ASSESSORS' CONSOLIDATED REPORT ON PIONEER HI-BRED'S APPLICATION FOR DIRECT USE AS FOOD AND FEED, OR FOR PROCESSING OF CORN MIR604

EXECUTIVE SUMMARY

On July 19, 2017, Syngenta Philippines Inc. submitted corn MIR604 for direct use as food and feed, or for processing, as original application 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- Biotech Team (BAI-BT), concurred that corn MIR604 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 the conditions set by DENR 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 corn MIR604 will not pose any significant risk to the 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 corn MIR604.

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, except for the SEC expert, the complete dossier submitted by Bayer. The SEC expert, on the other hand, was provided with a questionnaire on socio-economic, ethical and cultural considerations that have been addressed by Pioneer in relation to their application.

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

STRP ASSESSMENT AND RECOMMENDATIONS

Based on the documents submitted by the applicant:

A. Host Organism

2 of the STRPs agree that corn is a source of key nutrients like protein, fat, carbohydrates and dietary fiber. They also stated that it is a staple of the human diet for centuries. However, while not disagreeing the fact that corn contains key nutrients, one of the assessors stated that it is not specifically consumed as a source of key nutrients, to which another STRP has also concurred.

Meanwhile, the 3 STRPs agree that it is a source of a few anti-nutrients, including phytic acid, raffinose and trypsin inhibitor, however, these are not considered nutritionally significant. They also agree that there are no significant native toxins nor allergens are reported to be associated with corn.

All the STRPs also concur that corn is indeed used as a staple cereal by some 20% of the population in the Philippines (FNRI, 1993). These food products are said to be derived from wet milling, dry milling, and distillery as soups, edible oil, flakes, chips, etc. Human consumption of corn-based ingredients are primarily from high-fructose corn syrup, starch, sweeteners, cereals, oil and alcohol. Further, the STRPs all agree that field corn is also used mainly for animal feed. Corn silage/forage is used mainly as feed for ruminants, corn gluten meal, corn gluten feed and distillers dried grains derived as by-products of wet and dry milling are also important components of livestock feed (Morris, 1998).

All STRPs agree that since the Philippines belongs to cluster G09, the estimated consumption of corn is 16.736 g/kg bw/day for general population and 32.518 g/kg bw/day for children (GEMS 2012), however one the STRPs stated that this estimation might be a little bit of an overestimate, but then the STRP also stated that for risk assessment purposes, it is better to estimate it on a higher consumption.

B. Transgenic Plant

All the STRPs agree that MIR604 is reported to be not materially different in composition, safety and other relevant parameters from the conventional corn in the market, as per its (a) food approvals in the following countries: United States, Argentina, Australia/New Zealand, Brazil, Canada, China, Colombia, European Union, Indonesia, Japan, Korea, Malaysia, Mexico, Philippines, Russia, South Africa, Taiwan, and Vietnam; and its (b) feed approvals in the following countries: USA, Argentina, Brazil, Canada, China, Colombia, European Union, Japan, Korea, Malaysia, Mexico, Philippines, Russian Federation, South Africa, Taiwan, Turkey, and Vietnam.

They also agree that the consumption patter by population subgroups will not change upon the introduction of MIR604 corn in the market. They stated that the corn consumed as food is not likely to change as it is today.

C. Donor Organism

All STRPs agree that the *mcry3A* and *pmi* sequences have been adequately described. All the protein encoding sequences found in the original gene construct have been described with respect to source and potential pathogenic or allergenic properties. They also agree that all potentially inserted regulatory sequences of promoters, enhancers, termination signals of the *mcry3A* expression cassette, *pmi* expression cassette and the pZM26 transformation plasmid have been adequately described.

They also concurred on the information about the introduced expressible sequences namely: (a) *mcry3A*; and (b) *pmi.* The gene mcry3A came from B. thuringiensis while the pmi gene came from E. coli, both proteins coming from those genes not known to be toxic nor allergenic.

D. Transformation System

All the STRPs agree that the information provided by the applicant is sufficient, citing that the transformation method used was *Agrobacterium tumefaciens*-mediated transformation, and that the target of genetic modification is the Nuclear DNA. They also agree that the experimental protocol of the transformation method was completely provided and that the genetic components listed are adequate.

The STRPs concurred that the transformation plasmid pZM26 used to produce event MIR604 was thoroughly discussed. The size, orientation and location of all genetic elements were clearly presented. Further, the oligonucleotide primers used for PCR analysis as well as the sites of the restriction endonucleases used in the analysis of the endonuclease of the inserted DNA were presented. The gene expression cassettes for *mCry3A* and *pmi* were included in the DNA region between the left and right borders of the transformation plasmid. The *mCry3A* cassette consisted of the *mCry3A* coding region regulated by the MTL promoter and terminator NOS. The *pmi* cassette, on the other hand, is regulated by ZMUbiInt promoter and by NOS terminator.

They also concurred that there were no carrier DNA and/or helper plasmids used in the transformation.

E. Inserted DNA

All the STRPs agree that the molecular characterization data generated indicated that there was only a single intact insertion of the *mcry3A* and *pmi.* Data from southern analysis also showed that MIR604 contains only a single copy of the MTL and ZmUbInt promoters and that the genetic elements of the vector backbone are not present in Event MIR604 hybrids. The consensus sequence data for the event MIR604 T-DNA insert have sufficiently demonstrated the overall integrity of the insert and that contiguousness of the functional elements within the insert as intended in pZM26 have been maintained.

All the STRP also concur on the fact that sequence analysis revealed some truncation occurred at the RB and LB ends of the T-DNA insert during the transformation process that resulted in Event MIR604. The RB portion of the T-DNA insert was truncated by 44bp while the LB end of the T-DNA insert was truncated by 43 bp. These deletions have no effect on the efficacy of the T-DNA insert and this phenomenon has also been previously observed in Agrobacterium transformation. Further, they also agree that from the ORF analysis of all 6 potential reading frames at both the 5' and 3' T-DNA to genome junctions showed the presence of one putative novel ORF. This putative ORF is 258bp. The 240bp of this ORF is terminator sequence and no promoter elements has been found. Thus, transcription of this putative ORF is unlikely.

F. Genetic Stability

All the STRPs agree that the information provided by the applicant was adequate relative to the genetic stability of the trait. They cited that the multigenerational stability of the introduced trait was assessed using Southern analysis. Three generations (BC4, BC5 and BC6) of Event MIR604 corn were used. Results show that the hybridization pattern of several generations of Event MIR604 corn was identical using a mcry3 probe. This demonstrate that the T-DNA insert from pZM26 incorporated into Event MIR604 is stable over several generations.

The STRPs also concur that segregation of 6 generations was assessed wherein T0, T1, T2, T3, T4 and T5 generations were individually analyzed for the presence of *mCry3A* and *pmi*. Real time PCR

was used to confirm the Mendelian Inheritance ratios of the *mCry3A* and *pmi* by determining the segregation ratios. The zygosity of the plant were confirmed by progeny test and assaying by PCR. Chi-square analysis of the segregation data was performed to test the hypothesis that the MIR604 insert is inherited in a predictable manner according to Mendelian principles. The expected Mendelian Inheritance ratio of positive and negative plants for a hemizygous trait in these populations is 3:1.

G. Expressed Material

All the STRPs concur on the information provided by the applicant that the concentration of *mCry3A* and *pmi* proteins in several corn plant tissues derived from MIR604 corn were determined by ELISA. Quantifiable levels of MCRY3A protein were detected in all Event MIR604-derived plant tissues (leaves, roots, whole plants and kernel) analyzed except pollen. On the other hand, PMI protein was detected in most of the Event MIR604-derived plant tissues analyzed, however, at very low levels.

Furthermore, all the STRPs concur that PMI catalyzes the reversible interconversion of mannose-6-PO4 to fructose-6-PO4 and requires Zn for activity. The PMI reaction is specific for mannose-6-PO4 and fructose-6-PO4 with a Keq near 1.0. Plant cells expressing *pmi* gene are capable of survival and growth in the presence of mannose as the only or primary carbon source. Expression of *pmi* gene in transformed plants does not appear to adversely affect plant morphology, growth or agronomic characteristics (Metabolic Pathways, Syngenta, 2017). On the other hand, *mCry3A* is not an enzyme and therefore, does not affect plant metabolism. PMI and MCRY3A are not expected to interact within, nor affect together the metabolism of the corn plant.

H. Toxicological Assessment

All the STRPs have agreed that the simulated mammalian gastric fluid (SGF) containing pepsin was used to evaluate the susceptibility of mCry3A protein and PMI protein to proteolytic degradation. No intact mCry3A or immune-reactive fragments were detected following digestion in SGF for 2 min. as assessed by SDS-PAGE followed by Western blot analysis. Results showed the very fast action of SGF: no remaining fragments of the mCry3A was detected after 2 min. Meanwhile, Pepsin in SGF assay was used to evaluate the susceptibility of PMI to proteolytic degradation. No intact PMI (molecular weight of approximately 42 kDa) or degradation products were detected by Western blot analysis following incubation in SGF for 1 minute.

They also concurred that the effect of temperature on mCry3A protein and PMI protein was determined by incubating the test substance for 30 minutes at a range of temperature followed by a bioassay against western corn rootworm larvae. Results show that at 95oC, mCry3A was completely inactivated. At 4oC, 25oC and 37oC, there was little or no effect on mCry3A bioactivity, whereas at 65oC, there was some reduction in the bioactivity. On the other hand, temperatures of 25oC and 37oC showed no significant effect on PMI enzymatic activity. At 55oC, a loss of approximately 32% enzymatic activity in comparison to the control assay was observed. At 65oC, very little enzymatic activity remained while there was a complete loss of enzymatic activity at 95oC after 30 mins. A loss of approximately 39% immunoreactivity in comparison to the control assay was observed by ELISA after treatment at 65oC and 95oC for 30 mins.

Further, they also agreed that no known or putative mammalian toxins showed homology with amino acid sequence of mCry3A and PMI. The assessment of the mCry3A amino acid sequence using a comprehensive similarity search of a non-redundant NCBI Protein Database and a toxin-specific database created from the NCBI protein listing support the conclusion that the mCry3A sequence shows no biologically relevant similarity to any known or putative mammalian toxins. Further, a comparison of MIR604 PMI protein to sequences in two databases (NCBI and Syngenta Toxin Database) was performed using the Basic Local Alignment Search Tool (BLASTP) program. Results

from both database comparisons confirm that MIR604 PMI is not a toxic protein, nor does MIR604 PMI share significant sequence similarity with other known or putative toxins.

They also cited that the information on the acute oral toxicity study of mCry3A and PMI in mice conducted is adequate. No test substance-related mortalities occurred during the study, and no clinical signs attributable to the test substance were observed. There were no treatment-related effects on body weights, food consumption, or organ weights, nor were any treatment-related effects observed following macroscopic or microscopic examination of tissues. Results show that mCry3A is not acutely toxic to mice. Consequently, results also showed that the administration of 2072 mg PMI protein/kg bodyweight as a single dose, produced no effects that were considered to be related to treatment with the test substance. No treatment-related effects were found.

In addition, mCry3A expressed in recombinant E. coli (Test substance MCRY3A-0102) was compared by analysis of various functional and biochemical parameters to mCry3A protein produced in transgenic corn Event MIR604. Based on these results, it can be concluded that mCry3A protein from recombinant E. coli and MIR604-derived corn are substantially equivalent and that the microbial test substance, MCRY3A-0102, is a suitable surrogate for mCry3A protein produced in MIR604. This was also done for PMI where in PMI produced in the recombinant E. coli (MIR604-PMI-0105) is biochemically and functionally equivalent to the PMI produced in MIR604 corn, indicating that the PMI produced in the E. coli (MIR604-PMI-0105) is a suitable surrogate for the PMI in MIR604 corn.

I. Allergenicity Assessment

All the STRPs have agreed that the simulated mammalian gastric fluid (SGF) containing pepsin was used to evaluate the susceptibility of mCry3A protein and PMI protein to proteolytic degradation. No intact mCry3A or immune-reactive fragments were detected following digestion in SGF for 2 min. as assessed by SDS-PAGE followed by Western blot analysis. Results showed the very fast action of SGF: no remaining fragments of the mCry3A was detected after 2 min. Meanwhile, Pepsin in SGF assay was used to evaluate the susceptibility of PMI to proteolytic degradation. No intact PMI (molecular weight of approximately 42 kDa) or degradation products were detected by Western blot analysis following incubation in SGF for 1 minute.

They also concurred that the effect of temperature on mCry3A protein and PMI protein was determined by incubating the test substance for 30 minutes at a range of temperature followed by a bioassay against western corn rootworm larvae. Results show that at 95oC, mCry3A was completely inactivated. At 4oC, 25oC and 37oC, there was little or no effect on mCry3A bioactivity, whereas at 65oC, there was some reduction in the bioactivity. On the other hand, temperatures of 25oC and 37oC showed no significant effect on PMI enzymatic activity. At 55oC, a loss of approximately 32% enzymatic activity in comparison to the control assay was observed. At 65oC, very little enzymatic activity remained while there was a complete loss of enzymatic activity at 95oC after 30 mins. A loss of approximately 39% immunoreactivity in comparison to the control assay was observed by ELISA after treatment at 65oC and 95oC for 30 mins.

Further, they also agreed that no known or putative mammalian toxins showed homology with amino acid sequence of mCry3A and PMI. The assessment of the mCry3A amino acid sequence using a comprehensive similarity search of a non-redundant NCBI Protein Database and a toxin-specific database created from the NCBI protein listing support the conclusion that the mCry3A sequence shows no biologically relevant similarity to any known or putative mammalian toxins. Further, a comparison of MIR604 PMI protein to sequences in two databases (NCBI and Syngenta Toxin Database) was performed using the Basic Local Alignment Search Tool (BLASTP) program. Results from both database comparisons confirm that MIR604 PMI is not a toxic protein, nor does MIR604 PMI share significant sequence similarity with other known or putative toxins.

Moreover, the STRPs has concurred that the mCry3A proteins from sources (microbially produced and plant produced) were demonstrated to have the predicted MW of ca. 67,700 Da and immunologically cross-reacted with the same anti-mCry3A antibody. No evidence of post-translational glycosylation of mCry3A protein from either source was observed. Comparisons of the biological activity of E. coli-expressed and corn-expressed mCry3A protein in a larval diet bioassay using western corn rootworm showed very similar activities (LC50 values). On the other hand, the TSRPs also agree on this information that PMI from MIR604 corn plant material and microbially produced PMI test substance (MIR604-PMI-0105) showed the same mobility in the Western blot analysis and confirmed an apparent molecular weight consistent with the predicted molecular weight of approximately 42.9 kDa. Both proteins cross-reacted with anti-PMI antibodies, as shown by the Western blot analysis, confirming the identity and integrity of the PMI proteins from both sources. No apparent functional change in PMI as expressed in MIR604 corn and no evidence of glycosylation was found given that there was a replacement of glutamine with histidine which resulted in the substitution of an acidic residue for a basic residue.

Assuming that the protein constitutes ~10% of the total kernel weight (ILSI, 2004), then the mCry3A protein comprises ~0.00159% of the total protein in the kernels while the PMI protein comprises ~0.00028% of the total protein in the kernels.

J. Nutritional Data

All the STRPs concurred that in terms of grain samples the results showed that the actual average carbohydrate levels in the transgenic and control values differed by only 1.0 – 1.5%. A statistically significant difference was observed in protein levels in the 2003 grain, with average % dry weight of protein in the transgenic grain which was only 4-7% higher than in the non-transgenic control. In 2002 grain samples the differences in protein were not significant. Other scattered statistically significant differences are noted but none are consistent across hybrid pairs and growing seasons. While for forage samples, no significant differences were noted in the moisture content of samples in 2002 between transgenic and control. Moisture levels in the 2003 transgenic samples were on ca. 3% higher than in the control samples. All proximate values are within ranges reported in the literature for the analytes for both forage and grain and that the differences were not biologically relevant.

The STRPs also agree that in terms of micronutrient levels in grain, a small number of statistically significant differences are not consistent across hybrid pairs and growing seasons for both calcium and zinc. For forage, the level of potassium in the transgenic samples was ca. 9 -10% higher than in the control samples. Due to lack of literature data, the observed difference, the measured values maybe are normal variation within a typical corn population. Data for forage are available in the literature only for calcium and phosphorous the differences noted between transgenic and control samples were isolated and inconsistent. Selenium and sodium levels were at or below the limit of quantitation in most of the forage samples.

While for the analysis of vitamin composition in 2003 show values for cryptoxanthin, pantothenic acid, vitamin C and several tocopherols in the grain. Data not consistent across growing seasons or consistently associated with the transgene. A statistically significant difference for gamma-tocopherol was observed for both hybrid pairs grown in 2003. Average gamma-tocopherol levels were 9-18% lower in the transgenic grain than in the control grain.

On the other hand, the STRPs also agree that the information on amino acid levels of grain samples from both the 2002 and 2003 locations were analyzed for eighteen amino acids. Several statistically

significant differences between the 2003 transgenic and control grain are highlighted, but these differences were not detected in 2002 grain samples. The degree of difference between the average values for the transgenic and control samples ranges from 1-10%.As for the levels of fatty acids, the five most abundant fatty acids were measured in grain grown in 2002 and 2003. Statistically significant differences in oleic acid levels were observed in both 2002 and 2003, but the results of the F-Test Probability for Location x Genotype interaction indicate a high degree of inconsistency at different locations in the relative values of the hybrid pairs C/D in 2002 and E2/E4 in 2003. Other sporadic, statistically significant differences, especially for hybrid pair E4 and E2, are not consistent across the other hybrid pairs, across growing seasons, or in the direction of the difference.

Lastly, statistically significant differences are noted for both ferulic acid and p-coumaric acid, with lower levels of both in the transgenic samples as compared to the control. These substances are however, not biologically relevant and their values are very low.

K. Recommendation

Find scientific evidence that the regulated article applied for human food and animal feed / for processing use is as safe as its conventional counterpart and shall not pose any significant risk to human and animal health.

BPI-PPSSD ASSESSMENT AND RECOMMENDATION

Corn MIR604 was developed by Syngenta, Philippines, Inc., through the use of recombinant DNA technology. The said event was developed through *Agrobacterium tumefaciens* – mediated transformation of corn cells with pZM6 plasmid vector carrying the *mcry3A* gene encoding mCry3A protein that confers protection against target insect pests and pmi gene encoding PMI protein that catalyzes the isomerization of mannose-6-phosphate to fructose-6-phosphate.

Host Organism (*Zea mays* L.)

Corn (*Zea mays* L.) has been widely consumed as staple food for humans and feed ingredient for animals. It is used in food products such as oil, grit, meal, flour, ethanol, syrup and starch as well as feeds such as hulls, gluten and hominy (OECD, 2002). Humans consume corn mostly in the form of corn-based ingredients such as high fructose corn syrup, starch, sweeteners, cereals, oil and alcohol. In terms of the feeds, it is commonly consumed in the form of corn silage (forage), gluten meal, gluten feed and distillers dried grains. In 2014, the daily per capita consumption index of corn in the Philippines is 60.08 grams/day, while the daily per capita calories supply is 213.88 grams (PSA, 2015).

Corn is a source of key nutrients such as amino acids, fatty acids, carbohydrates, vitamins, minerals, and fiber (OECD, 2002). It is also known to contain anti-nutrients such as phytic acid, 2,4-Dihydroxy-7-methoxy-2H-1,4-benzoaxin-3(4H)-one (DIMBOA), raffinose, trypsin and chymotrypsin inhibitors, and secondary plant metabolites such as furfural, ferulic acid and p-coumaric acid. These anti-nutrients and secondary metabolites have been historically present in corn at levels that would not cause the food to be unsafe.

History of safe use was attributed to corn. It is known to produce no significant amount of toxins and anti-nutrients. It is not a common allergenic food; however, some reports had stated gastrointestinal and respiratory allergenic reactions.

Transgenic Plant

MIR604 corn has been reviewed and approved for food and/or feed use in many countries including Argentina (Food and Feed, 2012), Australia (Food, 2006), Brazil (Food and Feed, 2014), Canada (Food and Feed, 2007), China (Food and Feed, 2008), Colombia (Food and Feed, 2012), European Union (Food and Feed, 2009), Indonesia (Food, 2011), Japan (Food and Feed, 2007), Malaysia (Food and Feed, 2016), Mexico (Food, 2007), New Zealand (Food, 2006), Philippines (Food and Feed, 2007), Russian Federation (Food and Feed, 2007), South Africa (Food and Feed, 2011), South Korea (Food and Feed, 2007), Taiwan (Food, 2007), Thailand (Food, 2013), United States of America (Food and Feed, 2007), and Vietnam (Food and Feed, 2016) (ISAAA, 2017). Based on the documents provided by the developer, the introduction of MIR604 is not likely to change the consumption pattern by population subgroups (Syngenta, n.d.).

Donor Organisms

History of safe use has been attributed to both donor organisms, *Bacillus thuringiensis subsp. tenebrionsis*, the donor organism for *mcry3A* gene, and *Escherichia coli*, the donor organism for *pmi,* as both are not known to be toxic and allergenic and are being used as donor organisms for several other approved GM crops (Syngenta, n.d.).

Transformation System

The transformation method is through *Agrobacterium tumefaciens* – mediated transformation with plasmid vector pZM6 (Syngenta, n.d.). The target of genetic modification is the nuclear DNA. The plasmid vector contains the gene expression cassettes for *mcry3A* and *pmi* which were included between the left and right border of the plasmid. The *mcry3A* cassette consisted of *mcry3A* coding region regulated by MTL promoter and NOS terminator. The pmi cassette is regulated by ZMUbiInt promoter and NOS terminator.

The vector backbone is composed of Spec- *Streptomycin adenylyltransferase, aadA* gene from *E. coli* that confers the resistance to erythromycin, streptomycin and spectinomycin; used as a bacterial selectable marker. VS1ori, consensus sequence for the origin of replication and partitioning region from plasmid pVS1 of Pseudomonas. It also serves as origin of replication in *Agrobacterium tumefaciens* host. *ColE1ori,* permits the replication *E. coli. virG*, is part of the two-component regulatory system for the vir regulation in *Agrobacterium*. repA- pVS1 replication protein from *Pseudomonas,* also a part of pVS1 replicon that is functional in gram-negative plant (De Framond, 2014).

Inserted DNA

Southern blot analysis and T-DNA insert sequencing demonstrated that MIR604 corn contains one intact copy of the T-DNA insert at a single locus (Syngenta, n.d.). The integrity and order of genetic elements within each insertion site was demonstrated through Southern Blot Analyses.

Genomic DNA for Southern Blot Analysis show that MIR604 contains single copy of the MTL and ZmUbInt promoters and the mcry3A, pmi, MTL and ZmUbInt probes the KpnI digest resulting to a single hybridization band demonstrating that single copy of each element is present in the event (Syngenta, n.d.). The overall integrity of the insert was demonstrated by the consensus sequence data for the event MIR604 T-DNA insert and that contiguousness of functional elements within the insert as intended in pZm26 was maintained.

Based on the documents provided by the developer, truncations occur at the right border and left border ends of the insert during transformation process (De Framond, 2014). The right border was truncated by 44 base pairs while the left border was truncated by 43 base pairs. This event was previously observed in agrobacterium transformation and theses has no effects on the efficacy of the insert. Four other base pair changes was observed in the event, two occurred within the MTL promoter and the other two occurred within the *pmi* coding sequence. ORF analysis indicated that six potential reading frames showed presence of one putative novel ORF (De Framond, 2014). The ORF is 258 base pair, starting at the NOS terminator up to the T-DNA to genome junction into the 3' flanking sequence. The ~240 bp of this putative ORF is terminator sequence and no promoter elements found. The transcription of this putative ORF is unlikely.

No plasmid vector backbone sequence is present in MIR604 corn as demonstrated by Southern blot analysis of MIR604 genomic DNA digested with KpnI restriction enzyme on a backbone specific probe. The lack of hybridization showed the absence of any pZM26 vector backbone sequence being incorporated in the event during transformation process (De Framond, 2014).

Genetic Stability

The multigenerational stability of the introduced trait was demonstrated through Southern Blot Analyses of three generations (BC4, BC5 and BC6) of MIR604 Corn (Syngenta, n.d.; De Framond, 2014). Results showed that the hybridization bands specific to the MIR604 insert were identical in lanes containing DNA from corn grown from 3 generations indicating that mcry3A and pmi genes are stably inherited from one generation to the next.

Five generations (T0, T1, T2, T3, T4 and T5) were analyzed for the presence of mcry3A and pmi genes using PCR to confirm the Mendelian Inheritance ratio of the genes through its segregation ratio (Syngenta, n.d.; De Framond, 2014). Zygosity of the plants were confirmed by progeny test and PCR. Chi-square analysis of the segregation data was performed to test the hypothesis that MIR604 insert is inherited according to Mendelian Pronciple with the expected ratio of 3:1. The critical value to reject the hypothesis at 5% level is 3.84 Chi-squared value of less than 3.84 was obtained and it showed that mcry3A and pmi were inherited following Mendelian principle.

Expressed Material

The Cry proteins such as mCry3A have no metabolic role in plants. They act as proteins that bind to the specific receptors on the brush border membrane of the midgut epithelium of target insects and insert to the membrane which leads to the pore formation and disruption of the transmembrane gradients and cell integrity (English and Slatin, 1992). PMI is involved in the catalysis of isomerization of mannose-6-phosphate to fructose-6-phosphate (Syngenta, n.d.).

The protein levels were quantified using the enzyme-linked immunosorbent assay (ELISA). The concentration of mCry3A in MIR604 are as follows (Joseph and Hill, 2006). The concentration of mCry3A and PMI in MIR604 whole plant, leaves, roots, pollen and kernels ranges from below limit of detection (LOD) to 23 ug/g fresh weight, and from below limit of quantitation (LOQ) to 2.6 ug/g fresh weight, respectively.

Toxicological and Allergenicity Assessment

The novel proteins, mCry3A and PMI, were subjected to digestibility, heat inactivation, oral toxicity and amino acid sequence comparison studies to determine its potential to cause toxicity or allergenicity to humans (Syngenta, n.d.). The biochemical and functional equivalence between mCry3A and PMI proteins produced by MIR604 and test substances MIR604-0102 and MIR604-PMI-0105 were determined through comparing the protein samples in terms of immunoreactivity,

molecular weight and enzymatic activity. Results showed that the microbially-produced and plant-produced proteins were biologically and functionally equivalent (Song, 2009).

Digestibility study using Simulated Gastric Fluid (SGF) with pepsin demonstrated that mCry3A and PMI proteins are readily degraded within 2 minutes of incubation with SGF, in presence of pepsin at pH 1.2, a characteristic of most non-toxic proteins (Syngenta, n.d.; Joseph and Gerson, 2005; Nelson, 2009).

The thermal stability of the mCry3A and PMI protein was evaluated by heating protein solutions for 30 min at 4, 25, 37, 65, and 95 °C (Syngenta, n.d.; Joseph, 2003; Li et al., 2006). PMI enzymatic activity was determined through ELISA. The immunoreactivity of PMI was determined through sandwich ELISA. Results showed that mCry3A was completely inactivated upon incubation at 95°C for 30 minutes. Complete loss of immunoreactivity and enzymatic activity of PMI was observed upon incubation at 95 °C for 30 minutes.

Amino acid sequence comparison of mCry3A and PMI proteins to toxins and allergens using FASTA and BLASTp showed no structurally relevant similarity exists between the novel proteins and any known toxic, allergens or other biologically active proteins that would be harmful to human or animal health (Bailey, 2016; AIS-FRA-17-13 BIA).

Acute oral toxicity study of mCry3A and PMI proteins showed no clinical observations related to toxicity, no treatment-related effects on body weight, food consumption and no macroscopic or microscopic findings was observed in treated mice (Johnson, 2003; Barnes, 2005). The NOEL for mCry3A and PMI were considered to be >2377 and >2072 mg/kg body weight.

Based on the documents provided by the proponent, mCry3A and PMI proteins have distinct modes of action and are not likely to interact (Syngenta, n.d.). mCry3A confers resistance against larval corn rootworm damage while PMI was introduced as a selectable marker which catalyzes the isomerization of mannose-6-phosphate to fructose-6-phosphate. mCry3A have no metabolic role in plants. They act as proteins that bind to the specific receptors on the brush border membrane of the midgut epithelium of target insects and insert to the membrane which leads to the pore formation and disruption of the transmembrane gradients and cell integrity (English and Slatin, 1992). PMI is involved in the catalysis of isomerization of mannose-6-phosphate to fructose-6-phosphate (Syngenta, n.d.).

The mCry3A and PMI proteins constitute 0.000159% and 0.00028% of the total weight in kernels of MIR604 (Syngenta, n.d.).

The developer provided documents for serum screening of PMI (Bailey, 2016). In the 8 amino acid seach, a sigle match between PMI and a-parvalbumin frpm RANA sp. was observed. This has been previously reported, however, cross reactivity study between PMI and a-parvalbumin protein using serum from single individual known to have demonstrated IgE-mediated allergy to this specific a-parvalbumin show that IgE does not recognize any portion of PMI as an allergic epitope. The results supports that PMI shares no biologically relevant amino acid sequence to a known toxins

Results of the digestibility, heat inactivation, amino acid sequence comparison and acute oral toxicity studies indicates that mCry3A and PMI proteins being expressed in MIR604 corn are not toxic or allergenic to humans (Syngenta, n.d.).

Nutritional Data

Compositional analysis was provided by the developer indicating the nutritional data of MIR604 corn in comparison with the conventional corn (Syngenta, n.d.). The compositional analyses were done in two growing season, in 2002 where two transgenic and 2 non transgenic samples were grown in 3 locations; in 2003, one control and transgenic samples were planted in 7 locations while another 1 transgenic and non-transgenic samples were grown in 9 locations. Results of the analysis indicated that there are no differences in the proximate, fiber, mineral, amino acid, fatty acid, vitamins and anti-nutrient of MIR604 and the conventional corn that can be considered biologically relevant.

Conclusion

For the transgenic MIR604 corn, enough evidence is provided to support the equivalence of the genetically modified crop, in terms of the nutritional composition and food safety, with the conventional soybean other than resistance to target insect pests. After reviewing the provided material of Syngenta Philippines, Inc., it is therefore concluded that MIR604 corn is as safe as its conventional counterpart.

BAI ASSESSMENT AND RECOMMENDATIONS

Based on the documents submitted by the applicant, BAI made the following assessment:

A. Host Organism

Corn kernel is composed of starch, protein and oil. It contains vitamins, minerals, amino acids, fatty acids and organic acids. (OGTR, 2008). Meanwhile, Anti-nutrients in corn are ferulic acid, p-coumaric acid, furfural, inositol, phytic acid, raffinose and trypsin inhibitor. However, there are no generally recognized levels that are considered harmful.

BAI also added that Maize per se does not have toxicants, (OGTR, 2008) and is not considered highly allergenic but some allergic reactions have been reported in scientific literatures such as food and inhalation allergies. (OGTR, 2008)

Further, People consume corn kernels in processed/cooked forms, while it is fed to animals as fresh or dry forage, silage and grains.In the Philippines, consumption pattern is 16.736 g/kg bw/day for the general population and 32.518 g/kg bw/day for children.

B. Transgenic Plant

BAI concurred that MIR 604 has been approved for food in the US, Argentina, Australia, New Zealand, Canada, China, Colombia, EU, Indonesia, Japan, Korea, Mexico, Philippines, Russian Federation, Belarua, Khazakhstan, Aouth Africa, Taiwan and Vietnam; and has also been approved for feed use in the US, Argentina, Brazil, Canada, China, Colombia, EU, Japan, Korea, Malaysia, Mexico, Philippines, Russian Federation, South Africa, Taiwan, Turkey and Vietnam.

In addition, Consumption patterns are not expected to change with introduction of MIR604 corn in the market.

C. Donor Organism

BAI concurred that all protein-encoding sequences were described. Sources and sequences do not possess pathogenic or allergenic properties, and that all inserted regulatory sequences were also adequately described.

They also concurred that the following organisms as sources of the inserted genes and regulatory sequences are not known to be toxic or allergenic: (a) Zea mays; (b) Bacillus thuringensis subsp

tenebrionis; (c) Agrobacterium tumefaciens; (d)E. coli; (e) Pseudomonas; and that these encoded proteins are not known to be toxic or allergenic: (a) Modified Cry3A protein; (b) Phosphomannose isomerase enzyme

D. Transformation System

BAI has agreed that the information provided by the applicant is sufficient. They agree that the transformation method used is Agrobacterium-mediated transformation method and that the target of genetic modification is the plant genome. They also agree that complete experimental protocol and all genetic components were listed and adequately described.

In addition, they agree that the plasmid vector pZM26 is 13,811 bp in size and that no carrier DNA and/or helper plasmids were not used.

E. Inserted DNA

BAI concurred that the information on southern hybridization and sequencing of the T-DNA insert demonstrated the presence of one copy of the transgenes at a single integration site and that the overall integrity and contiguousness of the functional elements within the insert is as intended as confirmed by consensus sequence data.

They also agree that during the transformation process, sequence data revealed that the RB portion of the T-DNA wastruncated by 44bp and the LB end was truncated by 43 bp but these deletions have no effect on the efficacy of the T-DNA insert. Four base pair changes were also noted – two within the MTL promoter and two within the pmi coding sequence – but these substitutions have not resulted in any apparent changes in gene expression, and that ORF analysis of all six potential reading frames at both the 5' and 3' T-DNA to genome junctions showed the presence of one putative novel ORF. However, transcription of this putative ORF is unlikely.

Furthermore, they concur that mcry3A is expressed only in Zea mays, MIR604 and all its stacks events and that southern analysis demonstrated the absence of vector backbone sequences in Event MIR604 hybrids.

F. Genetic Stability

BAI is in agreement with the information that southern analysis using a mcry3A showed identical hybridization patterns for all three generations tested indicating multi-generational stability of the T-DNA insert.

In addition, they also agree that six generations of backcrosses were tested using immuno-detection strip, ELISA and TaqMan PCR. Segregation pattern follow Mendelian inheritance ratio of 3 positive: 1negative in the populations studied. These results confirm a single insert number.

G. Expressed Material

BAI has concurred that across all growth stages, mean mCry3A levels in leaves and roots ranged from 3-23 ug/g and 2-24 ug/g fresh weight, respectively while in kernels, levels detected were 0.6-1.4 ug/g fresh weight. NomCry3A protein was detectable in pollen. PMI levels, however, were low in leaves, roots and kernels ranging from below 0.05 ug/g to 0.4 ug/g fresh weight but pollen yielded 1.9-2.6ug/g. These were measured using ELISA.

They also stated that in whole plants, mCry3A is 0.9-11 ug/g fresh weight concentration while PMI level is less 0.05 ug/g fresh weight as determined by ELISA.

Additionally, BAI agree that in transformed plants, PMI catalyzes the reversible inter-conversion of mannose-6-phospate and fructose-6-phosphate. During transformation, PMI is used as selectable marker and mannose as the selective agent.mCry3A does not have a metabolic role.

They also agree that both PMI and mCry3A are not expected to interact within, nor affect together the metabolism of the corn plant.

H. Toxicological Assessment

BAI has concurred that the information provided by the applicant on the digestibility of both MCRY3A and PMI are adequate.
MCRY3A digestion in Pepsin: T50=2 minutes, SDS-PAGE and Western blot analysis did not show any fragment remaining after digestion at 2 min. Meanwhile, PMI digestion in Pepsin: T50 is less than 1 minute. SDS-PAGE and Western Blot did not show any fragment remaining at 1 minute of digestion.

BAI also concurred that bioassay against corn rootworm showed that there was only slight reduction in bioactivity of the mCry3A protein but was completely inactivated after incubation at 95C for 30 minutes. On the other hand, there was a complete loss of PMI enzymatic activity at 95C after 30 minutes and complete loss of reactivity at 65C. Enzymatic activity was measured in a coupled enzyme activity assay and the immunoreactivity was determined using a Sandwich ELISA.

Furthermore, a comprehensive similarity search in the NCBI Entrez Protein Database and the Syngenta Toxin database yielded no biologically relevant amino acid sequence similarity of MIR604 mCry3A and MIR604 PMI to any known or putative toxins.

BAI also concur that acute oral gavage for both MCRY3A and PMI was done using 10 mice. No treatment-related adverse effects were observed at the dose of 2,377 mg mCry3A protein/kg body weight and at the dose of 2,072 mg PMI/kg body weight.

In addition, equivalency of mCry3A from MIR604, PMI from MIR604 and the E-coli derived protein was demonstrated using SDS-PAGE, western blot, use of glycosylation detection kit and bioassay.
PMI protein from E. coli showed the same expected results as PMI from MIR604 using SDS-PAGE, Western blot, ELISA and continuous coupled spectrophotometric assay.

I. Allergenicity Assessment

BAI has concurred that the information provided by the applicant on the digestibility of both MCRY3A and PMI are adequate.
MCRY3A digestion in Pepsin: T50=2 minutes, SDS-PAGE and Western blot analysis did not show any fragment remaining after digestion at 2 min. Meanwhile, PMI digestion in Pepsin: T50 is less than 1 minute. SDS-PAGE and Western Blot did not show any fragment remaining at 1 minute of digestion.

BAI also concurred that bioassay against corn rootworm showed that there was only slight reduction in bioactivity of the mCry3A protein but was completely inactivated after incubation at 95C for 30 minutes. On the other hand, there was a complete loss of PMI enzymatic activity at 95C after 30 minutes and complete loss of reactivity at 65C. Enzymatic activity was measured in a coupled enzyme activity assay and the immunoreactivity was determined using a Sandwich ELISA.

Furthermore, a comprehensive similarity search in the NCBI Entrez Protein Database and the Syngenta Toxin database yielded no biologically relevant amino acid sequence similarity of MIR604 mCry3A and MIR604 PMI to any known or putative toxins.

Lastly, BAI has concurred that the prevalence in food of both mCry3A protein and PMI protein comprises approximately 0.00159% and 0.00028% of the total protein in the kernels respectively.

J. Nutritional Data

BAI has agreed with the information provided that for both grain and forage, Between MIR604 and non-transgenic control, there were no significant differences in moisture, protein, total fat, ash, carbohydrates, starch and fiber content (% dw); and that there were no significant differences in moisture, protein, total fat, ash, carbohydrates, NDF and ADF (% dw), respectively. In addition, all data from MIR604 are within the ranges reported in literatures for grain and forage.

Moreover, for grain, there was no significant difference between mineral, vitamin, amino acid and fatty acid content of MIR604 and non-transgenic control; while for forage, there was no significant difference between mineral content of MIR604 and non-transgenic control.All data derived from MIR604 are within the reported ranges in literatures for grain and forage and that there were no statistical differences.

Meanwhile, ferulic acid and p-coumaric acid levels are lower in the transgenic corn than in the non-transgenic control. There are no significant differences in the levels of the other anti-nutrients. Data from MIR604 fall within the ranges in literatures and that the statistical differences in the levels of ferulic acid and p-coumaric acid are not biologically relevant.

K. Recommendation

Find scientific evidence that the regulated article applied for human food and animal feed / for processing use is as safe as its conventional counterpart and shall not pose any significant risk to human and animal health

DENR ASSESSMENT AND RECOMMENDATION

After thorough and scientific review and evaluation of the documents provided by the Bureau of Plant Industry (BPI) on the application of Syngenta Philippines Inc. for direct use for feed, food or processing of Corn MIR604, here under are the observations and appropriate actions:

1. From the evaluation of the application submitted by the proponent, including the scientific evidences from the provided references and literature, as well as other related studies, the Committee finds that the direct use of the regulated article whether for food, feed and/or for processing will not cause any adverse effect on the environment (land, air, water) and non-target organisms, to wit:
    a. Genetic stability in the transgenic crop is ensured such that no unintended horizontal gene transfer shall occur to unrelated species.
    b. The protein product produced by the transgenic crop will degrade upon exposure to the natural environment and general conditions (i.e high temperatures (60 C and above), varying pH, enzyme digestion, etc); and
    c. The protein product will not increase the weediness potential of the transgenic crop
2. The data evaluated support the conclusion that the regulated article is as safe as conventional counterpart.
3. The project description report (PDR) discussed 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 of the proponent. Upon evaluation of the submitted PDR and environmental risk assessment (ERA), the Committee notes that 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 germination;
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 as per BPI's inspection in the port area.

The DENR-BC finds scientific evidence that the regulated article applied for Direct Use as Food and feed or for processing is as safe as its conventional counterpart and is not expected to pose any significant risk to the environment and to non-target organisms.

Based on the above considerations and with the submitted sworn statement and accountability of the proponent, we hereby submit our evaluation relative to Syngenta Philippines Inc MIR604 application for biosafety permit for food, feed, and/or processing**.**

DOH ASSESSMENT AND RECOMMENDATION

After a thorough review and evaluation of the documents provided by the proponent, Syngenta Philippines Inc., through the Bureau of Plant Industry (BPI), in support of their application for approval for Direct Use for Food and Feed or for Processing (FFP) of Corn MIR 604. I/We,

Find 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:

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 allergic reaction
3. The regulated article is as safe as food or feed derived from conventional corn varieties
4. The regulated article is not materially different in nutritional composition from that of the non-transgenic corn or the conventional corn.
5. It is suggested that the BPI ensure the following:
    a. Clear instructions that the product is only for the purpose of direct use as food, feed or for processing, 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, this recommendation is being submitted to the BPI related to the processing and issuance of a biosafety permit for Direct Use as FFP of Corn MIR604.

SEC ASSESSMENT AND RECOMMENDATIONS

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

A. Socio-economic issues
As per the SEC Expert, the data cited by Syngenta sourced from Philippine Statistics Authority (PSA 2017) attest to the significance of corn and corn products in terms of production, consumption and trade.

The SEC expert also stated that, from another source, it is shown that the Philippines' corn production and consumption in the last five years fluctuated in terms of growth rate but a positive growth rate in general is observed. However, comparing the production and consumption data, Table 1 shows that in the last five years (2013-2017), the production has been less than the consumption. This observation implies the need to increase corn production or import corn and corn products to meet the demand for consumption through importation.

Table 1. Philippines production, import, and export volume for corn and corn products from 2012 to 2016.

Commodity	**Unit**	**Year**				
		2012	**2013**	**2014**	**2015**	**2016**

Corn Production	Metric Ton	7,406,830	7,377,293	7,770,603	7,518,756	7,218,817
Corn Import	Metric Ton	179,219	359,640	589,650	458,968	817,577
Import Percent Total*	%	2	5	7	6	10
Corn Export	Metric Ton	862	751	906	667	475
Corn Starch Import	Metric Ton	30,709	12,365	8,507	20,760	29,300
Groats and Corn Meal Import	Metric Ton	2,186	2,307	2,829	3,476	4,234
Corn Oil Import	Metric Ton	2,096	1,333	1,410	1,965	1,576
Corn Flour Import	Metric Ton	382	473	195	341	835
Groats and Corn Meal Export	Metric Ton	822	385	52	114	95
Corn Starch Export	Metric Ton	2	2	0	0	0
Bran Sharps and Other Corn Milling Residues Export	Metric Ton	0	19	0	0	0

*Import Percent Total = (Corn Import/(Corn Production + Corn Import)) x 100

For the past four years, the following are the data on the area (ha) devoted to the production of GM corn:

2013-795,000
2014-831,000
2015-702,000
2016-812,000

The data presented above show the importance of GM corn in the Philippines.

Further, the SEC expert cited from the businessdiary.co.ph which reported on August 30, 2016 that the Philippines used to import one million metric tons of corn annually. It was added that the successful distribution of Bacillus thuringiensis (Bt) corn has beefed up the country's corn production leading to a potential export of 50,000 to 100,000 metric tons of grains possible to South Korea and Malaysia.

Table 2 shows the Philippines' importation of corn 2013-2017. Just like the production and consumption of corn, its importation in the last five years fluctuated.

Table 2. Philippines' importation of corn (in 1,000MT) in 2013-2017

Year	Corn Import (1,000MT)	% Growth Rate
2013	741	705.43
2014	623	-15.92
2015	742	19.10
2016	606	-18.33
2017	400	-33.99

(Source: https://www.indexmundi.com/agriculture/?country=ph&commodity=corn&graph=imports)

B. Recommendation
The SEC expert has recommended for the approval and issuance of the biosafety permit of the GM product.