation method and the target of genetic modification is the genomic DNA.

The transformation vector PV-GHBK04 contains well-characterized DNA segments required for selection and replication of the plasmid vector in bacteria and transfer of the T-DNA into plant cells. The plant expression vector was assembled and then transformed into *E. coli* and mated into the ABI *Agrobacterium* strain by the tri-parental conjugation system, using the helper plasmid pRK2013. When the plant tissue is incubated with the ABl:plasmid vector conjugate, the T-DNA vector is transferred to the plant cells.

The T-DNA, which includes the *cry1Ac*, *nptII*, and *aad* genes, was transferred into the genome of individual cotton cells thereby allowing selection on kanamycin. After a few days, the residual *Agrobacterium* cells were killed using different antibiotics. Plants were regenerated with modifications of those as described by Trolinder and Goodin (1987). Subsequently, the cotton tissues were treated to stimulate regeneration of transgenic cells into shoots and ultimately plantlets were grown in soil and assayed for insecticidal activity.

E. Inserted DNA

Genetic analyses demonstrated that two T-DNA copies inserted in a head-to-tail arrangement into the cotton genome to produce MON 531. One T-DNA insert, of approximately 8.2 Kb in size, contains a full length cry1Ac gene and

nptII gene (without the ori322 region) and the second insert, of approximately 1.7 Kb maximum size, contains a 3' portion of the cry1Ac gene that cannot be insecticidally active since it does not contain the insecticidally active 5' region of the cry1Ac gene. The two inserts were shown to be linked and this is supported by segregation data from commercial backcrossed lines.

F. Genetic Stability

The stability of the inserted DNA was determined using Southern blot analysis performed on DNA from the R_5 generation and R_6 generation of cotton MON 531, as well as two commercial cotton lines of Bollgard® cotton event 531. DNA from non-transgenic cotton line Coker 312 control. MON 531 from the R_5 generation or R_6 generation, and two commercial lines were digested with *XmnI* probed with the 7S 3' transcriptional termination sequence. Plasmid PV-GHBK04 was used as a positive control. Each of the blots exhibited identical banding patterns in the MON 531 R_5 or R_6 generation, showing physical stability of the inserted and surrounding cotton genomic DNA.

G. Expressed Material

MON 531 produces two functional proteins, Cry1Ac and NPTII, which respectively providing protection to Lepidopteran insects and acting as a selectable marker for plant transformation. Cry1Ac and NPTII protein levels in tissues derived from MON 531 were determined by enzyme-linked immunosorbent assay (ELISA). The levels of the Cry1Ac and NPTII proteins in seeds and leaves were measured in tissues collected from MON 531 samples produced in the United States field trials in 1992. Cry1Ac and NPTII proteins are produced at low levels in the various tissues of the cotton event 531.

Cry1Ac and NPTII proteins were detected in MON 531 but were not detected, as expected, in the Coker 312 parental line. The mean levels of the Cry1Ac protein were 1.56 and 0.86 µg/gram fresh weight in leaf and raw cottonseed, respectively. The mean levels of the NPTII protein were 3.15 and 2.45 µg/gram fresh weight, respectively for leaf and raw cottonseed.

It can be concluded that the Cry1Ac and NPTII protein expression levels measured in each tissue are comparable.

The Cry1Ac and NPTII do not have a metabolic role in the cotton other than rendering the transgenic cotton insect resistant. It is only active against Lepidopteran insect. The Cry proteins have been found toxic only in the gut of specific lepidopteran insect species and not to mammals

H. Toxicological Assessment and Allergenicity Assessment

Bioinformatics analyses demonstrated that the Cry1Ac and NPTII proteins do not share structurally or immunologically relevant amino acid sequence similarities with known toxins or allergens. The Cry1Ac and NPTII proteins represent a very small portion of the total proteins in MON 531 seed.

The Cry1Ac and NPTII proteins in MON 531 are rapidly degraded by simulated gastrointestinal fluids and no adverse effects were observed in

mice to which the Cry1Ac and NPTII proteins at dose levels up to 4200 mg/kg and 5000 mg/kg were respectively administrated.

I. Nutritional Data

The proximates (protein, fat, ash, carbohydrate, calories, and moisture) were measured and observed no statistically significant difference between MON 531 and Coker 312 control at the 95% confidence level, confirming that the levels of these components in Bollgard® cotton are comparable to those of conventional cotton. Amino acid analysis was performed on Bollgard® cotton MON 531 and the Coker 312 control cotton seeds, and no meaningful differences were detected between MON 531 and Coker 312 in terms of amino acid composition. Upon statistical analysis, the levels of glutamic acid, valine, methionine, isoleucine, tyrosine, lysine, and histidine in MON 531 were significantly different on a per dry weight of protein in cotton seed, but were within the published literature range for commercial cotton varieties, and thus these differences were not considered meaningful. The fatty acid composition of oil extracted from seeds was measured in both MON 531 and Coker 312 and there are no meaningful differences between MON 531 and Coker 312 in terms of their fatty acid compositions.

The antinutrients (gossypol and cyclopropenoid fatty acids) were measured. No statistically significant differences were observed in gossypol levels between cotton seeds from MON 531 and Coker 312. Levels of cyclopropenoid fatty acids showed no statistically differences between MON 531 and Coker 312.

In all cases, the results of the analyses demonstrate that the MON 531 seeds are not different from parental control cotton seeds, and that seed from Bollgard® cotton is compositionally equivalent to, and as nutritious as, seed from the parental cotton variety and other commercial cotton varieties.

B. <u>DENR BC (for Safety of Event to the Environment)</u>

After a comprehensive review and evaluation of the documents including the scientific evidences from provided references and literature submitted by Monsanto Philippines, Inc, on its application for Direct Use as FFP of <u>Cotton MON531)</u> 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 non-target organisms. Conventional cotton cultivars do not possess any characteristic of being weedy, such as seed dormancy, persistence in soil and seed banks, germination under adverse environmental conditions, rapid vegetative growth, short life cycle, and high seed output and dispersal rate (Keeler, 1985 and Keeler et al., 1996). The transgenic crop will not increase its weediness potential in case the seeds spill out into the environment because the protein product produced by the transgenic crop will degrade upon exposure to the natural environment and general conditions that is, high temperatures (75°C and

above), varying pH, enzyme digestion, etc. (Heman, Berry, Mueller, & Bell, 2012).

2. CrylAc and NPTII proteins show no toxicity based on amino acid sequence homology. There is no biological relevant identity with putative protein toxins. Likewise, these proteins are rapidly denatured and inactivate its functions in a simulated mammalian digestion (Keck & Mitsky, 1994).

3. The project description report (PDR) discusses the specified environmental management plan indicating the possible risk and harm to the environment and non-target organisms 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 considering that cottons have no potential to persist in an unfavorable environment (Keeler et al., 1996).

Based on the review and evaluation, the DENR-BC considered the regulated article safe to the environment and biodiversity.

C. <u>DOH-BC (for Environmental Health Safety)</u>

After a thorough review and evaluation of the documents provided by the proponent, the DOH-BC found that the regulated article applied for Direct Use for Food and 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 of the DOH-BC:

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.

1. Dietary exposure to the regulated article is unlikely to result in allergic reaction.
2. The regulated article is as safe as food or feed derived from conventional coin varieties.
3. The regulated article is not materially different in nutritional composition from that of the non-transgenic com or the conventional com.
4. 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.

D. <u>SEC Expert (for Socio-economic, ethical and cultural Consideration)</u>

Cotton is widely produced and consumed and is a significant component of global trade of agricultural commodities. However, Philippines is producing a very minimal quantity of cotton. The Philippines textile industry is highly dependent on imported cotton as local production is very negligible relative to the demand of the textile industry. Thus, granting a permit to import Cotton, including MON 531 will help stabilize prices of cotton in particular and textile in general.

In terms of consumption, the granting of permit to import this GM cotton will help stabilize domestic consumption due to stable supply and pries. In terms of global trade, it will have an insignificant effect considering that Philippines is a small country. Its importation is relatively small compared to total global trade of cotton products.

The importation of MON 531 Cotton will not drastically affect the current patterns of consumption, production and trade of cotton. As mentioned earlier, Philippine is an insignificant producer of cotton, thus rely greatly in imported cotton to meet domestic demand. Granting permit to import MON 531 cotton may help stabilize supply and prices of cotton. Thus, consumption will likewise be stabilized. With regards to trade, domestic trade may improve due to availability of supply and stable prices but global trade will not be affected since Philippine import of cotton is very minimal relative to global trade.

The SEC expert recommended for issuance of permit for MON531.