Executive Summary

Rice event GR2E (IR-ØØGR2E-5) was developed through the use of recombinant-DNA techniques to express elevated levels of provitamin A (mainly β-carotene) in the rice endosperm, which is converted in the body to vitamin A. 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.

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. The PMI protein, encoded by the *pmi* gene, is also expressed in a number of previously authorized maize lines, including MIR604, MIR162, 3272, and 5307.

Molecular characterization of the introduced DNA within event GR2E confirmed the presence at a single insertion site of one copy of the transfer-DNA (T-DNA) region derived from plasmid pSYN12424 that was stably inherited over multiple generations as a single genetic locus according to Mendelian rules of inheritance. In addition, nucleotide sequencing of the entire inserted DNA, including portions of the 5' and 3' flanking rice genomic sequence, confirmed that the T-DNA was inserted without modifications, deletions, or rearrangements, except for small truncations at the 5' and 3' termini of 23 bp and 11 bp, respectively. There were also no new novel open reading frames created as a consequence of the DNA insertion that would have the potential to encode a protein with any significant amino acid sequence similarity to known and putative toxins or allergens.

As predicted, expression of the *Zm*PSY1 and CRTI proteins was limited to the rice endosperm as assessed by western immunoblot analysis, while expression of the PMI protein was detected in all tissue types, including grain, bran, hulls, stem, leaves, and roots. In order to estimate potential human and animal dietary exposure to the *Zm*PSY1, CRTI, and PMI enzymes expressed in GR2E rice, the concentrations of these proteins in grain and straw, which represent the only potential pathways of dietary exposure, were determined in samples collected from four field locations over two growing seasons. For each protein, the highest measured concentrations were in samples of dough-stage grain, ranging between *ca*. 308–359 ng/g and between *ca*. 54–68 ng/g for *Zm*PSY1 and CRTI, respectively. Across the four locations, the highest concentrations of *Zm*PSY1 and CRTI measured in samples of mature grain were *ca*. 245 ng/g and 30 ng/g, respectively. The highest concentrations of PMI in samples of mature GR2E rice grain and straw were *ca*. 1891 ng/g and *ca*. 796 ng/g fresh weight tissue, respectively.

The maximum potential human daily dietary exposures to *Zm*PSY1, CRTI, and PMI proteins from GR2E rice were estimated to be less than *ca*. 4.5, 0.85, and 30 μg/kg body weight, respectively, based on the highest concentrations of these proteins determined in dough-stage grain and a maximum rate of rice consumption of 12.5 g/kg body weight, as reported for children in Bangladesh.

A tiered "weight-of-evidence" approach was followed in assessing the safety of the *Zm*PSY1, CRTI, and PMI proteins expressed in GR2E rice.

The *Zm*PSY1 and CRTI proteins did not display significant amino acid sequence similarity with known allergens nor were there any primary sequence structural alerts for potential toxicity based on similarity searches against a database of known and putative protein toxins. Both *Zm*PSY1 and CRTI were rapidly and completely digested *in vitro* in the presence of simulated gastric fluid containing pepsin, and the enzymatic activity of both proteins was completely destroyed following treatment at temperatures well below those used during cooking.

A tier-1 assessment of potential hazards associated with the *Zm*PSY1 protein, which considered the food crop source of the gene, 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.

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.

Based on its presence in a wide range of foods derived from genetically engineered maize lines, and on the extensive history of prior regulatory reviews, additional characterization of the PMI protein was unnecessary. Previously submitted safety studies reviewed in the context of other genetically engineered plant events are directly applicable to the safety assessment of PMI protein expressed in GR2E rice.

The genetic modification resulting in GR2E rice was only intended to increase levels of provitamin A (primarily β-carotene) in the rice endosperm. To confirm the intended effect and the lack of any meaningful unintended consequences of the genetic modification, compositional parameters were compared between GR2E rice and control, unmodified, rice. Compositional analyses were performed on samples of rice grain and straw obtained from PSB Rc82 rice containing event GR2E and near-isogenic control PSB Rc82 rice that was grown in side-by-side trials at four separate sites in the Philippines during 2015 and again in 2016. The compositional assessment included analyses for proximates, fibre, and minerals in samples of straw, and analyses for proximates, minerals, vitamins, amino acids, fatty acids, vitamins, and key anti-nutrients in grain samples. Samples of processed bran derived from GR2E and control rice were also analyzed for proximates, fibre, and minerals.

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) acid, a minor fatty acid component, measured in grain samples (not including the intended difference in provitamin A levels). With the exception of β-carotene and related carotenoids, the compositional parameters measured in samples of

GR2E rice, including stearic acid, 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 unexpected, unintended consequence of the genetic modification.

The purpose of this evaluation of GR2E rice was to determine whether the use of GR2E rice in food could raise any new safety concerns relative to conventional rice, and was not intended to address questions related to the efficacy of GR2E rice in helping combat vitamin A deficiency (VAD) in affected population sub-groups.

Collectively, the data presented in this submission have not identified potential health and safety concerns, and support the conclusion that food derived from provitamin A biofortified GR2E rice is as safe and nutritious as food derived from conventional rice varieties.