RISK ASSESSMENT REPORT FOR GENETICALLY MODIFIED PLANT FOR DIRECT USE AS FOOD AND FEED, OR FOR PROCESSING OF PROVITAMIN A BIOFORTIFIED GR2E RICE

<table>
<tr><th colspan="2">BASIC INFORMATION</th></tr>
<tr><td>Applicant(s)

Philippines Rice Research Institute (PhilRice) and the International Rice Research Institute (IRRI)</td><td>Event Code/Identification

GR2E (OECD Unique Identifier: IR-ØØGR2E-5)</td></tr>
<tr><td>Official Address(es)

PhilRice Central Experimental Station
Maligaya, Science City of Munoz, 3119 Nueva Ecija
Philippines

International Rice Research Institute
Pili Drive, UPLB, Los Baños, 4031, Laguna
Philippines</td><td>Nature/Identity of Transgene(s)

Zmpsy1 (phytoene synthase)
crtI (carotene desaturase I; syn phytoene desaturase)
pmi (phosphomannose isomerase)</td></tr>
<tr><td>Telephone No.
Roel Suralta (PhilRice): +63444560112
Donald J. MacKenzie (IRRI): +1 (202) 695-0436</td><td rowspan="3">Brief Description of Phenotypic Effect(s) of the Transgene
Provitamin A Biofortified Rice</td></tr>
<tr><td>Fax No.
Roel Suralta (PhilRice): +63444560112
Doanld J. MacKenzie (IRRI) : +1 (202) 478-0755</td></tr>
<tr><td>Email Address
Roel Suralta (PhilRice): rrsmfs@yahoo.com
Donald J. MacKenzie (IRRI): d.mackenzie@irri.org</td></tr>
<tr><td>Host Organism
Oryza sativa L.</td><td rowspan="3">Method of Transformation Used
Agrobacterium tumefaciens-mediated transformation</td></tr>
<tr><td>Donor Organism(s)
Zea mays (maize; corn)
Pantoea ananatis
Escherichia coli</td></tr>
<tr><td>Proposed Use
Production of rice for human consumption (e.g., milled rice and derived products, such as bran, germ, starch, flour, and oil) and rice by-products for use in livestock feeds in countries of production</td></tr>
<tr><td>Date Received</td><td>Status</td></tr>
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I. THE HOST ORGANISM (Scientific name: Oryza sativa L.)
QUESTIONS *(Please provide references used for each questions)*
1. Is it a source of key nutrients? The main nutrients provided by rice are carbohydrates (starch) and protein. Rice grain is a source of B vitamins (brown rice) but not fat-soluble vitamins (A, D, and K). Rice is not considered a significant source of micro-nutrient minerals.

References:
OECD (2016). Revised consensus document on compositional considerations for new varieties of rice (Oryza sativa): key food and feed nutrients, anti-nutrients and other constituents. Organisation for Economic Cooperation and Development, Paris. ENV/JM/WRPR(2016)47

Supporting dossier section 2.1.3

2. Is it a source of anti-nutrients?

Rice is generally not considered a significant source of anti-nutritional factors and these have not historically been present in rice-based foods at levels that would pose safety concerns. Anti-nutrient factors in the bran include phytic acid and digestive enzyme inhibitors (e.g., trypsin inhibitor).

References:
OECD (2016)
Supporting dossier section 2.1.3

3. Is it a source of toxicants?

No.

4. Is it a significant source of allergens?

Rice is not considered by allergists to be a common allergenic food. Rare cases of food allergy have been reported. There are two putative rice food allergens, Oryza trypsin alpha-amylase inhibitors (14–16 kDa) and Oryza glyoxalase I (33 kDa).

Reference:
OECD (2016)
Supporting dossier section 2.1.4

5. Is it used as food? If yes, describe the final form of the consumed food product (raw vs. processed, etc).

Rice has a long history of use as food dating back at least 4000 years. 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.

Reference:
OECD (2016)
Supporting dossier sections 2.1, 2.1.2

6. What is the usual consumption pattern of the product by population subgroups?

Rice is a staple for nearly half of the world's seven billion people. However, more than 90 percent of this rice is consumed in Asia, where it is a staple for most the population. Rice provides up to 50 percent of the dietary caloric supply and a substantial part of the protein intake for about 520 million people living in poverty in Asia.

In the Philippines, mean rice consumption by children and adults is approximately 112 g/day and 279 g/day, respectively.

Reference:
OECD (2016)
Supporting dossier sections 2.1.1 and 8.3

7. Is it used as feed? If yes, describe the final form of the consumed feed product (raw *vs*. processed, etc).

In monsoon Asia, rice grain (paddy) is seldom used for animal feeding because of its high cost, although damaged grain and portions considered unfit for human consumption (e.g., sweepings from warehouses and mills), are available for that purpose. By-products from mills are more generally available and constitute the most important feed resource in all rice-producing countries like the Philippines. The by-products include: rice bran, rice polishings and rice mill feed (sweepings).

Reference:
OECD (2016)

II. THE TRANSGENIC PLANT

1. If used as food, list countries that have approved the transgenic plant as food. Provide summary of existing documents, references or opinions of regulatory bodies.
As of the date of this application, GR2E rice has not yet been approved in any country for use in food. To date, regulatory submissions have been made to the US Food and Drug Administration, Food Standards Australia New Zealand, and Health Canada.

2. Will consumption patterns by population subgroups be changed as a result of introducing the novel food?
There are no anticipated changes in consumption amounts, or consumption patterns by population subgroups, as a consequence of the introduction of GR2E rice.

3. If used as feed, list countries that have approved the transgenic plant as feed. Provide summary of existing documents, references or opinions of regulatory bodies.
As of the date of this application, GR2E rice has not yet been approved in any country for use in livestock feed.

III. THE DONOR ORGANISMS (Scientific names: *Zea mays, Pantoea ananatis, Escherichia coli*)

1. Have all protein-encoding sequences found in the original gene construct been described with respect to source and potential pathogenic or allergenic properties?
Yes.

Reference:
Supporting dossier sections 2.2 and 3.2

2. Have all potentially inserted regulatory sequences (promoters, enhancers, termination signals etc) been adequately described?
Yes.

Reference:
Supporting dossier sections 2.2 and 3.2

3. Enumerate all introduced expressible sequences, including antisense.
Zmpsy1 (phytoene synthase)
crtI (carotene desaturase I; syn phytoene desaturase)
pmi (phosphomannose isomerase)

4. List all donor organisms. Indicate if known to be toxic or allergenic.

Donor Organism	Known Toxicity	Known Allergenicity	Reference
Zea mays (maize, corn) Source of *Zmpsy1* gene	None	Food allergy to maize is relatively rare, and the only significant reported food allergen is a nonspecific lipid transfer protein	Supporting dossier section 2.2.1
Pantoea ananatis Source of *crtI* gene	Strains of *P. ananatis* have been found to be pathogenic on a broad range of plant hosts	None	Supporting dossier section 2.2.2
Escherichia coli Source of *pmi* gene	*E. coli* is a normal inhabitant of the intestinal flora of humans and animals, where it	None	Supporting dossier section 2.2.3

	generally does not cause disease		

5. List all proteins encoded by the expressible sequences. Indicate if known to be toxic or allergenic.

Newly Expressed Protein	Known Toxicity	Known Allergenicity	Reference
*Zm*PSY1	None	None	Supporting dossier section 6.1
CRTI	None	None	Supporting dossier section 6.2
PMI	None	None	Supporting dossier section 6.3

IV. THE TRANSFORMATION SYSTEM

1. What is the transformation method used?
Agrobacterium-mediated transformation.

Reference:
Supporting dossier section 3.1

2. What is the target of genetic modification?
Nuclear DNA.

3. Is experimental protocol completely provided?
Yes.

Reference:
Supporting dossier section 3.1

4. List all the genetic components used. This should include all coding and non-coding regions together with the recombinant plasmid map and its components, description(s) or citation(s) for isolation and source, description and characterization for each region.
Provided in supporting dossier section 3.2, including table 4 and figures 3 and 4.

5. Where used, describe Carrier DNA and/or helper plasmids.
Not applicable.

V. THE INSERTED DNA

1. How many are the insertion sites? How was this demonstrated? Is this sufficient?
Single site of insertion as demonstrated by Southern blot analysis.

Reference:
Supporting dossier section 4.1
Submitted report IR2015-07003

2. How was integrity and order of genetic elements within each insertion site demonstrated?
Southern blot analyses were performed to investigate the number of sites of insertion of the pSYN12424 T-DNA and the integrity of introduced genetic elements. The nucleotide sequence of the entire pSYN12424 T-DNA insert present in GR2E rice, including a portion of the 5' and 3' flanking host genomic region, was determined in order to demonstrate overall integrity of the insert, contiguity of the functional elements, and to detect any individual base-pair changes.

Reference:
Supporting dossier section 4.1 and 4.2
Submitted report IR2015-07003 and IR2015-08001

3. Were there any truncations, deletions, rearrangements identified/determined? If yes, briefly describe. Was this satisfactorily demonstrated?
There were no truncations, deletions, or rearrangements of any gene coding or regulatory elements. There were small deletions of 23 bp and 11 bp in the right and left border sequences (RB, LB), respectively, of the T-DNA. A common occurrence for *Agrobacterium*-mediated transformations.

Reference:
Supporting dossier section 4.7
Submitted report IR2015-08001

4. If truncations, deletions, or rearrangements occurred, how was the potential for creating novel chimeric ORFs tested?
Bioinformatics analyses were conducted to evaluate any ORFs created as a consequence of the T-DNA insertion to assess their potential to encode amino acid sequences with significant similarity to known toxins or allergens.

Reference:
Supporting dossier section 4.7
Submitted report IR2015-08001

4. Has the main transgene been expressed in other approved GM crops? If yes, please enumerate these crops.
The *Zmpsy1* and *crtI* genes have not been expressed in other approved GM crops. PMI has been reviewed multiple times by regulatory authorities in at least 21 countries in the context of reviewing the food and/or feed, or environmental, safety of a number of genetically engineered maize events, including MIR604, MIR162, 3272, and 5307.

Reference:
Supporting dossier section 6.3 and table 18.

5. Were there any plasmid backbone sequences present? How was this determined? Was this sufficient?
Southern blot analysis of *Asc*I+*Xma*I-digested genomic DNA obtained from event GR2E in Kaybonnet germplasm was performed to demonstrate the lack of integration of any sequences derived from the pSYN12424 plasmid backbone.

Reference:
Supporting dossier section 4.3
Submitted report IR2015-07003

VI. GENETIC STABILITY

1. How was the multigenerational stability of the introduced trait assessed? How many generations were tested?
Southern blot analysis to demonstrate stability of the inserted DNA and carotenoid analysis of milled GR2E rice to demonstrate functional expression of the introduced trait. Stability was tested across four (4) generations of GR2E rice, including three different genetic backgrounds in three backcross generations.

Reference:
Supporting dossier sections 4.4 and 4.5
Submitted reports IR2015-07003 and IR2016-07001

2. How was segregation assessed? How many generations of backcrosses were tested? If any other method was used to show stability of segregation, describe and explain why such method was used.
Were the results from segregation analysis consistent with reported inserted number?
Inheritance pattern was investigated using a PCR-based zygosity test to determine segregation of the inserted DNA. Three different segregating backcross generations (BC_4F_2, BC_5F_1, and BC_5F_2) in each of three different genetic backgrounds (PSB Rc82, BRRI dhan 29, and IR64). The results from these analyses were consistent with Southern

hybridization data indicating the stable integration of the pSYN12424 T-DNA at a single site within the GR2E rice genome and segregation of the introduced DNA as a single genetic locus according to Mendelian rules of inheritance.

Reference:
Supporting dossier section 4.6
Submitted report IR2015-08003

VII. EXPRESSED MATERIAL

1. Indicate Level of Expression of Novel Protein in Different Plant Parts. How were they measured? (roots, stem, foliage, seed, pollen) (µg/g fwt)

Protein	Test Method	Roots	Stem	Foliage	Mature Seed (Grains)	Pollen
*Zm*PSY1	ELISA (µg/g fwt)	ND	<LOD	ND	0.245	ND
	Western blotting	<LOD	<LOD	<LOD	+	ND
CRTI	ELISA (µg/g fwt)	ND	<LOD	ND	0.03	ND
	Western	<LOD	<LOD	<LOD	+	ND
PMI	ELISA (µg/g fwt)	ND	0.80	ND	1.89	ND
	Western	+	+	+	+	ND

Concentrations shown are highest levels measured in mature grain or straw from any site and year.
ND = Not determined. Not a pathway of dietary exposure for humans or livestock animals.
LOD = Limit of detection.

Reference:
Supporting dossier section 5
Submitted reports IR2016-04003, IR2015-08004, and IR201604004

2. Does the novel protein have a metabolic role? If Yes, describe.
Expression of the *Zm*PSY1 and CRTI enzymes in the rice endosperm complete the carotenoid biosynthetic pathway leading to the accumulation of ß-carotene in the endosperm.

Reference:
Supporting dossier section 1.4.2 and figure 1.

VIII. TOXICOLOGICAL ASSESSMENT

A. Identify Novel Protein 1: *Zm*PSY1 (phytoene synthase from *Zea mays*)

1. Digestibility

-What enzyme was used in the digestibility study? What is the estimated T_{50} Result?

-If there is, what is the largest size of fragments remaining after digestion? How was this determined?

Digestibility of the *Zm*PSY1 protein was investigated using the standardized *in vitro* pepsin digestion model. No intact *Zm*PSY1 protein was detected following 30 seconds exposure to pepsin in simulated gastric fluid (SGF) pH 1.2. The estimated T_{50} is <30 seconds. No fragments were detected by western immunoblot analysis.

Reference:
Supporting dossier section 6.1.6
Submitted report IR2016-01002

2. Heat Inactivation

- What is the estimated T_{50} Result?

- How was this determined?

The thermal stability of the *Zm*PSY1 protein was evaluated by measuring enzymatic activity using an high-pressure liquid chromatography (HPLC) method to monitor the production of 15-*cis*-phytoene from *in situ* produced geranylgeranyl diphosphate derived from the enzymatic conversion of dimethylallyl diphosphate and isopentenyl diphosphate in the presence of active geranylgeranyl diphosphate synthase. The estimated T_{50} is 42°C.

Reference:
Supporting dossier section 6.1.7
Submitted report IR2015-12002

3. Amino Acid Sequence Comparison

- Is there homology with known toxins?

- If yes, which ones?

-What is the percentage of sequence similarity?

There were no sequence homology structural alerts for potential toxicity of the *Zm*PSY1 protein.

Reference:
Supporting dossier section 6.1.3
Submitted report IR2016-01005

4. Acute Oral Gavage

- Was this performed?

- If yes, report NOEL (mg/kg body weight)

A tier-1 assessment of potential hazards associated with the *Zm*PSY1 protein, which considered the food crop source of the gene, the known expression of *Zm*PSY1 in yellow maize grain and history of likely human and animal exposure, lack of significant amino acid sequence similarity with known toxins and allergens, susceptibility to heat inactivation, and rapid digestibility concluded that further hazard characterization by animal toxicity testing was unnecessary.

Reference:
Supporting dossier section 6.1.8

5. Source of the Test Protein

- What is the source of test protein?

- If not plant, was equivalency demonstrated?

The *Zm*PSY1 protein was produced in an *Escherichia coli* over-expression system for use in pepsin digestibility and heat stability studies, and as a calibration standard for quantitative ELISA. Based on a combination of physiochemical and enzyme activity analyses, the microbial-expressed *Zm*PSY1 protein was functionally equivalent to the *in planta* expressed *Zm*PSY1, and was a suitable surrogate protein for conducting relevant safety studies.

Reference:
Supporting dossier section 6.1.5
Submitted report IR2016-02005

B. Identify Novel Protein 2: CRTI (phytoene desaturase from *Pantoea ananatis*)

1. Digestibility

-What enzyme was used in the digestibility study? What is the estimated T50 Result?

-If there is, what is the largest size of fragments remaining after digestion? How was this determined?

Digestibility of the CRTI protein was investigated using the standardized *in vitro* pepsin digestion model. No intact CRTI protein was detected following 30 seconds exposure to pepsin in simulated gastric fluid (SGF) pH 1.2. The estimated T_{50} is <30 seconds. No fragments were detected by western immunoblot analysis.

Reference:
Supporting dossier section 6.2.6
Submitted report IR2016-07003

2. Heat Inactivation

- What is the estimated T_{50} Result?

- How was this determined?

The thermal stability of the phytoene desaturase (CRTI) protein was evaluated by measuring enzymatic activity using a spectrophotometric assay to monitor the conversion of liposome-incorporated 15-*cis*-phytoene to all-*trans*-lycopene. The estimated T_{50} is 51°C.

Reference:
Supporting dossier section 6.2.7
Submitted report IR2015-12001

3. Amino Acid Sequence Comparison

- Is there homology with known toxins?

- If yes, which ones?

-What is the percentage of sequence similarity?

There were no sequence homology structural alerts for potential toxicity of the CRTI protein.

Reference:
Supporting dossier section 6.2.3
Submitted report IR2016-01004

4. Acute Oral Gavage

- Was this performed?

- If yes, report NOEL (mg/Kg body weight)

Due to the non-food source of the *crtI* gene, acute oral toxicity testing of CRTI protein in mice was conducted as a further assurance of safety, which demonstrated a lack of any observable adverse effects at a dose of 100 mg/kg body weight, which represents at least a 115,000–fold margin of exposure relative to any realistically conceivable human dietary intake.

Reference:
Supporting dossier section 6.2.8
Submitted reports IR2016-04001 and IR2016-03002 (test substance characterization)

5. Source of the Test Protein

- What is the source of test protein?

- If not plant, was equivalency demonstrated?

The CRTI protein was produced in an *Escherichia coli* over-expression system for use in pepsin digestibility, heat stability, and acute oral toxicity studies, and as a calibration standard for quantitative ELISA. Based on a combination of physiochemical and enzyme activity analyses, the microbial-expressed CRTI protein was functionally equivalent to the *in planta* expressed CRTI, and was a suitable surrogate protein for conducting relevant safety studies.

Reference:
Supporting dossier section 6.2.5
Submitted report IR2016-02004

C. Identify Novel Protein 3: PMI (phosphomannose isomerase from *Escherichia coli*)

1. Digestibility

-What enzyme was used in the digestibility study? What is the estimated T50 Result?

-If there is, what is the largest size of fragments remaining after digestion? How was this determined?

The susceptibility of PMI to proteolytic degradation in simulated mammalian gastric fluid (SGF) containing pepsin was evaluated using SDS-PAGE and western blot analysis. The results from this study demonstrated that PMI was readily degraded with no intact protein or degradation products detected following digestion for one minute.

Reference:
Supporting dossier section 6.3.5
Syngenta submitted Letter of Access for previously submitted reports.

2. Heat Inactivation

- What is the estimated T_{50} Result?

- How was this determined?

The heat stability of PMI protein was investigated by measuring enzymatic activity following pre-incubation for 30 minutes at temperatures ranging from 25–95°C. PMI enzymatic activity was below that limits of quantification following pre-incubation at temperatures of 65°C and above.

Reference:
Supporting dossier section 6.3.6
Syngenta submitted Letter of Access for previously submitted reports.

3. Amino Acid Sequence Comparison

- Is there homology with known toxins?

- If yes, which ones?

-What is the percentage of sequence similarity?

There were no sequence homology structural alerts for potential toxicity of the PMI protein.

Reference:
Supporting dossier section 6.3.3
Syngenta submitted Letter of Access for previously submitted reports.

4. Acute Oral Gavage

- Was this performed?

- If yes, report NOEL (mg/Kg body weight)

Microbial-expressed PMI protein (89.5 percent purity) administered as a single oral gavage dose at 0 or 2000 mg/kg body weight followed by a 14-day observation period was well tolerated in male and female Crl:CD-1(ICR) mice with no signs of toxicity. The tested dose represents a 69,000–fold margin of exposure relative to any conceivable human exposure due to consumption of GR2E rice.

Reference:
Supporting dossier section 6.3.7
Syngenta submitted Letter of Access for previously submitted reports.

5. Source of the Test Protein

- What is the source of test protein?

- If not plant, was equivalency demonstrated?

Not applicable. See Syngenta submitted Letter of Access for previously submitted reports relevant to PMI protein.

1. Are these proteins expressed independently of each other and is the functional activity of these proteins maintained?

Yes, the *Zm*PSY1, CRTI, and PMI proteins are independently expressed. Functional activity of both the *Zm*PSY1 and CRTI proteins is evidenced by the accumulation of β-carotene in GR2E rice endosperm, which would not occur if either of these proteins was inactive. Functional expression of the PMI protein was evidenced by the ability to select transformed plantlets in tissue culture on media containing only mannose as a carbon source.

2. Describe, if these are expressed in the same cell organelle

The *Zm*PSY1 and CRTI proteins are targeted to the plastid, which is where the plant carotenoid biosynthetic pathway is localized. The PMI protein is expressed in the cytoplasm.

3. Describe, if and how they interact to express the phenotype(s)

There are no direct interactions between the *Zm*PSY1, CRTI, and PMI proteins.

4. Describe, if they interact in a metabolic pathway

Only the *Zm*PSY1 and CRTI enzymes interact in a metabolic pathway. The sequential activities of the *Zm*PSY1 and CRTI enzymes complete the carotenoid biosynthetic pathway in the GR2E rice endosperm. The condensation of two molecules of geranylgeranyl diphosphate to yield the first carotenoid, C_{40} 15-*cis*-phytoene, is catalyzed by *Zm*PSY1.

CRTI catalyzes consecutive modifications of phytoene, including desaturation and isomerization, to form all-*trans*-lycopene. The endogenous rice β-cyclase enzyme is responsible for the production of β-carotene from all-*trans*-lycopene.

Reference:
Supporting dossier sections 6.1.1 and 6.2.1

IX. ALLERGENICITY ASSESSMENT

A. Identify Novel Protein 1: ZmPSY1 (phytoene synthase from Zea mays)

1. Digestibility

-What enzyme was used in the digestibility study? What is the estimated T_{50} Result?

-If there is, what is the largest size of fragments remaining after digestion? How was this determined?

Digestibility of the *Zm*PSY1 protein was investigated using the standardized *in vitro* pepsin digestion model. No intact *Zm*PSY1 protein was detected following 30 seconds exposure to pepsin in simulated gastric fluid (SGF) pH 1.2. The estimated T50 is <30 seconds. No fragments were detected by western immunoblot analysis.

Reference:
Supporting dossier section 6.1.6
Submitted report IR2016-01002

2. Heat Inactivation

- What is the estimated T_{50} Result?

- How was this determined?

The thermal stability of the *Zm*PSY1 protein was evaluated by measuring enzymatic activity using an high-pressure liquid chromatography (HPLC) method to monitor the production of 15-*cis*-phytoene from *in situ* produced geranylgeranyl diphosphate derived from the enzymatic conversion of dimethylallyl diphosphate and isopentenyl diphosphate in the presence of active geranylgeranyl diphosphate synthase. The estimated T_{50} is 42°C.

Reference:
Supporting dossier section 6.1.7
Submitted report IR2015-12002

3. Amino Acid Sequence Comparison

- **Is there homology with known allergens?**
- **If yes, which ones?**

To assess the potential for allergenic cross-reactivity, the 410-amino acid sequence encoded by the *Zmpsy1* gene was compared to a peer-reviewed database of 1956 known and putative allergen and celiac protein sequences residing in the FARRP16 dataset at the University of Nebraska. No identity matches of > 35 percent over 80 residues were observed and there were also no matches of eight contiguous identical amino acids.

Reference:
Supporting dossier section 6.1.4
Submitted report IR2016-02002

4. Prevalence in Food

- Percent of Total Protein?

Highest concentration of *Zm*PSY1 protein in mature grain: 0.245 µg/g FWT; total protein content in grain was 8.1% DB; moisture content was 12.26%. Therefore, *Zm*PSY1 was 0.00034% of total protein.

0.245 µg/g * 100%/(100%-12.26%) * (1/81000 µg/g) * 100% = 0.00034%

Reference:
Supporting dossier sections 5 (*Zm*PSY1 protein concentration in mature grain) and 7.1 (crude protein content and moisture content of mature grain)

5. Serum Screening

- Was this performed? If yes, report results.

Not applicable. Serum screening is only indicated if gene source is major allergen source or if there is significant sequence similarity to known allergens. Neither of these situations apply.

B. Identify Novel Protein 2: CRTI (phytoene desaturase from *Pantoea ananatis*)

1. Digestibility

-What enzyme was used in the digestibility study? What is the estimated T_{50} Result?

-If there is, what is the largest size of fragments remaining after digestion? How was this determined?

Digestibility of the CRTI protein was investigated using the standardized *in vitro* pepsin digestion model. No intact CRTI protein was detected following 30 seconds exposure to pepsin in simulated gastric fluid (SGF) pH 1.2. The estimated T_{50} is <30 seconds. No fragments were detected by western immunoblot analysis.

Reference:
Supporting dossier section 6.2.6
Submitted report IR2016-07003

2. Heat Inactivation

- What is the estimated T50 Result?

- How was this determined?

The thermal stability of the phytoene desaturase (CRTI) protein was evaluated by measuring enzymatic activity using a spectrophotometric assay to monitor the conversion of liposome-incorporated 15-*cis*-phytoene to all-*trans*-lycopene. The estimated T_{50} is 51°C.

Reference:
Supporting dossier section 6.2.7
Submitted report IR2015-12001

3. Amino Acid Sequence Comparison

- **Is there homology with known allergens?**
- **If yes, which ones?**

To assess the potential for allergenic cross-reactivity, the 492-amino acid sequence encoded by the *crtI* gene was compared to a peer-reviewed database of 1956 known and putative allergen and celiac protein sequences residing in the FARRP16 dataset at the University of Nebraska. No identity matches of > 35 percent over 80 residues were observed and there were also no matches of eight contiguous identical amino acids.

Reference:
Supporting dossier section 6.2.4
Submitted report IR2016-02001

4. Prevalence in Food

- Percent of Total Protein?

Highest concentration of CRTI in mature grain: 0.03 µg/g FWT; total protein content in grain was 8.1% DB; moisture content was 12.26%. Therefore, CRTI was 0.00004% of total protein.

0.03 µg/g * 100%/(100%-12.26%) * (1/81000 µg/g) * 100% = 0.00004%

Reference:
Supporting dossier sections 5 (CRTI protein concentration in mature grain) and 7.1 (crude protein content and moisture content of mature grain)

5. Serum Screening

- Was this performed? If yes, report results

Not applicable. Serum screening only indicated if gene source is major allergen source or if there is significant sequence similarity to known allergens. Neither of these situations apply.

C. Identify Novel Protein 3: PMI (phosphomannose isomerase from *Escherichia coli*)

1. Digestibility

-What enzyme was used in the digestibility study? What is the estimated T_{50} Result?

-If there is, what is the largest size of fragments remaining after digestion? How was this determined?

The susceptibility of PMI to proteolytic degradation in simulated mammalian gastric fluid (SGF) containing pepsin was evaluated using SDS-PAGE and western blot analysis. The results from this study demonstrated that PMI was readily degraded with no intact protein or degradation products detected following digestion for one minute.

Reference:
Supporting dossier section 6.3.5
Syngenta submitted Letter of Access for previously submitted reports.

2. Heat Inactivation

- What is the estimated T50 Result?

- How was this determined?

The heat stability of PMI protein was investigated by measuring enzymatic activity following pre-incubation for 30 minutes at temperatures ranging from 25–95°C. PMI enzymatic activity was below that limits of quantification following pre-incubation at temperatures of 65°C and above.

Reference:
Supporting dossier section 6.3.6
Syngenta submitted Letter of Access for previously submitted reports.

3. Amino Acid Sequence Comparison

- **Is there homology with known allergens?**
- **If yes, which ones?**

To determine whether or not the PMI amino acid sequence showed biologically relevant amino acid sequence similarity to known or putative allergens, two different searches were performed against the FARRP AllergenOnline database, version 11.0, which contained 1,491 amino acid sequences of known and putative allergens. No identity matches of > 35 percent over 80 residues were observed. There was one eight-amino acid identity match to a known allergen, α-parvalbumin from Rana species CH2001 (unidentified edible frog).

Reference:
Supporting dossier section 6.3.4
Syngenta submitted Letter of Access for previously submitted reports.

4. Prevalence in Food

- Percent of Total Protein?

Highest concentration in mature grain: 1.89 µg/g FWT; total protein content in grain was 8.1% DB; moisture content was 12.26%. Therefore, PMI was 0.0027% of total protein.

1.89 µg/g * 100%/(100%-12.26%) * (1/81000 µg/g) * 100% = 0.0027%

Reference:
Supporting dossier sections 5 (PMI protein concentration in mature grain 1.89 µg/g) and 7.1 (crude protein content and moisture content of mature grain)

5. Serum Screening

- Was this performed? If yes, report results

Serum screening was performed to investigate the biological relevance of the single 8-amino acid sequence identity between PMI and α-parvalbumin from *Rana* species CH2001, a known allergen. No cross-reactivity between PMI and the serum from the single individual known to have demonstrated IgE-mediated allergy to this specific α-parvalbumin was found. The patient's serum did not recognize any portion of the PMI protein as an allergenic epitope. Therefore, the sequence identity between PMI and the α-parvalbumin from *Rana* species CH2001 is not biologically meaningful and has no implications for the potential allergenicity of PMI

Reference:
Supporting dossier section 6.3.4
Syngenta submitted Letter of Access for previously submitted reports.

6. Additional Comments

The safety and lack of allergenicity of the PMI protein has been previously reviewed by Philippines regulators during reviews of maize (corn) events MIR604, MIR162, 3272, and 5307. The amino acid sequence of the PMI protein expressed in GR2E rice is identical to the sequences of PMI protein expressed in events MIR162, 3272, and 5307.

X. NUTRITIONAL DATA

Compositional analyses were performed on samples of rice grain and straw obtained from event GR2E introgressed into PSB Rc82 (BC_5F_3 in 2015; BC_5F_4 in 2016) and near-isogenic control PSB Rc82 rice that were grown in side-by-side confined field tests at four separate sites in the Philippines (Batac City, Los Baños, Muñoz, and San Mateo) during 2015 (wet season) and again in 2016 (dry season). Three blocks (replicates) of each entry were established at each test site in a randomized complete block design. Grain and straw samples were collected from matured rice plants, the stage when typical grain harvest would occur. Grain samples were analyzed for key nutritional components, including proximates, fibre, polysaccharides, fatty acids, amino acids, minerals, vitamins, and anti-nutrients. Samples of straw and bran were analyzed for proximates and minerals.

Reference:
Supporting dossier section 7
Submitted reports IR2015-07001 and IR2016-05001

Proximate and Fibre Analysis: Straw

1. Comparison with SE Comparator (i.e., non-modified control, or other principal comparator) – Any significant differences? If yes, in which parameters?

There were no statistically significant differences in proximates and fibre between samples of straw obtained from GR2E and control PSB Rc82 rice.

Reference:
Table 19 in supporting dossier section 7.1

2. Comparison with Range of Commercial Varieties

- If comparison included a range of commercial varieties: How many?

- Were these grown under the same environmental conditions?

- Were the data derived from the test (transgenic) line within the observed range?

If not, which parameters were outside the range?

Not applicable.

3. Comparison with Range of Literature Values

- Were the data derived from the test (transgenic) line within the reported range?

If not, which parameters were outside range?

Except for moisture content, which is dependent on the extent of drying of straw following harvest, the mean values for all proximates and fibre in GR2E and control PSB Rc82 rice straw were similar to the ranges reported in the literature.

4. Biological Significance

- For any statistical difference, are they biologically relevant? If yes, note concern.

Not applicable, no statistically significant differences were observed between samples of GR2E and control PSB Rc82 rice.

Proximate and Fibre Analysis: Grain

1. Comparison with SE Comparator (i.e., non-modified control, or other principal comparator) – Any significant differences? If yes, in which parameters?

In the multi-year combined-sites analysis, comparisons of proximates, fibre, and polysaccharides in grain (paddy) samples derived from GR2E and control PSB Rc82 rice resulted in no statistically significant differences in any of the measured parameters.

Reference:
Table 20 in supporting dossier section 7.1

2. Comparison with Range of Commercial Varieties

- If comparison included a range of commercial varieties: How many?

- Were these grown under the same environmental conditions?

- Were the data derived from the test (transgenic) line within the observed range?

If not, which parameters were outside the range?

Not applicable.

3. Comparison with Range of Literature Values

- Were the data derived from the test (transgenic) line within the reported range?

If not, which parameters were outside range?

Except for crude fat, acid detergent fibre, neutral detergent fibre, and starch, the mean values of all components measured for samples of GR2E rice were within the reported ranges. For those values slightly outside the range, there were no statistically significant differences between GR2E rice and control PSB Rc82 rice.

4. Biological Significance

- For any statistical difference, are they biologically relevant? If yes, note concern.

Not applicable, no statistically significant differences were observed between samples of GR2E and control PSB Rc82 rice.

Proximate and Mineral Analysis: Bran

1. Comparison with SE Comparator (i.e., non-modified control, or other principal comparator) – Any significant differences? If yes, in which parameters?

No statistically significant differences were noted for any of the measured parameters between bran samples derived from GR2E and control PSB Rc82 rice grain.

Reference:
Table 21 in supporting dossier section 7.1

2. Comparison with Range of Commercial Varieties

- If comparison included a range of commercial varieties: How many?

- Were these grown under the same environmental conditions?

- Were the data derived from the test (transgenic) line within the observed range?

If not, which parameters were outside the range?

Not applicable.

3. Comparison with Range of Literature Values

- Were the data derived from the test (transgenic) line within the reported range?

If not, which parameters were outside range?

The measured values for these analytes were within the respective ranges reported in the literature except for crude fat and phosphorus, which were slightly higher in both GR2E and control PSB Rc82 rice.

4. Biological Significance

- For any statistical difference, are they biologically relevant? If yes, note concern.

Not applicable, no statistically significant differences were observed between bran samples produced from GR2E and control PSB Rc82 rice.

Key Nutrients: Straw Minerals

1. Comparison with SE Comparator (i.e., non-modified control, or other principle comparator – Any significant differences? If yes, in which parameters?

There were no statistically significant differences in concentrations of calcium and phosphorus measured in samples of GR2E and control PSB Rc82 rice straw across locations and growing seasons

Reference:
Table 22 in supporting dossier section 7.2

2. Comparison with Range of Commercial Varieties

- If comparison included a range of commercial varieties: How many?

- Were these grown under the same environmental conditions?

- Were the data derived from the test (transgenic) line within the observed range?

If not, which parameters were outside the range?

Not applicable.

3. Comparison with Range of Literature Values

- **Were the data derived from the test (transgenic) line within the reported range?**
- **If not, which parameters were outside range?**

Mean values were within ranges for these two minerals reported in the literature.

4. Biological Significance

- For any statistical difference, are they biologically relevant? If yes, note concern.

Not applicable, no statistically significant differences were observed between straw samples from GR2E and control PSB Rc82 rice.

Key Nutrients: Grain Minerals

1. Comparison with SE Comparator (i.e., non-modified control, or other principle comparator – Any significant differences? If yes, in which parameters?

Comparison of the mineral composition in samples of GR2E and control PSB Rc82 rice grain did not reveal any statistically significant differences in the concentrations of any measured analytes.

Reference:
Table 23 in supporting dossier section 7.2

2. Comparison with Range of Commercial Varieties

- If comparison included a range of commercial varieties: How many?

- Were these grown under the same environmental conditions?

- Were the data derived from the test (transgenic) line within the observed range?

If not, which parameters were outside the range?

Not applicable.

3. Comparison with Range of Literature Values

- **Were the data derived from the test (transgenic) line within the reported range?**
- **If not, which parameters were outside range?**

The mean concentrations of each of the minerals measured in samples from GR2E and control PSB Rc82 rice grain were within the ranges reported in the literature.

4. Biological Significance

- For any statistical difference, are they biologically relevant? If yes, note concern.

Not applicable, no statistically significant differences were observed between grain samples from GR2E and control PSB Rc82 rice.

Key Nutrients: Grain Amino Acids

1. Comparison with SE Comparator (i.e., non-modified control, or other principle comparator) – Any significant differences? If yes, in which parameters?

Across locations and growing seasons, there were no statistically significant differences in the concentrations of any amino acids between samples of GR2E and control PSB Rc82 rice.

Reference:
Table 24 in supporting dossier section 7.3

2. Comparison with Range of Commercial Varieties

- If comparison included a range of commercial varieties: How many?

- Were these grown under the same environmental conditions?

- Were the data derived from the test (transgenic) line within the observed range?

If not, which parameters were outside the range?

Not applicable.

3. Comparison with Range of Literature Values

- **Were the data derived from the test (transgenic) line within the reported range?**
- **If not, which parameters were outside range?**

Except for tryptophan, which was slightly lower and not statistically significantly different between GR2E and control PSB Rc82 rice, the mean concentrations of each of the amino acids measured in samples from GR2E and control PSB Rc82 rice grain were within the ranges reported in the literature.

4. Biological Significance

- For any statistical difference, are they biologically relevant? If yes, note concern.

Not applicable, no statistically significant differences were observed between grain samples from GR2E and control PSB Rc82 rice.

Key Nutrients: Grain Vitamins

1. Comparison with SE Comparator (i.e., non-modified control, or other principal comparator) – Any significant differences? If yes, in which parameters?

Except for β-carotene, which was intended to be elevated in GR2E rice, there were no statistically significant differences noted in the concentrations of any measured vitamins between GR2E and control PSB Rc82 rice in the combined-sites analysis over both growing seasons.

Reference:
Table 25 in supporting dossier section 7.4

2. Comparison with Range of Commercial Varieties

- If comparison included a range of commercial varieties: How many?

- Were these grown under the same environmental conditions?

- Were the data derived from the test (transgenic) line within the observed range?

If not, which parameters were outside the range?

Not applicable.

3. Comparison with Range of Literature Values

- **Were the data derived from the test (transgenic) line within the reported range?**
- **If not, which parameters were outside range?**

Except for pyridoxine (B6), folic acid (B9), and α-tocopherol, which were not statistically significantly different between GR2E and control PSB Rc82 rice, the mean concentrations of each of the vitamins measured in samples from GR2E and control PSB Rc82 rice grain were within the ranges reported in the literature. As previously noted, the intended elevation of β-carotene in GR2E rice grain was significantly outside the literature range.

4. Biological Significance

- For any statistical difference, are they biologically relevant? If yes, note concern.

The accumulation of β-carotene in GR2E rice grain was intended to be biologically relevant, and consumption of GR2E rice is intended to improve the vitamin A status of consumers at risk for vitamin A deficiency.

Key Nutrients: Grain Fatty Acids

1. Comparison with SE Comparator (i.e., non-modified control, or other principle comparator – Any significant differences? If yes, in which parameters?

In the combined-sites analysis over both growing seasons, the only statistically significant difference observed between GR2E and control PSB Rc82 rice samples was in the concentration stearic (C18:0) acid, which was approximately 6.5 percent higher for GR2E rice.

Reference:
Table 27 in supporting dossier section 7.5

2. Comparison with Range of Commercial Varieties

- If comparison included a range of commercial varieties: How many?

- Were these grown under the same environmental conditions?

- Were the data derived from the test (transgenic) line within the observed range?

If not, which parameters were outside the range?

Not applicable.

3. Comparison with Range of Literature Values

- **Were the data derived from the test (transgenic) line within the reported range?**
- **If not, which parameters were outside range?**

The mean concentrations of each of the fatty acids measured in samples from GR2E and control PSB Rc82 rice grain were within the ranges reported in the literature.

4. Biological Significance

- For any statistical difference, are they biologically relevant? If yes, note concern.

Stearic acid (C18:0) comprises approximately two percent of the total fatty acids in rice grain and is not an essential fatty acid. The small but statistically significant difference (*ca*. 6.5 percent) between stearic acid concentrations in samples of GR2E and control PSB Rc82 rice is unlikely to be biologically relevant.

Anti-nutrients Grain Phytic Acid and Trypsin Inhibitor

1. Comparison with SE Comparator (i.e., non-modified control, or other principal comparator) – Any significant differences? If yes, in which parameters?

There were no statistically significant differences in the concentrations of phytic acid or in the levels of trypsin inhibitor between samples of GR2E and PSB Rc82 control rice.

Reference:
Table 28 in supporting dossier section 7.6

2. What is the effect of processing on the level of anti-nutrient?
Trypsin inhibitor is affected by heat and activity is expected to be significantly reduced following cooking.

Reference:
OECD (2016)

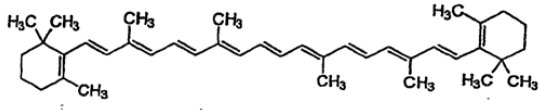
3. Comparison with Range of Commercial Varieties
- If comparison included a range of commercial varieties: How many?
- Were these grown under the same environmental conditions?
- Were the data derived from the test (transgenic) line within the observed range?
If not, which parameters were outside the range?
Not applicable.

4.Comparison with Range of Literature Values
- Were the data derived from the test (transgenic) line within the reported range?
Data on levels of phytic acid and trypsin inhibitor in conventional rice grain are limited or non-existent. Mean concentrations of phytic acid in grain samples from GR2E and control PSB Rc82 rice were both slightly outside the range reported in the ILSI Crop Composition Database, but were not significantly different.

5. Biological Significance
- For any statistical difference, are they biologically relevant? If yes, note concern.
Not applicable, no statistically significant differences were observed between grain samples from GR2E and control PSB Rc82 rice.

COMPOSITIONALLY ALTERED REGULATED ARTICLE

Does it contain a new substance?
Provitamin A carotenoids, primarily β-carotene ($C_{40}H_{56}$).



Trans Beta Carotene

What is the purpose of having the regulated article produce this new substance?
What is the benefit of this new substance?
Provitamin A carotenoids (e.g., β-carotene) are converted in the body to vitamin A (retinol). The intended nutritional effect of GR2E rice is to complement existing VAD control efforts by supplying up to 30–50 percent of the estimated average requirement (EAR) for vitamin A for preschool age children and pregnant or lactating mothers in high-risk countries, including Bangladesh, Indonesia, and the Philippines.

Reference:
Supporting dossier section 1.4

Is the new substance found in the edible portion of the plant? Which part/s of the plant?
Yes, rice endosperm.

Is the new substance known as (a) nutrient (b) pesticide (c) biologically active compound (d) allergen (e) toxin (f) anti-nutrient (g) others?
Nutrient.

If it is an allergen, explain why this allergenic substance is safe in the particular use the regulated article was intended for.
Not applicable.

If it is a toxin, explain why this toxic substance is safe in the particular use the regulated article was intended for.
Not applicable.

If it is an anti-nutrient, explain why this anti-nutrient is safe in the particular use the regulated article was intended for.
Not applicable.

IF THE SUBSTANCE IS A NUTRIENT

What is the known biological function of this substance?
Beta-carotene is a provitamin, i.e. a precursor of vitamin A (retinol), which is classified as an essential nutrient for humans. Beta-carotene is one of many carotenoids found in plants, fungi, and bacteria. Carotenoids are therefore predominantly obtained through foods of plant origin or food supplements.

Reference:
Supporting dossier section 8.1

Describe the history of its use as a nutrient.
See below.

Is this new substance a natural component of other food crops? Enumerate these crops.
Beta-carotene is regarded as the major carotenoid present in the human diet and is found in significant quantities in a variety of green leafy and yellow-coloured vegetables and orange-coloured fruit. In addition to information provided in Table 30 in the supporting dossier (section 8.1), concentrations of naturally occurring β-carotene in some selected foods are shown below.

Table 1: Concentrations of β-carotene in some selected foods

Food	β-carotene (µg/g)	Food	β-carotene (µg/g)
Grape leaves, raw	161.9	Carrots, raw	82.9
Kale, cooked, drained	81.7	Spinach, frozen, cooked	72.4
Turnip greens, raw	69.5	Kale, raw	59.3
Spinach, raw	56.3	Lettuce, green leaf, raw	44.4
Chard, Swiss, raw	36.5	Chives, raw	26.1
Apricots, raw	10.9	Squash, winter, raw	8.2
Cowpeas, young pods	7.9	Cabbage, red, raw	6.7
Mangos, raw	6.4	Peas, edible, raw	6.3

Source: USDA-ARS National Nutrient Database (accessed 25 August 2016)

Is the new substance equivalent in structure to those found in these food crops? Explain.
The chemical structure of β-carotene produced in GR2E rice is identical to that produced in other food crops and produced synthetically.

Does this new substance occur in the regulated article in the same amount as in other foods? If yes, please indicate the range of values in each food where this new substance occurs. If no, please indicate whether the amount is lower or higher compared to other food crops. Explain why the level of the new substance in the regulated article is safe.
The highest concentration of β-carotene measured in samples of milled rice from field-grown plants of PSB Rc82 containing event GR2E was 7.31 µg/g (see supporting dossier Table 26), which is slightly less than 10 percent of the level found naturally occurring in carrots (see Table 30 in supporting dossier section 8.1 and Table 1, above). Based on the highest rate of daily rice consumption reported for children in Bangladesh (12.5 g/kg body weight) and assuming an average adult body weight of 57.7 kg, the maximum daily intake of β-carotene from GR2E rice was estimated to be 5.3 mg (supporting dossier section 8.3). In context, the maximum daily intake of β-carotene from GR2E rice based on the above scenario is equivalent to about 100 g of raw Saluyot leaves, or slightly more than one medium carrot.

How much is the average consumption of the naturally occurring substance?
In well-nourished populations, such as in Europe and North America, the mean daily intake of β-carotene is in the range of 2.7–6.4 mg (supporting dossier section 8.1).

What is the effect of prolonged consumption of the naturally occurring substance?
Beta-carotene is an effective source of vitamin A in both conventional foods and vitamin supplements, and it is generally considered virtually nontoxic because humans tolerate high dietary dosages without apparent harm. There are no reports of adverse effects arising from the consumption of naturally-occurring β-carotene in food.

What is the amount of the new substance in the regulated article?
As noted above, the highest concentration of β-carotene measured in samples of milled rice from field-grown plants of PSB Rc82 containing event GR2E was 7.31 µg/g (see supporting dossier Table 26).

Is this substance known to be toxic or have adverse effects when ingested in excess of the average consumption?
There is no evidence that conversion of β-carotene to vitamin A contributes to vitamin A toxicity, even when β-carotene is ingested in large amounts, and there is much circumstantial evidence that supplementary intakes of 15–50 mg/day are without side effects except for discolouration of the skin related to hypercarotenemia in some subjects at high intakes. Dosages of β-carotene as high as 180 mg per day have been given to humans for several months during the treatment of light-sensitive skin diseases (e.g., protoporphyria) without observed adverse effects other than changes in skin colour, which are reversible upon reduction of β-carotene intake.

In 2012, the European Food Safety Authority (EFSA) Panel on Food Additives and Nutrient Sources added to Food (ANS) reviewed the safety of β-carotene supplements. The EFSA panel concluded that exposure to β-carotene from its use as a food additive and as a food supplement at a level below 15 mg per day does not give rise to concerns about adverse health effects in the general population. It also stated that no sensitive groups were identified from the available evidence at this exposure; therefore, the term general population encompasses all groups, including heavy smokers.

Reference:
Supporting dossier section 8.1 and 8.2, and references contained therein.

Does the new substance alter the level or concentration of other related compound/s?
As noted below, β-carotene in GR2E rice will undergo degradation over time via the same mechanisms for β-carotene degradation in other fruits and vegetables where it is found. A comprehensive compositional assessment of GR2E rice grain in comparison to its non-transformed parental counterpart did not reveal any biologically meaningful changes in the levels of other key nutrients or anti-nutrients.

How do the following factors affect the stability of the new substance and describe the method of determination: (a) heat (b) digestion (c) processing (d) others (e.g. light, oxidation, storage etc.)
The likely mechanisms of carotenoid, including β-carotene, degradation in conventional foods may involve: the reaction of carotenoids with atmospheric oxygen (autooxidation), light (photodegradation), and heat (thermal degradation), as well as degradation by the interactions of carotenoids with singlet oxygen, acid, metals, and free radicals. Upon storage, β-carotene in GR2E rice will undergo breakdown via these same mechanisms, and resulting in the same products, as in all other foods containing β-carotene.

Is the substance in the regulated article being used in food fortification? If yes, (a) at what level is it used? (b) How does this level compare with the level found in the regulated article?
Carotene, including β-carotene, is used as a substance to colour products such as juice, cakes, desserts, butter and margarine. It is approved for use as a food additive in the EU (listed as additive E160a), Australia and New Zealand (listed as 160a) and the US. Its use should be in amounts consistent with good manufacturing practice (i.e., no prescribed maximum limits but according to technical necessity).

Is this substance produced by a new pathway in the regulated article? If yes, (a) how many new proteins have been introduced to complete the pathway? (b) Are these proteins expressed in the edible parts of the regulated article? (b1) at what levels are they present? (b2) have these expressed proteins been assessed for toxicity and allergenicity potential? Is all the information required to answer b1 and b2 entered in appropriate section of this document? Two new enzymes, *Zm*PSY1 and CRTI, have been introduced into GR2E rice to complete the β-carotene biosynthetic pathway in the rice endosperm. Both proteins are expressed in the edible form of rice at maximal concentrations of 0.24 μg/g and 0.03 μg/g, respectively, in matured grains (section VII – Expressed Material). Both proteins have been assessed for potential toxicity and allergenicity, and all information relating to these topics is included in this document. Information on the potential toxicity of *Zm*PSY1 and CRTI is included in section VIII-A and –B, respectively, and information on the potential allergenicity of ZmPSY1 and CRTI is included in section IX-A and –B, respectively.
IF A SUBSTANCE IS KNOWN NON-PROTEIN PESTICIDE (NOT APPLICABLE FOR GR2E RICE)
Describe the following about the non-protein pesticide: (a) LD_{50} value, minimum pesticide residue level values, target pest (b) Known adverse effects to humans/animals and non-target organisms (c) Concentration/level in the edible portion of the plant
Is this new substance a natural component of other foods? If yes, enumerate these foods.
Is the new substance equivalent in structure to those found in these other foods? If not, explain.
Does this new substance occur in the regulated article in the same amount as in other foods? If yes, indicate the range of values in each food where this new substance occurs. If no, please indicate whether the amount is lower or higher compared to other food crops. Explain why the level of the new substance in the regulated article is safe.
How much is the average consumption of the naturally occurring substance?
What is the effect of prolonged consumption of the naturally occurring substance?
What is the amount of the new substance in the regulated article?
Is this substance known to be toxic or have adverse health effects when ingested in excess of the average consumption?
Does the new substance alter the level or concentration of other related compound/s? If yes, is there possible adverse health effect due to this alteration?
How do the following factors affect stability of the substance and describe the method of determination: (a) heat (b) digestion (c) processing (d) others (e.g. light, oxidation, storage, etc.)
Is this substance produced by a new pathway in the regulated article? If yes, (a) how many new proteins have been introduced to complete the pathway? (b) are these proteins expressed in the edible parts of the regulated article? If yes, (b1) at what levels are they present? (b2) have these expressed proteins been assessed for toxicity and allergenicity potential? Are all the information required to answer b1 and b2 entered in appropriate section of this document?
IF NEW SUBSTANCE IS A BIOLOGICALLY ACTIVE COMPOUND (NOT APPLICABLE FOR GR2E RICE)
Is this new substance a natural component of other foods? If yes, enumerate these foods.
Is this new substance equivalent in structure to those found in these food crops?
Does this new substance occur in the regulated article in the same amount as in other foods? If yes, enumerate the crops and corresponding values. If no, please indicate whether the amount is lower or higher compared to other food crops.
How much is the average consumption of the naturally occurring substance?
At what concentration does this compound cause characteristic bio-chemical or physiological changes to occur in humans and animals?
What is the concentration of this compound in the edible portion of the plant?
At what levels of consumption the regulated article be recommended for a person to achieve the desired biological function to the body?
What is the effect of prolonged consumption?
Is this substance known to be toxic or have adverse health effects when ingested in certain quantities or in overdose? Explain.
What measures are recommended to prevent overdosing?

How do the following factors affect stability of the substance and describe the method of determination: (a) heat (b) digestion (c) processing (d) others (e.g. light, oxidation, storage etc.)
Is this substance produced by a new pathway in the regulated article? If yes, (a) how many new proteins have been introduced to complete the pathway? (b) are these proteins expressed in the edible parts of the regulated article? If yes, (b1) at what levels are they present? (b2) have these expressed proteins been assessed for toxicity and allergenicity potential? Are all the information required to answer b1 and b2 entered in appropriate section of this document?