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Te Mana Kounga Kai – Ahitereiria me Aotearoa

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Supporting Document 2

Nutrition Risk Assessment Report (at Approval) – Application A1138

Food derived from Provitamin A Rice Line GR2E

Executive summary

This Application requests amending the *Australia New Zealand Food Standards Code* (the Code) to include food derived from a genetically modified rice line referred to as GR2E. GR2E produces provitamin A carotenoids, predominantly beta (β)-carotene, and is intended to complement existing vitamin A deficiency control efforts mainly in Asian countries. The risk assessment includes a hazard assessment, which considers the potential adverse effects associated with β-carotene intake, and a dietary intake assessment for β-carotene which assumes that all rice, rice bran and rice bran oil in the Australian and New Zealand markets are replaced with GR2E.

Vegetables, fruits and cereals are the major food categories contributing to the dietary intake of β-carotene ranging between 1 and 5 mg/day in Australia and New Zealand. Intake of β-carotene in foods or supplements, even in large amounts, has not been associated with hypervitaminosis A. In the absence of adverse effects such as vitamin A toxicity, there is no requirement to establish an Upper Level of Intake (UL). Carotenemia, a clinically benign condition involving yellow to orange skin pigmentation, can occur after intakes of large amounts of carotene rich foods or administration of β-carotene at high doses (≥ 30 mg/day) in supplement form. Daily intake of up to 50 mg β-carotene in supplemental form for several years did not result in any adverse effects in healthy people or people with different forms of cancer, except those with or at risk of developing lung cancer. A slight, but statistically significant, increased incidence of lung cancer and mortality rate primarily due to lung cancer and ischemic heart disease was shown in heavy smokers taking 20 mg β-carotene supplements per day for 5 to 8 years. This risk was shown to decline within four to six years after discontinuing β-carotene supplementation.

The dietary intake assessment concluded that the replacement of all rice (which includes rice, rice bran and rice bran oil eaten as is or in processed foods and mixed dishes) in the Australian and New Zealand markets with GR2E may result in a 2-13% (40-336 µg per day) increase in estimated intakes of β-carotene by Australian and New Zealand population groups. The increase in β-carotene intakes is equivalent to the amount of β-carotene from approximately 1 teaspoon or less of carrot juice. The increase in intakes would be lower in reality as it is unlikely that all rice or rice products consumed by the entire population would be derived from GR2E. Additionally, the potential population increases in β-carotene are over-estimates as the assessment assumes that the population consumes the mean amount

of rice over time. Also, given the difference between the mean and high (90th- percentile) intakes, the increase would be well within normal daily variation in β-carotene intakes. Based on a comparison of the doses resulting in no adverse effects in human studies and the relatively small increase in total dietary intake of β-carotene due to consumption of GR2E rice, it is concluded that GR2E rice consumption will not pose a nutritional risk to the Australian and New Zealand population.

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1 Introduction

This Application requests amending the *Australia New Zealand Food Standards Code* (the Code) to allow for the inclusion of a rice line that has been genetically modified to produce provitamin A carotenoids. This rice line has the OECD Unique Identifier IR-00GR2E-5 (hereafter referred to as GR2E). GR2E produces provitamin A carotenoids, predominantly beta (β)-carotene, and is intended to complement existing vitamin A deficiency control efforts by supplying 30 to 50% of the estimated average requirement for vitamin A for preschool age children and pregnant or lactating mothers in high-risk countries, including Bangladesh, Indonesia, and the Philippines.

This risk assessment includes a hazard assessment, which considers the potential adverse effects associated with β-carotene intake (including information on human studies with supplements), and a dietary intake assessment for β-carotene which assumes that all rice, and rice products including rice bran and rice bran oil in the Australian and New Zealand markets are replaced with GR2E.

2 Nutrition hazard assessment

2.1 Upper Level of Intake

The Upper Level of Intake (UL) is a Nutrient Reference Value (NRV) that defines the highest average daily nutrient intake level likely to pose no adverse health effects to almost all individuals in the general population. As intake increases above the UL, the potential risk of adverse effects increases (NHMRC and MoH 2006a)[1]. In Australia and New Zealand, a number of ULs for vitamin A have been established (as retinol equivalents) for different subpopulations, ranging from 600 µg/day for infants and children aged between 1 and 3 years to 3000 µg/day for adults (NHMRC and MoH 2006b). A UL was not established for β-carotene because excess intake has not been associated with vitamin A toxicity (NHMRC and MoH 2006b).

2.2 Biochemistry and physiology of provitamin A carotenoids

A large body of human and animal research suggests that oral intake of carotenoids may be beneficial to eye, prostate, skin and cardiovascular health (Shao and Hathcock 2006). Provitamin A carotenoids are converted to retinol (a form of vitamin A) in the intestinal mucosa of humans (Harrison 2012). Vitamin A is an essential nutrient for humans because it cannot be synthesised *de novo* within the body and so it must be obtained through the diet (Bendich and Langseth 1989; Bates 1995) as pre-formed vitamin A from animal-derived foods and provitamin A carotenoids from fruits and vegetables (ABS 2014a; Olson 2000). Of the provitamin A carotenoids, β-carotene, and to a lesser extent alpha (α)-carotene and β-cryptoxanthin, are the most important to human nutrition. As further detailed in Section 3.2 of this report, vegetables, fruits and cereals are the major food categories contributing to the dietary intake of β-carotene ranging between 1 and 5 mg/day in Australia and New Zealand.

Carotenoids are known to exist in different forms (*cis*- and *trans*-isomers) which may be interconverted by light, thermal energy or chemical reactions. In human blood serum, most of the β-carotene is present as the all-*trans* isomer, even after significant intakes of the 9-*cis* isomer over long periods, whereas the liver and adrenal tissue contain more of the 9-*cis* and 13-*cis* isomers of β-carotene (Woutersen et al. 1999; Rock 1997).

[1] Nutrient Reference Values, Australia and New Zealand: https://www.nrv.gov.au/

2.3 Carotenoids in GR2E rice

Based on dry weight of milled GR2E grain, β-carotene (consisting of all-*trans*-β-carotene and 9-*cis*-β-carotene) comprises approximately 73% of the total carotenoids (based on mean values) followed by all-*trans*-α-carotene (12%) and β-cryptoxanthin (5%) (Table 12, Supporting Document 1 (SD1)). Compositional differences between paddy rice and milled rice of GR2E are also given in SD1. Dehulling the rice grain through preliminary milling gives brown rice while further milling also removes the germ and bran layers to give white rice. Since a high proportion of vitamins, minerals and dietary fibre are found in the germ and bran layers, increased milling depletes the nutritional value of the grain. With the exception of provitamin A carotenoids, the compositional parameters measured in milled samples of GR2E were similar to, or within the natural variability range of, those components in conventional rice varieties as indicated in the Application and SD1. Therefore, this section will consider the potential hazards associated with the intake of β-carotene, the major carotenoid present in GR2E.

2.4 Bioavailability and bioconversion of provitamin A carotenoids

Compared to other provitamin A carotenoids, β-carotene is considerably more abundant in fruits and vegetables (Burns et al. 2003) and its extent of bioconversion to vitamin A is greater (Institute of Medicine (U.S.) 2001; Weber and Grune 2012). Intestinal absorption of carotenoids and bioconversion into vitamin A is homeostatically regulated and dependent on the vitamin A status and the dietary intake of preformed vitamin A (Lietz et al. 2010; Lobo et al. 2010). Recent studies suggest that the intestinal absorption process of carotenoids is likely to be mediated by specific epithelial transporters yet to be identified (Harrison 2012; During et al. 2002; Stahl et al. 1995; Gaziano et al. 1995; Reboul 2013). It has been reported that between 10 and 90% of dietary β-carotene is absorbed in humans, with absorption decreasing as the intake increases (Hickenbottom et al. 2002; Ho et al. 2007; Faulks et al. 1997; van Vliet et al. 1995). Carotenoids are transported in association with lipoproteins. About 75% of the β-carotene can be bound to low density lipoproteins (LDL) while the rest bind to high density lipoproteins (HDL) and very low density lipoproteins (VLDL) in the blood serum of fasting humans (Woutersen et al. 1999; Romanchik et al. 1995; Traber et al. 1994). Liver and adipose tissue are the main sites of carotenoid deposition in humans (Parker 1988; Kaplan et al. 1990).

The bioavailability of α- and β-carotenes as well as β-cryptoxanthin varies considerably as their release from the food matrices by mashing, cooking or pureeing of food and the extent of their absorption depend on factors such as the degree of processing of the food, the levels and types of dietary fat and the presence of other carotenoids in the food (Furr and Clark 1997; Yeum and Russell 2002; van het Hof et al. 2000). Dietary fat enhances the solubilisation of released carotenoids into lipid globules and therefore the absorption of α- and β-carotenes and β-cryptoxanthin; unsaturated fatty acids improve carotenoids bioavailability while absorption is higher from monounsaturated than from polyunsaturated fatty acids (Failla et al. 2014; Goltz et al. 2012; Hu et al. 2000). Dietary fibre decreases the bioavailability of carotenoids by disrupting micelle formation or entrapping carotenoids as well as by interacting with bile acids resulting in increased faecal excretion of fats and fat-soluble substances such as carotenoids (Yeum and Russell 2002; Rock and Swendseid 1992).

2.4.1 Studies with Golden Rice

The collective name ‘Golden Rice’ has been used to describe a number of genetically modified versions of rice containing provitamin A carotenoids (up to 37 µg β-carotene per gram of rice on dry weight basis) (Paine et al. 2005) and including GR2E. In an early study (Tang et al. 2009), Golden Rice, not GR2E, servings weighing between 65 and 98 g (130 to

200 g of cooked rice) and containing 0.99 to 1.53 mg deuterium-labelled β-carotene were fed to 5 healthy adults (3 women and 2 men, average age 59±11 years, average BMI 26±2.5) with 10 g butter, 50 g peeled cucumbers, 0.2 g salt, 5 g vinegar, and 500 mL water in a daily breakfast over 36 days. A reference dose of [$^{13}C_{10}$]retinyl acetate (0.4 to 1.0 mg) in oil was given to each volunteer one week before ingestion of the first Golden Rice meal. All of the subjects consumed standardised isoenergetic lunch meals, not containing Golden Rice or any of the labelled β-carotene or retinol, 4 hours after the breakfast. No information was given on other meals consumed by the study subjects. Blood samples were collected over the 36 days to study retinol levels in the blood. Golden Rice containing 1.53 mg β-carotene provided 0.24 to 0.94 mg retinol. The conversion was measured by calculating the area under the curve (AUC) of serum deuterium-labelled retinol formed from deuterium-labelled β-carotene present in the consumed Golden Rice with the AUC of [$^{13}C_{10}$]retinyl acetate taken as a reference. The bioconversion ratio (oral dose of β-carotene compared with the amount of vitamin A) of Golden Rice's β-carotene to retinol was calculated as 3.8±1.7 to 1 by weight, or 2.0±0.9 to 1 by moles. No adverse effects such as allergic reactions or gastrointestinal disturbance were observed or reported in the study subjects following the consumption of Golden Rice.

Compared with the bioconversion ratios of provitamin A carotenoids from fruits, green leafy vegetables, *Spirulina* and provitamin A-enriched cassava into retinol, reported as between 7.5 and 28 to 1 by weight, β-carotene from Golden Rice has a favourable bioconversion ratio (de Pee et al. 1998; Tang et al. 1999; Haskell et al. 2004; Tang et al. 2005; Khan et al. 2007; Wang et al. 2008; La Frano et al. 2013).

2.5 Adverse effects of provitamin A carotenoids

There are sufficient data from human RCTs for the hazard assessment of β-carotene, therefore studies performed in vitro and in laboratory animals were not considered as a part of this assessment. A hazard assessment of carotenoids other than β-carotene was not conducted because (i) other carotenoids comprise less than 30% of the total carotenoids in milled GR2E rice grain, (ii) the bioconversion of other carotenoids present in GR2E to vitamin A is less efficient compared with that of β-carotene and therefore pose low risk of hypervitaminosis A, and (iii) a dietary intake assessment for other carotenoids was not possible because of insufficient food composition data.

2.5.1 Randomised controlled trials of β-carotene supplementation

Several systematic reviews and meta-analyses of RCTs have evaluated the effects of β-carotene supplementation on a range of health endpoints. A meta-analysis of 8 RCTs included subjects aged from 40 to 84 years who were smokers, exposed to asbestos, at risk of developing cardiovascular disease (CVD), at risk of developing skin cancer, at risk of developing cataract or vision loss, as well as healthy subjects to assess the effect of vitamin E, β-carotene, or both, provided as supplements, on all-cause mortality and cardiovascular death (Vivekananthan et al. 2003). The daily intake of β-carotene ranged from 20 to 50 mg with intervention periods ranging from 1.4 to 12 years. Without observing a significant heterogeneity for any analysis, the meta-analysis concluded that β-carotene supplementation slightly but significantly increased the odds ratio (OR) of all-cause mortality (OR 1.07 [95% CI: 1.02, 1.11], p = 0.003, n = 138,113) and cardiovascular death (OR 1.1 [95% CI: 1.03, 1.17], p = 0.003, n = 131, 551). The pooled estimates in these meta-analyses were, however, not exclusively drawn from RCTs in which β-carotene was the only intervention and therefore the findings cannot be directly attributed to β-carotene.

Bjelakovic et al. (2012) updated their earlier systematic review of supplementation with antioxidants and mortality (Bjelakovic et al. 2008). Twenty-six RCTs with low risk of bias were analysed that included 173,006 subjects who were healthy or in a stable phase of

various diseases such as gastrointestinal, cardiovascular, neurological, ocular, dermatological, rheumatoid, renal or endocrine diseases. These subjects received 1.2 to 50 mg (mean dose 15.5 mg) of β-carotene either used singly or in combination with other antioxidants as supplements daily or on alternative days for an average of 3 years. No new studies providing only β-carotene were added to the meta-analysis in the updated systematic review. In the trials with low risk of bias, there was a small but statistically significant increase in mortality in the meta-analysis of β-carotene used singly or in combination with other antioxidants (risk ratio (RR) 1.05 [95% CI: 1.01, 1.09], p = 0.019). It is however not possible to separate any effect of β-carotene from that of the other antioxidants used in the interventions.

In 2013, Bjelakovic et al. published a meta-analysis of antioxidants and all-cause mortality in subjects who were healthy or in a stable phase of various diseases which was based on their previous systematic review and meta-analysis (Bjelakovic et al. 2012). It was concluded that β-carotene, considered to be administered singly in 7 of the included studies with 43,019 subjects, at doses ranging between 25 and 50 mg/day significantly increased all-cause mortality (RR 1.06 [95% CI: 1.02, 1.10]) when compared with placebo. The authors have not identified these 7 studies which seem to have included RCTs using β-carotene in combination with either vitamin C and/or selenium; both were disregarded by the authors as concomitant interventions. Due to the concerns around the interventions in the studies included in the meta-analysis, the reported effect estimate of the intervention in the 7 studies on all-cause mortality cannot be attributed to β-carotene alone.

Earlier publications have inversely correlated dietary β-carotene intake and blood levels of retinol with the risk of different types of cancer in humans (Peto et al. 1981). This finding resulted in studies investigating whether oral supplementation with β-carotene can reduce the incidence of different types of cancer. One of these RCTs was designed to test whether β-carotene reduced the risk of new cancers in 1805 subjects (70:30 males to females, average age 65 years) who were diagnosed with a non-melanoma skin cancer (Greenberg et al. 1990). Participants in the intervention group (n = 913) received capsules containing 50 mg β-carotene per day. The intervention lasted for five years and the follow-up period lasted for another five years. There was no difference between the intervention and the placebo groups in the incidence of the first new non-melanoma skin cancer with relative rate of 1.05 [95% CI: 0.91, 1.22] and no adverse health effects attributable to β-carotene supplementation were reported.

A community-based, placebo-controlled randomised trial that lasted for 4.5 years and enrolled 1621 residents of southeast Queensland aged between 20 and 69 years investigated the ability of β-carotene to prevent skin cancers as compared with sunscreen (Green et al. 1999). The study concluded that daily supplementation of 30 mg β-carotene did not affect the rate ratios of either basal-cell and squamous-cell types of skin cancer. Following up on the Green et al. (1999) study, Hughes et al. (2013) conducted a randomised, placebo-controlled trial on the subjects younger than 55 years of age (n = 903) in the 1621 subjects studied earlier. The study investigated whether the regular use of sunscreen compared with discretionary use or β-carotene supplements can retard skin aging. After 4.5 years of β-carotene supplementation at 30 mg/day, no overall effect on skin aging has been observed in the studied population. There was no difference in increases in microtopography grades among persons allocated to β-carotene or placebo (p = 0.6) and odds were consistent across photo-aging levels (p = 0.51). Therefore, the study did not identify an effect of β-carotene supplementation on skin aging.

A double-blinded, placebo-controlled RCT enrolled 29,133 male smokers aged 50 to 69 years, out of which 7,282 subjects received 20 mg/day β-carotene supplementation for a duration ranging between 5 and 8 years with a median of 6.1 years (ATBC Study Group 1994). Other groups in the trial received α-tocopherol alone or in combination with β-

carotene. At the end of the intervention phase, a higher incidence of lung cancer was observed among the men who received β-carotene than among those who did not (change in incidence 18% [95% CI: 3, 36%], p = 0.01). β-Carotene intake had no effect on the incidence of other cancers. Total mortality was 8% higher ([95% CI: 1, 16%], p = 0.02) among those men who received β-carotene than among those who did not, primarily due to more deaths from lung cancer and ischemic heart disease. Post-intervention follow-up for cause-specific deaths and all-cause mortality lasted for 6 and 8 years, respectively, and showed no significant difference in the risk ratio for lung cancer among β-carotene recipients compared with non-recipients (RR 1.06 [95% CI: 0.94, 1.20]) (ATBC Study Group 2003). No statistically significant overall difference in lung cancer incidence was observed between β-carotene recipients and non-recipients during the post-intervention follow-up period (RR 1.03 [95% CI: 0.91 to 1.20]). In addition, there were no delayed preventive effects on other cancers. Relative risk of death for β-carotene recipients compared with non-recipients was 1.07 [95% CI: 1.02, 1.12]. The higher mortality rate of β-carotene recipients observed compared with non-recipients at the end of the intervention period returned toward null within four to six years of follow-up after stopping the supplementation (ATBC Study Group 2003).

Two long-running RCTs investigated the potential effect of β-carotene supplementation in combination with antioxidants and/or with retinol on age-related macular degeneration and visual acuity (AREDS) (Age-Related Eye Disease Study Research Group 2001) or on lung cancer and cardiovascular disease (CARET) (Omenn et al. 1996b; Omenn et al. 1996a; Goodman et al. 1993). No statistically significant adverse health effect was associated with any of the formulations in the AREDS study but an increase in the incidence of lung cancer, the risk of death from lung cancer, cardiovascular disease from any cause in smokers and workers exposed to asbestos was observed in the CARET study. However, although these studies were done in thousands of subjects taking doses comparable to that used in the ATBC study, the findings from these studies of combined intervention cannot be ascribed to β-carotene alone.

Following the observations made in the ATBC and the CARET studies, the Physicians' Health Study (PHS); another large randomised, double-blind, placebo-controlled trial was conducted and enrolled 22,071 healthy male physicians with no history of cancer, myocardial infarction, stroke, or transient cerebral ischemia (Hennekens et al. 1996). The study tested the effect of aspirin with or without β-carotene supplementation on the prevention of cardiovascular disease and cancer. Subjects were treated and followed for an average of 12 years: 5 years combined with aspirin followed by 7 years without concomitant intervention. Half of the participants were never-smokers at enrolment, 39% were former smokers and 11% were current smokers. The aspirin component of the study was terminated five years after initiating the study due to a clear preventive effect of aspirin on myocardial infarction. Meanwhile, the β-carotene component continued uninterrupted for 12 years. The β-carotene intervention group (n = 11,036) received 50 mg β-carotene on alternate days, while the rest (n = 11,035) were in the placebo group. The study found that β-carotene had no effect on the risk of any malignant neoplasm (RR 0.98 [95% CI: 0.91, 1.06], p = 0.65). No significant effect for β-carotene, compared with placebo, was found for cases of lung cancer (82 vs. 88), deaths from cancer (386 vs. 380), all-cause death (979 vs. 968), cardiovascular disease (338 vs. 313), myocardial infarction (468 vs. 489), stroke (367 vs. 382) or the number of subjects with any one of the previous three endpoints (967 vs. 972). Statistical analyses restricted to former or current smokers showed that β-carotene did not result in significant early or late differences in any of these endpoints within these groups. The authors concluded that 12 years of β-carotene supplementation (5 years combined with aspirin followed by 7 years without concomitant intervention) at 50 mg on alternate days produced neither benefit nor harm in terms of the incidence of malignant neoplasms, cardiovascular disease, or death from all causes.

In a systematic review of treatment options for dermal photosensitivity, Minder et al. (2009)

retrieved 16 reports including 337 subjects in 12 retrospective case reports, 3 uncontrolled trials and 1 RCT that used β-carotene capsules as a treatment. The included studies ranged in duration from 4 months to 6 years, mainly through the summer periods, with oral doses ranging from 25 to 200 mg/day. Adverse health effects from the intake of β-carotene were not explicitly reported in the review. However, in the RCT included in the systematic review, no adverse effects were observed in the subjects. In this RCT, 11 subjects with dermal photosensitivity but otherwise healthy, aged between 19 and 35 years, were given 100 mg/day of β-carotene supplement for 4 months without adverse health effects (Corbett et al. 1977).

Oral doses ranging from 15 to 200 mg/day β-carotene were given for consecutive months, mainly during the summer period over a range of years varying for each enrolled subject, to adults and children with skin photosensitivity diseases such as erythropoietic protoporphyria and polymorphous light eruption (Mathews-Roth 1974; Thomsen et al. 1979). One of these observational studies included 53 subjects (1 to 53 years of age) receiving β-carotene supplements for several months, mainly during summer, each year over a range of 3 to 47 years (Mathews-Roth 1974) while another study included 36 subjects (4 to 68 years of age) mainly during the summer months over 5 years (Thomsen et al. 1979). It was reported that the subjects in these trials did not have abnormally elevated blood vitamin A concentrations, did not develop leukopenia or have any serious side effects from the doses of β-carotene supplements.

Carotenoids have antioxidant properties as these compounds, mainly β-carotene, are able to directly or indirectly quench singlet oxygen ($^1O_2$) without degradation and react with free radicals, such as peroxyl, hydroxyl and superoxide radicals (Kasperczyk et al. 2014; Li et al. 2010; Stahl 2011). Evans and Lawrenson (2012) prepared a systematic review of the evidence for the role of antioxidant vitamin and mineral supplements in slowing the progression of age-related macular degeneration (ARMD). In this systematic review, experimental arms of pure β-carotene supplementation were only used within two high-quality RCTs described above: the ATBC study which provided 20 mg β-carotene per day for 6.1 years to male smokers (ATBC Study Group 1994) and the PHS study which provided 50 mg every second day for 12 years to 11,036 males (11% are smokers, 39% former smokers) (Hennekens et al. 1996). None of these RCTs reported adverse effects on eye health or increased the risk of ARMD following the intake of β-carotene at 20 mg/day or 50 mg every second day in healthy non-smoking males or in male smokers. Kasperczyk et al. (2014) conducted a study to determine whether β-carotene administration reduces oxidative stress and influences antioxidant defence systems in workers chronically exposed to lead. The study comprised two randomly divided groups of 85 healthy males occupationally exposed to lead. Workers in the reference group (n = 50) did not receive any antioxidants, while workers in the CAR group (n = 35) received 10 mg/day of β-carotene as a supplement for 12 weeks. The authors reported significant reduction in oxidative stress parameters and elevated glutathione levels associated with β-carotene supplementation without observing any clinically significant side effects.

β-carotene levels in the blood of ≥ 2 mg/L have been associated with subcutaneous deposition of β-carotene mainly in the stratum corneum, in sweat and in sebum, causing yellow to orange pigmentation of the skin; a clinically benign condition known as carotenemia, also referred to as hypercarotenemia (Maharshak et al. 2003; Lascari 1981; Mathews-Roth 1990). Carotenemia has been reported to develop within 3 weeks of increasing the intake of carotenoids through food or supplements at 30 mg/day but was not observed at an intake at or below 12 mg/day. The skin colouring symptoms disappear gradually in 2 weeks after reducing the intake and this condition has not been associated with any adverse effects apart from harmless skin pigmentation (Diplock 1995; Micozzi et al. 1988). In contrast in the larger β-carotene intervention studies that lasted for several years, signs of carotenemia were not reported in all of the subjects. Skin yellowing was found in

approximately 15% of subjects receiving 50 mg of β-carotene on alternate days in the PHS study (Hennekens et al. 1996) and in 60% of subjects receiving 30 mg/day β-carotene alone or combined with retinol in the CARET study (Goodman et al. 1993). Carotenemia has also been reported in studies involving subjects with anorexia nervosa, diabetes mellitus, hypothyroidism or hyperlipidaemia and is thought to be associated with high β-carotene in the diet or an impaired conversion of β-carotene into vitamin A in the liver (Pops 1968; Hoerer et al. 1975; Maharshak et al. 2003). Clinical variation will lead to heterogeneity in the results if the intervention effect is influenced by the factors that vary across studies such as patient characteristics (Higgins and Green 2011). There is however an agreement that carotenemia is harmless and causes no symptoms other than pigmentation, nor is there normally any evidence of hypervitaminosis A (FAO/WHO 1974; Maharshak et al. 2003).

2.6 Conclusions of the nutrition hazard assessment

Vegetables, fruits and cereals are the major food categories contributing to the dietary intake of β-carotene ranging between 1 and 5 mg/day in Australia and New Zealand. Intake of β-carotene in foods or supplements, even in large amounts, has not been associated with hypervitaminosis A. In the absence of adverse effects such as vitamin A toxicity, there is no requirement to establish a UL. Carotenemia, a clinically benign condition involving yellow to orange skin pigmentation, can occur after intakes of large amounts of carotene rich foods or administration of β-carotene at high doses (≥ 30 mg/day) in supplement form. Daily intake of up to 50 mg β-carotene in supplemental form for several years did not result in any adverse effects in healthy people or people with different forms of cancer, except those with or at risk of developing lung cancer. A slight, but statistically significant, increased incidence of lung cancer and mortality rate, primarily due to lung cancer and ischemic heart disease, was shown in heavy smokers taking 20 mg β-carotene supplements per day for 5 to 8 years. This risk was shown to decline within four to six years after discontinuing β-carotene supplementation.

3 Dietary intake assessment

3.1 Approach to estimating dietary intakes of β-carotene

Baseline intakes of β-carotene for Australians aged 2 years and above were derived from the 2011-12 Australian National Nutrition and Physical Activity Survey (2011-12 NNPAS) using FSANZ's computer program (Harvest) and were adjusted to better predict longer term nutrient intakes using the 2nd day adjustment method. The 2nd day adjustment method is described in *Principles and Practices of Dietary Exposure Assessment for Food Regulatory Purposes* (FSANZ 2009). Usual intakes of β-carotene were not published by the Australian Bureau of Statistics (ABS) from the 2011-12 NNPAS.

Baseline β-carotene intakes for New Zealanders were taken from the published reports on the 2002 NZ CNS and 2008-9 NZ ANS. Both of these surveys published usual intakes that were derived using the PC-Side method (Ministry of Health 2003; University of Otago and Ministry of Health 2011). The impact of allowing GR2E in the Australian and New Zealand marketplaces was estimated by assessing the potential increase in β-carotene intakes assuming the mean consumer amount of rice (rice, rice bran and rice bran oil), is consumed as provitamin A rice (otherwise known as GR2E).

The data used in this assessment include:

- β-carotene concentrations in foods from the food composition datasets from national Australian and New Zealand nutrition surveys and from the Application;
- Food consumption data from the available Australian and New Zealand national nutrition surveys; and
- current (Baseline) β-carotene intakes from the available New Zealand national nutrition surveys.

The extraction of Australian Baseline β-carotene intakes and the consumption of rice by Australians and New Zealanders was undertaken using FSANZ's custom dietary modelling computer program, Harvest[2].

The general FSANZ methodology and approach to conducting dietary intake assessments is set out in *Principles and Practices of Dietary Exposure Assessment for Food Regulatory Purposes* (FSANZ 2009).

3.1.1 Food consumption data used

The food consumption data used for the dietary intake assessments were:

- 2002 New Zealand National Children's Nutrition Survey (2002 NZ CNS): a 24-hour recall survey of 3,275 New Zealand children aged 5-14 years, with a second 24-hour recall undertaken for 15% of respondents. Baseline β-carotene intakes in this assessment are those that were published with the 2002 NZ CNS (Ministry of Health 2003). They are adjusted intakes derived from the distribution of usual intakes of β-carotene using the PC-SIDE computer software.
- 2008–09 New Zealand Adult Nutrition Survey (2008–09 NZ ANS): a 24-hour recall survey of 4,721 New Zealanders aged 15 years and above, with a second 24-hour

[2] Harvest is FSANZ's custom-built dietary modelling program that replaced the previous program, DIAMOND, which does the same calculations just using a different software program.

recall undertaken for 25% of respondents. Baseline β-carotene intakes in this assessment are those that were published with the 2008-9 NZ ANS (University of Otago and Ministry of Health 2011). They are adjusted intakes derived from the distribution of usual intakes of β-carotene using the PC-SIDE computer software.

- 2011-12 Australian National Nutrition and Physical Activity Survey (2011-12 NNPAS), a component of the 2011-13 Australian Health Survey (2011-13 AHS): a 24-hour recall survey of 12,153 Australians aged 2 years and above, with a second 24-hour recall undertaken for 64% of respondents (ABS 2014b) . Baseline β-carotene intakes are adjusted intakes, calculated using the 2nd day adjustment method in FSANZ's computer program Harvest.

Dietary exposure assessments based on national nutrition survey (NNS) food consumption data provide the best estimation of actual consumption of a food and the resulting estimated dietary intake assessment for the Australian and New Zealand populations. However, it should be noted that NNS data do have limitations. The design of these nutrition surveys vary and the key attributes of each, including survey limitations, are set out in Appendix 1.

The mean consumer consumption of rice was derived by assuming that all brown and milled rice, rice products including bran and rice bran oil in the Australian and New Zealand food supplies is derived from GR2E (based on Day 1 consumption of rice from each of the national nutrition surveys). This included rice, rice bran and rice oil that were reported as consumed in their own right in the national nutrition surveys, or when used in processed foods (e.g. rice flour, puffed rice products, rice crackers) or where rice and rice flour is used in mixed dishes (e.g. fried rice, rice pudding, rice-based beverages, cakes and biscuits) based on FSANZ's recipe database in Harvest. Rice consumption was expressed in raw rice equivalents.

3.1.2 Population groups assessed

The hazard assessment did not identify any population age-sex based sub-groups for which there were specific concerns in relation to β-carotene. The population groups used for the dietary intake assessment are based on the Nutrient Reference Value (NRV) age groups and are outlined in Table 2.

3.1.3 Proposed foods and concentration data used

GR2E expresses elevated levels of provitamin A, mainly as β-carotene, in comparison to the standard rice (i.e. without the genetic modification). The rounded total β-carotene concentration (sum of all-*trans*-β-carotene and 9'-*cis*-β-carotene) in GR2E is 4.3 µg/g milled rice (dry weight). This concentration was converted to a raw rice basis by using a moisture content of 12.26% (as provided in the application). The final concentrations of β-carotene in both the standard rice and GR2E used for the dietary intake assessment are listed in Table 1. For the purposes of calculating the increase in β-carotene dietary intakes, it was assumed that all standard rice does not contain β-carotene pre-application, as reported in national food composition tables.

Table 1: β-carotene concentrations in Standard rice and GR2E

Food	Cooked or raw?	Country	β-carotene concentration (µg/kg rice)
Standard white rice	Raw	Australia[1]	0
	Boiled	Australia[1]	0
		New Zealand[2]	0
GR2E[3]	Raw		3,801

Sources:
[1] AUSNUT 2011-13 (FSANZ 2016)
[2] (New Zealand Institute for Plant & Food Research Limited and Ministry of Health 2015)
[3] A1138 application document (converted from dry weight to raw weight rice, moisture content = 12.26%)

3.1.4 Assumptions and limitations of the dietary intake assessment

The aim of the dietary intake assessment was to make the most realistic estimation of dietary β-carotene intake possible. However, where significant uncertainties in the data existed, conservative assumptions were generally used to ensure that the estimated dietary intake was not an underestimate of intake (for example, assuming that the population consumes the mean consumer amount of rice over time over-estimates potential population increases in β-carotene).

Assumptions made in the dietary intake assessment included:

- The current (Baseline) concentrations of β-carotene are as per the published nutrient composition data that accompany each national nutrition survey.
- The β-carotene nutrient intakes that were published or estimated for each national nutrition survey reflect the current intakes.
- GR2E replaces 100% of the rice and rice products including rice bran and rice bran oil consumed in Australia and New Zealand.
- There is no contribution to β-carotene intake through the use of complementary or other medicines (e.g. dietary supplements).

In addition to the specific assumptions made in relation to this dietary intake assessment, there are a number of limitations associated with the nutrition surveys on which this assessment is based. A discussion of these limitations is included in Section 6 of the *Principles and Practices of Dietary Exposure Assessment for Food Regulatory Purposes* (FSANZ 2009).

Table 2: Population groups used in the dietary intake assessment for β-carotene*

Country	Age group	Sex
Australia	2-3 years	Males
		Females
	4-8 years	Males
		Females
	9-13 years	Males
		Females
	14-18 years	Males
		Females
	19-30 years	Males
		Females
	31-50 years	Males
		Females
	51-70 years	Males
		Females
	71 years and above	Males
		Females
New Zealand	5-6 years	Males
		Females
	7-10 years	Males
		Females
	11-14 years	Males
		Females
	15-18 years	Males
		Females
	19-30 years	Males
		Females
	31-50 years	Males
		Females
	51-70 years	Males
		Females
	71 years and above	Males
		Females

* Age groups assessed may not exactly match the NRV age groupings due to the ages included in the different nutrition surveys.

3.2 Estimated population dietary intakes of β-carotene

3.2.1 Estimated β-carotene dietary intakes for Australians and New Zealanders

3.2.1.1 Australia

Baseline mean and P90 intakes of β-carotene for Australians aged 2–18 years were 1,000–1,449 µg/day and 1,877–3,095 µg/day, respectively. For Australians aged 19 years and above, Baseline mean and P90 intakes were 1,526–1,987 µg/day and 2,513–3,466 µg/day, respectively.

It is estimated that, with the mean consumption of GR2E over time, mean and P90 β-carotene intakes for Australian children aged 2–18 years may increase by 67–131 µg/day. For Australian adults aged 19 years and above, this increase is expected to be 40–183 µg/day. This increase is equivalent to the amount of β-carotene from less than ⅔ teaspoon of carrot juice[3]. Further details can be found in Table 3 below.

3.2.1.2 New Zealand

Baseline mean and P90 intakes of β-carotene for New Zealand children aged 5–14 years were 1,439–2,064 µg/day and 2,105–3,821 µg/day, respectively. For New Zealanders aged 15 years and above, Baseline mean and P90 intakes were 2,037–3,208 µg/day and 3,269–5,189 µg/day, respectively.

It is estimated that, with the mean consumption of GR2E over time, mean and P90 β-carotene intakes for New Zealand children aged 5–14 years may increase by 93–169 µg/day. The increase in β-carotene intakes is expected to be 106–336 µg/day for New Zealanders aged 15 years and above. These increases are equivalent to the amount of β-carotene from approximately ⅓–1 teaspoon of carrot juice. Further details can be found in Table 3 below.

3.2.2 Major food categories contributing to β-carotene dietary intakes

For Australians aged 2 years and above the major contributors to baseline β-carotene intakes were vegetable products and dishes (45%), cereal based products and dishes (13%), meat, poultry and game products and dishes (10%), fruit products and dishes (9%), soup (7%) and milk products and dishes (5%).

The major contributing food groups (≥5%) to Baseline β-carotene dietary intakes for New Zealand children aged 5-14 years (Ministry of Health 2003) and New Zealanders aged 15 years and above (University of Otago and Ministry of Health 2011) were vegetables (64% and 44% respectively), fruits (8% and 9% respectively), bread-based dishes (6% – for 15 years and above only) and grains and pasta (6% – for 15 years and above only).

3.3 Dietary exposure assessment summary

The replacement of all rice and rice products including rice bran and rice bran oil in the Australian and New Zealand markets with GR2E may result in a 2–13% (40–336 µg per day) increase in estimated intakes of β-carotene by Australian and New Zealand population groups. The increase in intakes would be lower in reality as it is unlikely that all rice or rice products consumed by the entire population would be derived from GR2E. Additionally, potential population increases in β-carotene will be over-estimated if it is assumed the

[3] 1 teaspoon of carrot juice contains approximately 318 µg β-carotene (FSANZ 2016)

population consumes the mean consumer amount of rice over time. The increase in β-carotene intakes is equivalent to the amount of β-carotene from approximately 1 teaspoon of carrot juice or less. In addition, given the difference between the mean and P90 intakes, the increase would be well within normal natural daily variation in β-carotene intakes.

Table 3: Estimated dietary intakes of β-carotene for Australian and New Zealand population groups

Country	Age Group	Sex	Estimated Baseline dietary intake of β-carotene (µg/day)		Mean consumer rice consumption$^{\Sigma}$	Estimated increase in dietary intake of β-carotene from GR2E consumption		
			Mean	P90	(g/day)	(µg/day)	% increase from Baseline	
							Mean	P90
Australia	2-3 years*	Males	1,121	2,469	21	80	7	3
		Females	1,000	1,877	18	67	7	4
	4-8 years*	Males	1,272	2,208	23	89	7	4
		Females	1,151	2,158	23	86	7	4
	9-13 years*	Males	1,328	2,471	26	101	8	4
		Females	1,479	2,705	27	103	7	4
	14-18 years*	Males	1,403	3,095	34	131	9	4
		Females	1,235	2,235	26	99	8	4
	19-30 years*	Males	1,526	2,513	48	183	12	7
		Females	1,664	2,962	34	128	8	4
	31-50 years*	Males	1,690	2,954	34	127	8	4
		Females	1,764	2,887	29	109	6	4
	51-70 years*	Males	1,688	2,759	34	129	8	5
		Females	1,987	3,466	21	81	4	2
	71 years and above*	Males	1,802	2,836	13	48	3	2
		Females	1,745	3,118	11	40	2	1

Country	Age Group	Sex	Estimated Baseline dietary intake of β-carotene (µg/day)		Mean consumer rice consumption[Σ]	Estimated increase in dietary intake of β-carotene from GR2E consumption		
			Mean	P90	(g/day)	(µg/day)	% increase from Baseline	
							Mean	P90
New Zealand	5-6 years[ɣ]	Males	1,541	2,920	30	113	7	4
		Females	1,439	2,105	24	93	6	4
	7-10 years[ɣ]	Males	1,968	3,829	33	125	6	3
		Females	1,726	3,396	30	115	7	3
	11-14 years[ɣ]	Males	2,064	3,581	44	169	8	5
		Females	1,909	2,831	25	93	5	3
	15-18 years[ʊ]	Males	2,037	3,523	63	238	12	7
		Females	2,057	3,433	38	143	7	4
	19-30 years[ʊ]	Males	2,664	5,011	88	336	13	7
		Females	2,082	3,269	58	222	11	7
	31-50 years[ʊ]	Males	3,118	5,033	61	233	7	5
		Females	2,789	4,105	54	206	7	5
	51-70 years[ʊ]	Males	2,754	5,126	57	217	8	4
		Females	3,179	5,189	40	152	5	3
	71 years and above[ʊ]	Males	3,208	4,910	37	142	4	3
		Females	2,947	4,445	28	106	4	2

Σ Rice consumption is derived from Day 1 of the national nutrition survey data and is for consumers only

* 2011-12 NNPAS – baseline β-carotene intakes are adjusted intakes, calculated using the 2nd day adjustment method in Harvest

ɣ 2002 NZ CNS (http://www.health.govt.nz/system/files/documents/publications/nzfoodnzchildren.pdf)

ʊ 2008-9 NZ ANS (http://www.health.govt.nz/system/files/documents/publications/a-focus-on-nutrition-ch4_0.pdf)

4 Risk characterisation and conclusion

This risk assessment considers the potential adverse effects associated with β-carotene intake (including information on human studies with supplements), and a dietary intake assessment for β-carotene which assumes that all rice, and rice products including rice bran and rice bran oil in the Australian and New Zealand markets are replaced with GR2E.

Vegetables, fruits and cereals are the major food categories contributing to the dietary intake of β-carotene ranging between 1 and 5 mg/day in Australia and New Zealand. Intake of β-carotene in foods or supplements, even in large amounts, has not been associated with hypervitaminosis A. In the absence of adverse effects such as vitamin A toxicity, there is no requirement to establish a UL. Carotenemia, a clinically benign condition involving yellow to orange skin pigmentation, can occur after intakes of large amounts of carotene rich foods or administration of β-carotene at high doses (≥ 30 mg/day) in supplement form. Daily intake of up to 50 mg β-carotene in supplemental form for several years did not result in any adverse effects in healthy people or people with different forms of cancer, except those with or at risk of developing lung cancer. A slight, but statistically significant, increased incidence of cancer and mortality rate primarily due to lung cancer and ischemic heart disease was shown in heavy smokers taking 20 mg β-carotene supplements per day for 5 to 8 years. This risk was shown to decline within four to six years after discontinuing β-carotene supplementation.

Assuming all rice and rice products are completely replaced with GR2E in Australia and New Zealand, although replacement in reality would be less than 100%, the estimated intakes of β-carotene by Australian and New Zealand population may result in a 2–13% (40–336 µg per day) increase in estimated intakes. The increase in β-carotene intakes is equivalent to the amount of β-carotene from approximately 1 teaspoon of carrot juice or less. The highest estimated 90th-percentile of β-carotene intake, including the contribution from GR2E consumption, is approximately 3.5 and 5.3 mg/day in the Australian and New Zealand populations, respectively. Based on a comparison of the doses resulting in no adverse effects in human studies and the relatively small increase in total dietary intake of β-carotene due to consumption of GR2E rice, it is concluded that GR2E rice consumption will not pose a nutritional concern to the Australian and New Zealand population.

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Appendix 1: Dietary Intake Assessments at FSANZ

A dietary intake or exposure assessment is the process of estimating how much of a nutrient or food chemical a population, or population sub group, consumes. Dietary intake of nutrients is estimated by combining food consumption data with food composition data. The process of doing this is called 'dietary modelling'.

Dietary intake = food chemical concentration x food consumption

FSANZ's approach to dietary modelling is based on internationally accepted procedures for estimating intake of nutrients (FSANZ 2009). Different dietary modelling approaches may be used depending on the assessment, the type of food chemical, the data available and the risk assessment questions to be answered. In the majority of assessments FSANZ uses the food consumption data from each person in the national nutrition surveys to estimate their individual dietary intake. Population summary statistics such as the mean intake or a high percentile intake are derived from the ranked individual person's intakes from the nutrition survey. In some cases, FSANZ will use the usual mean and high percentile intakes of nutrients that are published with the nutrition survey.

An overview of how dietary intake assessments are conducted and their place in the FSANZ Risk Analysis Process is provided on the FSANZ website at http://www.foodstandards.gov.au/science/riskanalysis/Pages/default.aspx.

FSANZ has developed a custom-built computer program 'Harvest' to calculate nutrient intakes and dietary exposures. Harvest is a newly built program and replaces the program 'DIAMOND' that had been used by FSANZ for many years. Harvest has been designed to replicate the calculations that occurred within DIAMOND using a different software package. Harvest was used for this assessment to extract the β-carotene intakes for Australians and to extract the consumption of rice for Australians and New Zealanders.

Further detailed information on conducting dietary intake assessments at FSANZ is provided in *Principles and Practices of Dietary Exposure Assessment for Food Regulatory Purposes* (FSANZ 2009), available at http://www.foodstandards.gov.au/science/exposure/documents/Principles%20_%20practices%20exposure%20assessment%202009.pdf.

A1.1 Food consumption data used

The most recent food consumption data available were used to estimate rice consumption for Australians and New Zealanders. The national nutrition survey (NNS) data used for these assessments were:

- The 2011-12 Australian National Nutrition and Physical Activity Survey (2011-12 NNPAS)
- The 2002 New Zealand National Children's Nutrition Survey (2002 NZ NCNS)
- The 2008/09 New Zealand Adult Nutrition Survey (2008-9 NZ ANS).

The design of each of these surveys varies somewhat and key attributes of each are set out below. Further information on the National Nutrition Surveys used to conduct dietary intake assessments is available on the FSANZ website at: http://www.foodstandards.gov.au/science/exposure/Pages/dietaryexposureandin4438.aspx

A1.1.1 2011–12 Australian National Nutrition and Physical Activity Survey (2011–12 NNPAS)

The 2011–12 Australian National Nutrition and Physical Activity Survey (NNPAS) undertaken by the Australian Bureau of Statistics is the most recent food consumption data for Australia. This survey includes dietary patterns of a sample of 12,153 Australians aged 2 years and above. The survey used a 24-hour recall method for all respondents, with 64% of respondents also completing a second 24-hour recall on a second, non-consecutive day. The data were collected from May 2011 to June 2012 (with no enumeration between August and September 2011 due to the Census). Day 1 24-hour recall data for consumers were used to derive the consumer consumption amounts for rice. These data were weighted for use in the calculation. Consumption and respondent data from the survey were incorporated into the Harvest program from the Confidentialised Unit Record Files (CURF) data set (ABS 2014b).

The NCI method of usual nutrient intake assessment was not used for this assessment as this method is used when comparing nutrient intakes against Nutrient Reference Values (NRVs) and there are none for β-carotene. For this application, the 2nd day adjustment method was used to estimate longer term nutrient intakes instead as this was more appropriate for the assessing β-carotene intakes for this application. Further detailed information on is provided in *Principles and Practices of Dietary Exposure Assessment for Food Regulatory Purposes* (FSANZ 2009), available at http://www.foodstandards.gov.au/science/exposure/documents/Principles%20_%20practices%20exposure%20assessment%202009.pdf.

A1.1.2 2002 New Zealand National Children's Nutrition Survey (2002 NZ NCNS)

The 2002 NZ NCNS was a cross-sectional and nationally representative survey of 3,275 New Zealand children aged 5-14 years. The data were collected during the school year from February to December 2002. The survey used a 24-hour food recall and provided information on food and nutrient intakes, eating patterns, frequently eaten foods, physical activity patterns, dental health, anthropometric measures and nutrition-related clinical measures. It was also the first children's nutrition survey in New Zealand to include a second day diet recall data for about 15% of the respondents, and dietary intake from both foods (including beverages) and dietary supplements. For the consumption of rice, only the Day 1 24-hour recall food consumption data for all consumers (excluding supplements) were used for this assessment. These data were used weighted in Harvest.

A1.1.3 2008-9 New Zealand Adult Nutrition Survey (2008-9 NZ ANS)

The 2008-9 NZ ANS provides comprehensive information on the dietary patterns of a sample of 4,721 respondents aged 15 years and above. The survey was conducted on a stratified sample over a 12 month period from October 2008 – October 2009. The survey used a 24-hour recall methodology with 25% of respondents also completing a second 24-hour recall. The information collected in the 2008 NZANS included food and nutrient intakes, dietary supplement use, socio-demographics, nutrition related health, and anthropometric measures. For the consumption of rice, only the Day 1 24-hour recall data for all consumers were used for this assessment. These data were used weighted in Harvest.