ASSESSOR'S CONSOLIDATED REPORT ON PHILRICE'S GR2E RICE APPLICATION FOR COMMERCIAL PROPAGATION

EXECUTIVE SUMMARY

On October 29, 2020, PHILRICE submitted rice GR2E for commercial propagation, 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 Scientific and Technical Review Panel (STRP), Bureau of Animal Industry and BPI-Plant Product Safety Services Division, concurred that rice GR2E is as safe for human food, animal feed and commercial propagation 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 since it poses no significant adverse effect to the environment.

The Department of Health – Biosafety Committee (DOH-BC), after a thorough scientific review and evaluation of documents related to Environmental Health Impact, concluded that rice GR2E 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 rice GR2E.

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 Section 15 of the JDC No.1, S2016, no regulated article shall be released for commercial propagation unless: (1) a Biosafety Permit for Commercial Propagation has been secured in accordance with this Circular; (2) it can be shown that based on field trial conducted in the Philippines, the regulated article does not pose greater risks to biodiversity, human and animal health than its conventional counterpart; (3) food and feed safety studies show that the regulated article does not pose greater risks to biodiversity, human and animal health than its conventional counterpart, consistent with CODEX Alimentarius Guidelines on the Food Safety Assessment of Foods Derived from the Recombinant-DNA Plants and protocols of the DOH and BAI on feeding trials; and (4) if the regulated article is a pest-protected plant, its transformation event that serves as plant-incorporated protectant (PIP) has been duly registered with the Fertilizer and Pesticide Authority (FPA).

The BPI Biotech Office provided the assessors the complete dossier submitted by PHILRICE. The SEC expert, on the other hand, was provided with special questionnaire on socio-economic, ethical and cultural considerations that have been addressed by PHILRICE in relation to their application.

INFORMATION ON THE APPLIED EVENT

Rice event GR2E (IR-ØØGR2E-5) was developed using recombinant-DNA techniques to express elevated levels of provitamin A (mainly beta carotene) in the rice endosperm, which is converted in the body to vitamin A. GR2E rice was produced by *Agrobacterium tumefaciens*-mediated transformation of embryogenic rice calli with plasmid pSYN12424 resulting in the introduction of the phytoene synthase (*psy1*) gene from *Zea mays* (Zmpsy1), the carotene desaturase I (*crtI*) gene from *Pantoea ananatis*, and the phosphomannose isomerase (*pmi*) gene from *Escherichia coli* as a selectable marker.

GR2E rice is intended to complement existing efforts to mitigate vitamin A deficiency by supplying consumers in societies whose diet is primarily rice-based with a portion of the estimated average requirement for vitamin A.

Countries Where Approvals Have Been Granted

Country	Food	Feed	Cultivation
Australia	2017		
Canada	2018		
New Zealand	2017		
Philippines	2019	2019	
United States	2018	2018	

Source: https://www.isaaa.org/gmapprovaldatabase/event/default.asp?EventID=528

STRP's Assessment

1. **Host Organism**
   a. Rice is a major source of carbohydrates, supplying 55%-75% of dietary energy in the Filipino diet. The carbohydrates are mainly in the form of starch, amylose, and amylopectin. It also contains non-starch polysaccharides, making rice a good source of dietary fiber with its numerous benefits in gut function apart from its beneficial metabolic effect. While rice contains small amounts of protein in the form of glutelin, nevertheless, rice is a significant source of protein in the Filipino diet owing to the large amount of rice consumed. Besides, the protein quality in rice is considered good because of the good proportion of the essential amino acids it contains, unlike the protein in wheat and corn. Rice also contains small amount of fat especially in the bran, mainly in the form of triglycerides, which may be processed into rice bran oil. Rice also contains water soluble vitamins especially thiamine, riboflavin and niacin, a large part of which is lost in milling [1].
   b. Rice is universally considered as safe, nevertheless, there are anti-nutritional factors, most of which are concentrated in the bran, such as phytic acid, trypsin inhibitors and hemagglutinin-lectins, oryza cystatin, and *alpha-amylase* subtilisin inhibitor. Apart from phytic acid, the other antinutritional factors are proteinaceous and are denatured in cooking [1].
   c. Arsenic, cadmium, mercury, and lead are four ubiquitous trace elements known to have a harmful effect on human health. These elements are naturally present at very low concentrations in the environment, and human bodies can detoxify them in limited amounts. Rice plants can take up these toxic elements from polluted soil or irrigation water [1].
   d. Rice is not considered a common cause of allergies by allergologists and pediatricians, even among infants and young children. While rice is not considered to be a common allergenic food, rare allergic reactions have been documented [1].
   e. Rice is the major staple especially in Asia including the Philippines. Most of the rice used for food in the Philippines and other Southeast Asian countries is boiled milled rice, although brown rice is being promoted for its better nutritional value. Glutinous rice is often prepared in many countries in Asia including the Philippines into many kinds of desserts. Rice flour is a pulverized product of milled rice, and it also finds itself in many desserts. Rice bran oil is made from rice bran, which may be used as cooking oil [2].
   f. The average household consumption of rice and rice products was 1263 g, which is 37.2% of the total food consumption. It is slightly higher in the rural areas (1335 g/day) than in the urban areas (1182 g/day), highest in the Cordilleras and in the poorest segment of the population (1288 g/day) (compared to 1165 g/d among the richest). Rice was reportedly consumed by 94.6% of households from infancy to old age [3].
   g. Rice grain (paddy) is seldom used for animal feeding because of its high cost. It is the by-products from mills such as rice bran, rice polishings and sweepings, that are used as feed. Rice straw is the most abundant feed resource in small farms [1][4].

2. **Prior Safety Approval outside of the Philippines:** GR2E rice has been approved in Australia, New Zealand, and Canada for use in food. It is approved in the US for food and feed [25]. It is not likely that the consumption patterns of the rice will change because of the introduction of the GR2E rice.

3. **Donor Organism**
   a. The polyubiquitin promoter and the gene known as *Zmpsy1* (source of *ZMPSY* protein) were isolated from the genome of corn (*Zea mays* L.*).* Corn was first domesticated in Mexico 8000 years ago. Corn or maize has a long history of safe use as food and feed.
   b. The source of the *crt1* gene (source of *CRT1* protein) is *Pantoea ananatis* (formerly *Erwinia uredovora*) belonging to the family *Enterobacteriaceae*. This organism is present in various natural environments like water and soil. Even strains of *P. ananatis* are known to be pathogenic on a wide range of plant hosts including humans, it was found based on genome analysis that potential molecular determinants of the pathogen's underlying pathogenicity showed the absence of many factors that are very relevant to the pathogenicity and virulence arsenal of related plant and animal pathogens plus animal toxins, hemolysins, phytotoxins and possible effectors.
   c. The gene *pmi* (source of PMI protein) was taken from the bacterium *Escherichia coli* belonging to the family *Enterobacteriaceae* strain K12 which is a non-pathogenic strain. This strain was already engineered to make it safe for laboratory use.

4. **Transformation System**
   The *Agrobacterium*-mediated transformation of the japonica rice cultivar Kaybonnet using plasmid pSYN12424 [26].

5. **Food and Feed Safety**
   a. Following exposure to SGF containing pepsin for 30 seconds, the earliest time point sampled during digestion, no intact ZmPSY1 protein (ca. 42 kDa) was evident as assessed by either SDS-PAGE or western immunoblot analysis. Enzyme activity was also irreversibly destroyed upon heat treatment, with 50% loss of activity following pre-incubation at ca. 42^0C for 15 minutes and complete loss of activity following pre-incubation at 50^0C for 15 minutes. Lastly, the ZmPSY1 query sequence did not return any entries with E-values less than 1×10^{-5}. Based on the results of this study, there are no sequence homology structural alerts for potential toxicity of the ZmPSY1 protein. The ZmPSY1 query sequence was also evaluated for any eight contiguous identical amino acid matches to the allergens contained in the FARRP database, and no matches were found [5][6][7].
   b. CRTI protein was rapidly and completely digested when incubated in SGF containing pepsin. Following 30 seconds of incubation, the earliest time point sampled during digestion, no intact CRTI protein was evident as assessed by either SDS-PAGE or western immunoblot analysis. Enzyme activity was irreversibly destroyed upon heat treatment, with 50% loss of

activity following pre-incubation at ca. 51^0C for 15 minutes and complete loss of activity following pre-incubation at 55^0C for 15 minutes. A search using CRTI query sequence returned three protein accessions from the toxin database with an E-score less than 1×10^{-5}. The three sequence alignments were to the N-terminal regions of L-amino acid oxidase (*LAAO*) enzymes from three species of venomous snakes. The limited sequence similarity between the *P. ananatis CRTI* protein and the three L-amino acid oxidase accessions retrieved from the toxin database was due to homology between the N-terminal motifs involved in FAD binding and was not considered to be a structural alert for potential toxicity. The N-terminal sequence similarity was also observed between the native rice phytoene desaturase and a range of L-amino acid oxidases. No identity matches of >35% over 80 residues were also observed and there were also no matches of eight contiguous identical amino acids in the FAARP Allergen database. Oral administration of CRTI protein to male and female mice at 100 mg/kg did not result in mortality or other evidence of acute oral toxicity, based on evaluation of body weight, clinical signs, and gross pathology [9][8][10].

c. Simulated mammalian gastric fluid containing pepsin was used for the digestibility study. The results showed that the PMI protein was readily degraded with no intact protein or degradation products detected after digestion for one minute. The T_{50} for heat stability could be estimated at less than 1 minute at 37^0C. The enzymatic activity was measured following pre-incubation for 30 minutes at temperatures ranging from 25–95°C. Two different searches were performed against the FARRP AllergenOnline database. No identity matches of >35% over 80-mer residues were observed. There was one 8-mer residue identified to match to a known allergen of an unidentified edible frog. The acute toxicity of test substance, PMI-0105 containing the active ingredient phosphomannose isomerase, was administered as a single oral gavage to male and female mice at 0 to 2000 mg active ingredient/kg BW. Under conditions of the study, all mice survived, and no clinical, physiologic, and anatomical findings were observed to indicate toxicity [11].
d. The major constituents, or proximates, of rice straw and grain are carbohydrates, protein, fat, and ash. Fiber is the predominant form of carbohydrate present in rice straw. Fiber is measured by NDF method, which measures the insoluble fiber [12]. There were no statistically significant differences in proximates and fiber between samples of straw obtained from GR2E and control PSB Rc82 rice. Except for moisture content, which is dependent on the extent of drying of straw following harvest, the mean values for all proximates and fiber in rice straw were similar to the ranges provided in literature [13].
e. Although there was a statistically significant difference in the mean concentration of crude fiber between samples of GR2E and PSB Rc82 rice grain, the difference was relatively small (10.5%) and unlikely to be biologically meaningful. In the combined-sites analysis, comparisons of proximates, fiber, and sugars in grain (paddy) samples derived from GR2E and control PSB Rc82 rice grown during the dry season, resulted in no statistically significant differences in any of the measured parameters

[11][12][13].

f. No statistically significant differences were noted for proximates, fiber, and minerals calcium and phosphorus between bran samples derived from GR2E and control PSB Rc82 rice grain. Except for crude fat and phosphorus, the measured ranges for these analytes were within the range reported in the literature [11][12][13].
g. There were no statistically significant differences in concentrations of calcium and phosphorous measured in samples of GR2E and PSB Rc82 rice straw [11][12][13].
h. A comparison of amino acid composition of event GR2E and control PSB Rc82 rice (grown during the rainy and dry season) grain showed no statistical differences in the concentrations of any amino acids between samples of Gr2E and PSB Rc82 [11][12][13].
i. Apart from β-carotene, which was intended to be elevated in GR2E rice, the only statistically significant difference between samples of GR2E and PSB Rc82 control rice was in the concentration of Vitamin B3 (niacin), which was elevated in GR2E rice grain samples by ca. 16% [11][12][13].
j. The concentrations of several fatty acids occurring in trace amounts (caprylic, capric, lauric, pentadecanoic, heptadecanoic, eicosadienoic, eicosatrieenoic, arachidonic, erucic and nervonic acids) in both GR2E and PSB Rc82 control rice grain samples were below the limit of quantification and are not reported. In the combined 4 locations over 2 seasons, 2015 and 2016, the only statistically significant difference between GR2E and control PSB Rc82 rice samples was in the concentration stearic acid, which was approximately 6.7% higher for GR2E rice [11][12][13].
k. There was no statistically significant difference in the concentrations of phytic acid or in the levels of trypsin inhibitor samples of GR2E and PSB Rc82 control rice [11][12][13].
l. The expression of the ZmPSY1 and CRTI enzymes in the endosperm tissue of GR2E rice results in the production of all-trans-lycopene from geranylgeranyl diphosphate. Lycopene is the substrate of two competing endogenous cyclases namely: ε-cyclase and β-cyclase, which acts simultaneously leading to the formation of α-carotene. On the other hand, the action of β-cyclase alone forms β-carotene. The principal carotenoid produced in GR2E rice endosperm is all-trans-β-carotene [12][13].
m. Beta-carotene is a precursor of vitamin A (retinol), which is an essential nutrient for humans. β-carotene is synthesized by the action of phytene synthase on geranylgeranyl diphosphate, followed a series of desaturations and isomerizations, leading to the synthesis of all-trans-lycopene and ultimately all-trans-β-carotene. Beta-carotene is the major carotenoid present in the human diet and is found in significant quantities in dark green leafy vegetables such as kangkong, malungay leaves, spinach, saluyot, and camote leaves, as well as yellow-colored vegetables and orange-colored fruit such as mango, orange, dalanghita, and carrots. Consumption of these vegetables is encouraged to prevent vitamin A deficiency [10][14][15].
n. The concentration of all-trans- β-carotene in milled rice from event GR2E and control rice grown at four locations in the Philippines over two growing seasons in 2015 and 2016 ranged from 1.96-7.31 ug/g DW, which is close to

the concentration of β-carotene in many of the common naturally occurring vegetables and fruits in the Filipino diet. Based on the mean rice intake of Filipinos found in the FNRI surveys, the potential β-carotene intake in mg/d from GR2E rice, (for example, mean of 0.82 mg in children and 2.39 mg/day at the 9th percentile) would be safe when compared with the concentrations of naturally occurring vegetables and fruits usually consumed in the Filipino diet. However, the potential β-carotene intake from GR2E rice would be a significant addition to the β-carotene intake in the usual Filipino diet [16][17][18][19].

o. There are no reports of harmful effects from the consumption of β-carotene and is considered non-toxic. β-carotene when consumed regularly in large amounts may cause the yellowing of the skin (carotenemia) which is harmless and disappears when the intake of the carotenoid is discontinued [20].
p. GR2E rice is intended to complement existing efforts to mitigate vitamin A deficiency by supplying consumers in societies whose diet is primarily rice-based with a portion of the estimated average requirement for vitamin A.

6. Environmental Safety

a. Rice (*Oryza sativa* L.) is a self-pollinated annual crop. The low degree of outcrossing in rice is due to wind pollination and not due to pollinators. However, the rice pollen is short-lived with most pollen grains losing viability after approximately 3-5 minutes under typical environmental conditions [21][22].
b. Species in the *Oryza* genus can be grouped according to the compatibility of their genomes. *Oryza sativa* has an AA-type genome, which means that its chromosomes can pair correctly at meiosis with other AA-type genome species. Despite this, hybrids between AA genome rice species can be difficult to obtain and have been reported to show low fertility [21][22].
c. The only sexually compatible cultivated species in the Philippines is other cultivated rice (*O. sativa*). The modified organism, GR2E rice, is capable of interbreeding with its conventional counterpart. However, rice is essentially self-pollinating, rice pollen is only viable for 3–5 minutes, and the chances of interbreeding are very small [22].
d. Agronomically, GR2E rice is unchanged from conventional rice. There are no expected changes in cultivation practices that could have adverse environmental impact. Agronomically, GR2E rice is unchanged from conventional rice. The local cultivation practices currently for conventional rice varieties, including the application of fertilizer, crop protection products, and labor, are directly applicable to the cultivation of those varieties containing the GR2E event.
e. Some differences in relative root and shoot lengths between GR2E and non-transformed control rice were noted but are unlikely to be biologically meaningful and were not indicative of any changes that would affect seedling establishment or vigor [23].
f. There were no observed unintended changes in either gross pollen morphology or in pollen viability between GR2E and conventional rice. Thus, the rate of out-crossing between Gr2E and sexually compatible plants

has not been changed because of altered pollen viability.[23].

g. There will be no adverse environmental consequences arising from the potential introgression of the provitamin A trait from GR2E rice into other rice or sexually compatible species as the trait does not confer a fitness advantage that would enable progeny to become more weedy, invasive of natural or managed ecosystems, or otherwise alter their impact on biodiversity [23].
h. The data collected for GR2E showed no significant increase in seed production or changes in seed characteristics that would increase seed dispersal [24].
i. No changes in seed dormancy or ratooning were noted between GR2E rice and control, non-transformed rice during the post-harvest monitoring of field trial sites. There were also no significant differences detected in percent germination, total seedling length, or in SVI between GR2E and control in germination tests conducted at 16°C or 26°C. The lack of difference between GR2E and control rice in germination rates at two different temperature regimes was another indicator that there were no changes in seed dormancy because of the genetic modification [23].
j. Since the GR2E rice does not have the potential to produce any pesticidal compounds unlike in *Bt*-corn that have *Cry* or *Vip* insecticidal proteins, there are no target organisms that can be affected. Thus, in addition, there is no scientific basis for expecting any non-target organism to be affected. The observations for damage due to opportunistic disease infection or pest infestations support the conclusion that there were no meaningful changes in susceptibility of transgenic GR2E rice compared to the non-transgenic comparator [19].

STRP's Conclusion

Find scientific evidence that the regulated article applied for propagation is as safe as its conventional counterpart and is not expected to pose any significant risk to human and animal health and the environment.

BAI's Assessment

1. **Toxicological Assessment**
   a. It reported that the SDS-PAGE and Western Immunoblot analysis for ZmPSY1 Protein revealed no intact fragments of the protein remaining after digestion at 30 seconds. Furthermore, samples of ZmPSY1 were subjected to heat treatment over a temperature incubation range of 30-65° C for 15 minutes after which the enzymatic activity was measured by monitoring the production of 15-cis-phytoene using the HPLC method. It was also confirmed that a FASTA36 bioinformatic alignment search was performed against a toxin database (UniProt Knowledgebase) and there were no sequence homology structural alerts for potential toxicity. For this protein, no animal toxicity studies were performed since sufficient data has been derived to rule out potential for toxicity.
   b. Pepsin was used in the digestibility study for CRTI protein. The estimated T50 is <30 seconds. There was no fragment remaining after digestion for 30 seconds as demonstrated by SDS-PAGE and Western immunoblot analyses. It was reported that the thermal stability was determined by measuring enzymatic activity using spectrophotometric assay to monitor conversion of 15-cis-phytoene to all-trans-lycopene. T50 is 51° C. Meanwhile, a search of the UniProt Knowledgebase returned sequence similarities with 3 L-amino acid oxidase (LAAO) accessions form the database due to homology between N-terminal motifs involved in FAD binding and was not considered to be a structural alert for potential toxicity. The three sequence alignments were from three species of venomous snakes.
   c. The BAI reported that pepsin was used in the digestibility study of PMI protein. T50 is less than 1 minute. SDS-PAGE and western blot analysis demonstrated that PMI was completely degraded. Heat stability of PMI was also evaluated. This was determined by measuring enzymatic activity. T50 is 65°C. The BLASTP search of the NCBI Entrez Protein Database identified 1384 protein sequences as having significant sequence similarity to PMI amino acid sequence but none were known or putative toxins.

2. **Allergenicity Assessment**
   a. There are no identity matches of >35% over 80 residues were observed using the FARRP16 allergen dataset for both ZmPSY and CRT1 proteins. There were also no eight contiguous amino acid matches observed.
   b. Meanwhile, using the FARRP AllergenOnline database, no identity matches of >35% over 80 residues were observed for the PMI protein. A sequence similarity search for any eight contiguous identical amino acid matches yielded a match to a α-parvalbumin from Rana species CH2001 (unidentified edible frog). Sequence identity is not biologically meaningful and has no implication for the potential allergenicity of PMI.

3. **Nutritional Data**
   a. For the proximate and fiber analysis of straw, no statistically significant differences in proximate and fiber between samples of straw obtained from

GR2E and control PSB rice. With the exception of moisture content, which is dependent on the extent of drying of straw following harvest. The mean values for all proximate and fiber in rice straw were similar to the ranges reported. Furthermore, for the proximate and fiber analysis of grain, there is also no statistically significant differences identified in grain samples derived from GR2E and control PSB Rc82 rice. The data derived from the transgenic line are within the reported range. For those values slightly outside the range, there were no statistically significant differences between GR2E rice and control PSB Rc82 rice.

b. No statistically significant differences were identified between samples of GR2E and control PSB Rc82 rice as a result of the proximate and mineral analysis of bran. In addition, values measured from the analytes were within respective ranges reported in the literature except for crude fat and phosphorus which were slightly higher in both GR2E and control PSB Rc82.

c. For the analysis of straw minerals, there were no statistically significant differences identified between sample GR2E and control PSB Rc82 as to concentration of calcium and phosphorus. On the other hand, there were also no statistically significant differences observed in the mineral composition between the samples of GR2E grain and the control.
d. A comparison of amino acid composition of event GR2E and control PSB Rc82 rice grain showed no significant differences. In addition, the data derived from the transgenic line are within the reported range except for tryptophan which was slightly lower and not statistically different from sample and the control.
e. Among all vitamins tested, no significant differences were observed in vitamins composition between the sample and the control except for Beta carotene which was intended to be elevated and no statistical difference from the sample and the control. In addition, the data derived from the transgenic line are within the reported range of literature except for pyridoxine (B6), folic Acid (B9) and α-tocopherol which were not statistically significantly different between sample and control.
f. For the analysis of fatty acids in grain, the BAI reported that no statistically significant differences were identified between sample GR2E and control PSB Rc82 rice as to concentration of fatty acids except in the concentration of stearic acid, which was approximately 6.5 % higher for GR2E rice. In addition, the data derived from the transgenic line of fatty acid are within the reported range in literature. Stearic acid comprises approximately two percent of the total fatty acids in rice grain and is not essential fatty acid. The small but statistically significant difference between stearic acid concentrations in samples of GR2E and control PSB Rc82 rice is unlikely to be biologically relevant.
g. No statistically significant differences were observed in phytic acid composition or in the levels of trypsin inhibitor between the sample and the control. In addition, the data derived from the transgenic line are within the reported range of literature and mean concentrations of phytic acid in grain samples from sample and control rice.

BAI's Conclusion

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

BPI-PPSSD's Assessment

1. **Toxicological and Allergenicity Assessment**
   a. Digestibility study using Simulated Gastric Fluid (SGF) with pepsin demonstrated that ZmPSY1 is readily degraded within 2 minutes of incubation with SGF, in presence of pepsin at pH 1.2, a characteristic of most non-toxic proteins (PhilRice and IRRI, 2017). The same were observed in CRTI and PMI proteins upon incubation for 30 and 60 seconds, respectively.

   b. Heat inactivation of ZmPSY1 was evaluated by monitoring the production of 15-cis--phytoene from *in situ* generated precursor geranylgeranyl diphosphate. Functional activity of CRTI was determined by monitoring the conversion of liposome-incorporated phytoene to all-trans-lycopene Results both analyses showed complete loss of activity for ZmPSY1 and CRTI was observed after incubation at 50°C for 15 minutes and 55°C for 15 minutes, respectively. The heat stability of PMI was determined through monitoring its immunoreactivity using Enzyme Linked Immunosorbent Assay (ELISA) [27]. Results of analysis provided by the developer showed that the enzymatic activity of PMI was below the limit of quantitation upon incubation at 65°C and above. This indicates that upon cooking or subjection to high temperatures, PMI is readily denatured. PMI is a transgenic protein in several maize events approved for food and feed under Department of Agriculture Administrative Order No. 8.
   c. Amino acid sequence comparisons of ZmPSY1, CRTI and PMI protein were provided by the proponent and were confirmed using the similar Bioinformatics tools, BLASTp and FASTA. ZmPSY1 protein was confirmed to have no significant homology to any known toxin or allergen.
   d. PMI protein was confirmed to have one eight-amino acid identity match to α-parvalbumin, a known allergen from unidentified edible frog, Rana species. Sensitive serum screening methodology indicated no cross-reactivity between PMI and the serum from the single individual known to have demonstrated IgE-mediated allergy to this specific α-parvalbumin. This indicates that the sequence identity between PMI and α-parvalbumin is not biologically relevant and has no implications for the potential allergenicity of PMI.
   e. CRTI protein were confirmed to have 32-37% identity to sequence alignments from three species of venomous snakes, *Bungarus multicinctus*, *B. fasciatus* and *Daboia russelii* in 81 amino acid overlap. However, limited sequence similarity between CRTI protein from *P. ananatis* and the three LAAOs was due to homology between N-terminal motifs involved in FAD binding and was not considered to be a structural concern for potential toxicity. N-terminal sequence similarity was also observed between the native rice phytoene desaturase and a range of L-amino acid oxidases. Also, literatures had stated that snake venom LAAOs are non-toxic via oral route. Weight of evidence approach indicating that oral ingestion of CRTI protein

will not pose hazard to human or animal health and that the enzyme activity of CRTI protein is irreversibly destroyed upon heat treatment at temperatures lower than cooking or processing.

f. Acute oral toxicity study of CRTI and PMI indicated no clinical signs of toxicity nor mortality, no gross lesions found in mice at necropsy, no treatment-related effects on body weight, food consumption or haematology parameters and no macroscopic or microscopic findings were observed. The No Observed Effect Level for CRTI and PMI proteins were 100 and 2000 mg/kg body weight, respectively. The developer did not provide an acute oral gavage study for ZmPSY1 protein. However, weight of evidence approach indicated that ZmPSY1 is not likely to act as a toxin.

g. In terms of the prevalence in food, ZmPSY1, CRTI and PMI constitute 0.00034%, 0.00004% and 0.0027% of the total protein of the grain.

h. Serum screening was performed in Rana species CH2001(unidentified edible frog). Results indicated that the patient's serum did not recognize any portion of the PMI protein as an allergenic epitope.

2. Nutritional Data

a. Compositional analysis was provided by the developer indicating the nutritional data of GR2E in comparison with the non-transgenic rice (PSBRc82), and range of literature values. The trials were conducted in four (4) locations in the Philippines during one dry and one wet season. Results of the analysis indicated that there are no differences in the proximate, fiber, mineral, amino acid, fatty acid, vitamins and anti-nutrient of GR2E rice and the non-transgenic rice that can be considered biologically relevant except for the fortification with β-carotene which is the induced trait in GR2E rice.

b. GR2E rice grain contains β-carotene and other carotenoids such as β-cyptoxanthin, all trans-α-carotene, and 9'-cis- β-carotene. The purpose of having the regulated production this new substance is to enhance nutritional quality of rice through introduction of β-carotene, a precursor of vitamin A to complement Vitamin A Deficiency (VAD) in the Philippines.

c. Carotenoids are one of the most widespread groups of pigments in nature. They are essential in human diet as antioxidants and protective agents against various diseases [28]. B-carotene is a precursor of vitamin A which is an essential nutrient in humans required for normal functioning of visual system, maintenance of cell function, epithelial integrity, production of red blood cells, immunity and reproduction. Other carotenoids found in GR2E are not converted into Vitamin A in significant amounts [29]. Animals convert beta-carotene to Vitamin A enzymatically through either β-carotene 15,15'-oxygenase 1 or β-carotene 9',10'-oxygenase 2. The other carotenoids can also serve as precursors to Vitamin A although the conversion is reportedly less efficient than that observed for β -carotene.

d. β-carotene is a safe source of vitamin A in human body commonly found in cellular tissues of fruits and vegetables such as carrots, spinach, mango, lettuce, spinach, sweet potato, onion, eggplant, and other food crops that have been part of human diet. Beta-carotene and the other carotenoids are also found in the grain. These substances are also naturally present in the

leaves of the rice plant. B-carotene and other carotenoid structures in GR2E rice are identical to those that are synthetically produced and also those naturally found in other food crops.

e. The β-carotene content of carrot is 78.6 μg/g; onion is 11.2 μg/g; mango is 7.8 μg/g; and eggplant is 0.8 μg/g. The amount of β-carotene in GR2E is 1.96-7.31 μg/g dry weight. Total carotenoid content ranges from 3.5-10.9 μg/g dry weight.
f. The Linus Pauling Institute provides the carotenoid content of various foods in terms of cups. Conversion tables assume that 1 cup is equivalent to 340 g. Based on data presented, the amount of beta-carotene, cryptoxanthin, and 9-cis-carotene that can be consumed by an average adult is much less than that obtainable from raw or cooked carrots.
g. The potential β-carotene intake of average Filipino adults is 2.04 mg/day. The average Filipino commonly consumes at least one medium-sized mango per day (approximately 100 g edible portion) which contains 1.17 mg β-carotene. This indicates that the daily intake of β-carotene from daily consumption of rice (278.6 g/ day) is equivalent to consuming two medium sized mangoes.
h. No adverse effects upon prolonged consumption of β-carotene in food have been reported. The proponent indicated in the dossier that standard toxicological tests have been performed on β-carotene, as recently reviewed by EFSA (2012). Results of the analyses showed no evidence of permanent harmful effects. Excess ingestion of carotenoids can lead to hypercarotemia in light-skinned individuals, but this condition is reversible once ingestion rates are reduced. Diplock (1995) also reported that supplementary intakes of 15-50 mg β-carotene /day has no side effects except for discoloration of the skin related to hypercarotenemia in some subjects at high intakes which are reversible upon reduction of b-carotene intake.
i. β-carotene is not known to be toxic or have adverse effects when ingested in excess of the average consumption. Diplock (1995) reported no evidence that the conversion of β-carotene to vitamin A contributes to vitamin A toxicity even when ingested in large amounts.
j. Heat, digestion, processing, interaction with atmospheric oxygen, light and storage may lead to degradation of β-carotene. Stability of β-carotene in GR2E rice during storage had been assessed. Results of analysis identified trace amounts of β-cyclocitral, ionene, 5,6-epoxy-β-ionone, β-ionone, dihydroactinidiolide and 4-oxo-β-ionone (FASEB Journal). Effect of storage temperature on the stability of β-carotene in rice varieties showed a decrease in the carotenoids levels in rice upon storage within two weeks at room temperature and at four weeks at 4^0C.

3. Other Concerns

a. History of safe use was attributed to the other carotenoids present in GR2E rice such as β-cryptoxanthin, α-carotene, all-trans-β-carotene and 9-cis-β-carotene.
b. Some β-carotene degradation products that may be found in GR2E rice may pose certain toxicological effect to human but with considerably high concentrations. β-carotene degradation products may be present in GR2E but

not at that level which may alter human health. It has to be considered that the daily intake of β-carotene in GR2E rice is equivalent to the daily intake of β-carotene in other food crops that has a long history of safe use as presented in the Risk Assessment Report.

c. There is a wide variation in conversion factors for vitamin A reported not only between different studies but also between individuals in a particular study. These findings show that the vitamin A value of individual plant foods rich in provitamin A carotenoids may vary significantly.
d. The increase in incidence of neoplasia in male smokers having a high risk of lung cancer cannot be directly attributed as a possible effect of supplementation of β-carotene through GR2E rice. The rapid non-enzymatic oxidative cleavage of β-carotene could be a result of heavy oxidative stress such as smoking, asbestos and photo irradiation in the skin and eyes. Such activities are known to have carcinogenic effects on human body. It was also indicated in the Risk Assessment Report that the structure β-carotene in GR2E rice is identical to those found in other food crops. This indicates that any effects of the β-carotene cleavage products in GR2E rice on the respiratory burst and human neutrophils can also be found in carrots, mango, cabbage, lettuce and other commonly consumed food crops that have a long history of safe use.
e. Intake of high amount of β-carotene is considered safe but can become toxic to liver when taken in combination with alcohol. However, it has to be considered that the daily intake of β-carotene in GR2E rice is equivalent to the daily intake of β-carotene in other food crops that has a long history of safe use as presented in the Risk Assessment Report. Hence, normal consumption of GR2E rice does not indicate high intake of β-carotene which could lead to toxicity upon combination with alcohol.

BPI- PPSSD's Conclusion

For the Provitamin A Biofortified Rice Event GR2E, enough evidence was considered to support the substantial equivalence of the genetically modified crop in terms of nutritional composition and food safety, with the conventional rice other than the biofortification with β-carotene. After reviewing the provided materials and taking into consideration the other concerns pertaining to the genetic modification of GR2E rice, the BPI-PPSSD found scientific evidence that GR2E rice is as safe as its conventional counterpart.

DENR- BC's Assessment

1. The regulated article (GR2E) showed no significant difference from the conventional rice counterpart in terms of biologically relevant components, aside from the introduced traits. Rice has a history of safe use while GR2E had previously been approved for direct use as food in Australia, Canada, New Zealand, Philippines, and the USA, and as feed in the Philippines and USA [37][38].
2. The inserted genes *crtI*, *zmpsy1*, and *pmi* exhibited stable integration into the genome of the host plant, and a stable inheritance was observed across its multiple generations. The protein products have also been assessed for safety and have no similarity with any known toxin [39][40].
3. The host plant is widely cultivated species, and a limited degree of crossing-pollinating with other cultivated rice may occur. The regulated article, however, is less likely to pose a significant risk since rice is generally self-pollinating and possible cross-pollination is limited by simultaneous flowering and close proximity [41].
4. No danger to non-target organisms can be observed since the regulated article contains no added insecticidal nor herbicidal traits. The added trait was only for the production of beta-carotene, a Vitamin A precursor, thus discounting the likelihood of Vitamin A overdose due to GR2E consumption by wildlife; and
5. Hybrids between cultivated and wild/distantly-related rice varieties are hardly viable due to cross-sterility [22]. The proponent reported that there were no unintended changes observed in the pollen morphology and viability in GR2E rice compared to conventional rice and that there is a negligible likelihood of adverse environmental consequences arising from the potential introgression of the provitamin A trait from the GR2E rice into other rice or sexually compatible species. An independent study confirmed that the introduced trait does not confer either an increased fitness compared to the cultivated varieties nor any advantage that would enable progeny to become weedier, become more invasive of natural or managed ecosystems, or otherwise alter their impact on biodiversity [41]. On the contrary, another study found that the introduced gene contributes to the interruption of endogenous genes, which results in lower fitness of the plant compared to its conventional counterpart, such as in terms of growth disturbance [42].

DENR-BC's Conclusion

After a comprehensive review and evaluation of the documents and scientific evidence from literature submitted by PHILRICE relative to its application for commercial propagation of Golden Rice (GR2E), the DENR-BC considered that the regulated article poses no significant adverse effect to the environment.

DOH-BC's Assessment

1. Rice is the common name for the plant *Oryza sativa L.*, which has a long history of use as food dating back at least 4000 years. Rice is used in various forms including whole and milled grain, flour, and bran. The husks may be used for fertilizers and animal feed as well as for fiber production. Numerous varieties of rice have been developed from subspecies *indica*, *japonica,* and *javanica*. Over 90 percent of rice production and consumption is in Asia, with around five percent from the Americas, three percent from Africa and another one percent from Europe and Oceania. The crop is well adapted to diverse growing conditions from cool climates to deserts (with irrigation) and can perform well in areas with saline, alkaline, or acid-sulphate soils. The biology of rice has been extensively described in various publications from international organizations [22] and government agencies [30][31].
2. GR2E rice differs from standard rice since it contains extra genes added through genetic modification to ensure the production of provitamin A in the rice grains. Once absorbed into the body, provitamin A (beta-carotene) is converted into vitamin A as needed.
3. A FASTA36 bioinformatic alignment search using the ZmPSY1 amino acid sequence as the query sequence was performed against a toxin database to identify possible significant sequence similarity with known or potential toxins. The toxin database search using the ZmPSY1 query sequence did not return any entries with an E-score less than 1 x 10-5 [32]. Based on the results of this analysis, there were no sequence homology structural alerts for potential toxicity of the ZmPSY1 protein.
4. A FASTA36 bioinformatic alignment search using the CRTI amino acid sequence as the query sequence was performed against a toxin database to identify possible significant sequence similarity with known or potential toxins. A search using the CRTI query sequence returned two protein accessions from the toxin database with an E-score less than I x I 0-5. The two sequence alignments were to the N-terminal regions of L-amino acid oxidase (LAAO) enzymes from two species of venomous snakes: *Bungarus multicinctus* (many-banded krait, also known as the Taiwanese krait or the Chinese krait) and *B. fasciatus* (banded krait). As previously noted, snake venom LAAOs are non-toxic via the oral route and the N-terminal sequence similarity was also observed between the native rice phytoene desaturase and a range of L-amino acid oxidases. An analysis of potential toxicological hazards associated with the CRTI protein that considered the lack of primary sequence structural alerts for toxicity, the defined mode of action of CRTI including its lack of thermal stability, and the rapid pepsin digestibility of CRTI, did not identify any concerns. Based on these considerations, it can be predicted that CRTI protein is unlikely to be acutely

toxic by the oral route.

5. The BLASTP program was used to search the National Center for Biotechnology Information Entrez® Protein Database to determine whether the PMI amino acid sequence showed significant similarity to known or putative toxins [33]. The threshold value for determining significance of matches was based on searches conducted with randomly shuffled sequences of the amino acids comprising PMI. There were 1384 protein sequences identified as having significant sequence similarity to PMI amino acid sequence; however, none of these proteins were known or putative toxins. These results support the conclusion that there are no primary sequence structural alerts for potential toxicity associated with the PMI protein.
6. Potential identities between the ZmPSY1 query sequence and proteins in the allergen database were evaluated with the FASTA35 sequence alignment tool using the default parameters. Based on the results of this analysis, the lack of potentially significant sequence similarity between the ZmPSY1 protein and known and putative allergens indicates that it is not a known allergen and is unlikely to be cross-reactive to known allergens.
7. Potential identities between the CRTI query sequence and proteins in the allergen database were evaluated with the FASTA35 sequence alignment tool using the default parameters. Based on the results of this analysis, the lack of potentially significant sequence similarity between the *Pantoea ananatis* CRTI protein and known and putative allergens indicates that it is not a known allergen and is unlikely to be cross-reactive to known allergens.
8. Bioinformatics analyses showed that the PMI protein did not display significant sequence similarity to known or putative protein toxins [33], nor did it display any biologically meaningful sequence similarity to known or putative allergens [34].
9. The ZmPSYI protein was rapidly degraded in simulated gastric fluid containing pepsin at pH 1.2. No intact ZmPSY1 or ZmPSY1-derived fragments were detected by western immunoblot labelling after 30 seconds of exposure to pepsin-containing SGF. The CRTI protein was rapidly degraded in simulated gastric fluid containing pepsin at pH 1.2. No intact CRTI or CRTI-derived fragments were detected after 30 seconds exposure to pepsin-containing SGF as assessed by Coomassie blue staining and western immunoblot labelling following SOS-PAGE analysis. The PMI protein was rapidly degraded in simulated gastric fluid containing pepsin at pH 1.2. No intact PMI or PMl-derived fragments were detected after one-minute exposure to pepsin-containing SGF as assessed by western immunoblot labelling following SOS-PAGE analysis [35].
10. No acute toxicity testing conducted for ZmPSY1. Additional hazard characterization through acute oral toxicity testing of CRTI protein in mice demonstrated a lack of any test substance-related adverse effects at a dosage of I 00 mg/kg body weight, which represented a 115,000-fold margin of exposure relative to any conceivable human exposure. Additional hazard characterization through acute oral toxicity testing of PMI protein in mice demonstrated a lack of any test substance-related adverse effects at a dosage of 2000 mg/kg body

weight [36].

11. Among the 69 compositional components that were assessed in samples of GR2E and control PSB Rc82 rice grain, and 10 components that were assessed in straw samples, the only statistically significant difference observed from the multi-year combined-site analysis was for stearic (C18:0) fatty acid measured in grain samples (not including the intended difference in beta-carotene levels). With the exception of pro-vitamin A carotenoids, the compositional parameters measured in samples of GR2E rice were within or similar to the range of natural variability of those components in conventional rice varieties with a history of safe consumption. Overall, no consistent patterns emerged to suggest that biologically meaningful changes in composition or nutritive value of the grain or straw had occurred as an unintended consequence of the genetic modification.

Based on the evaluation of available literature and dossier documents presented, that rice varieties containing event GR2E are unlikely to pose an altered risk to human health relative to conventional rice, and that food derived from GR2E rice are as safe as food derived from conventional rice varieties.

DOH-BC's Conclusion

After a thorough review and evaluation of the documents provided in support of their application for approval for Commercial Propagation of Rice GR2E, the DOH Biosafety Committee find that the regulated article applied for Commercial Propagation (CP) is as safe as its conventional counterpart and shall not pose any significant risk to human health.

SEC Expert's Assessment

1. Rice is among the top 3 most important crops produced in the Philippines, providing 93% of the national requirements and the rest, 7% is imported.
2. The genetic modification resulting in GR2E rice is only intended to increase the levels of provitamin A (primarily β-carotene) in the rice endosperm. The current patterns of production, consumption as well as trade will not change with the introduction of this GM rice.
3. This provitamin Biofortified rice event (Golden Rice) that is being introduced simply aims to address problems of Vitamin A insufficiency and not productivity. Reports on the productivity performance of this GM shows that productivity is not affected.
4. Reports of evaluation of the performance of the GM rice show that there are no additional requirements or changes in farm management practices in producing it. Its cultivation practices do not differ from non-GM rice. The complementary inputs necessary to sustain productivity is similar to non-GM rice. Since cultural practices have not changed, the cost of production should not be affected.
5. If a premium is attached to the nutrient value of the provitamin, we would expect it to be more competitive than the non-GM seeds. Otherwise, competitiveness of small-scale farmers would not be affected. There should also be no change in the profitability of farms.

SEC Expert's Recommendation

The SEC expert recommends for the approval and issuance of the biosafety permit of rice GR2E for commercial propagation.

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