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Testbiotech comment on data for risk assessment of Provitamin A Biofortified Rice Event GR2E submitted to Food Standards Australia New Zealand by IRRI

Preliminary version

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Summary

In 2016, the International Rice Research Institute (IRRI) filed an application at the Food Standards Australia New Zealand (FSANZ) for the market approval of food derived from so-called Golden Rice for import in food (IRRI, 2016). In 2017, FSANZ gave the go-head for the approval for import and usage as food (FSANZ, 2017).

The rice is genetically engineered to produce provitamin A carotenoids; and the rice kernels are yellowish in colour. It is intended to be a fortified food with a high content of carotenoids, in particular, beta-carotene in the grains, to help combat vitamin A deficiency (VAD) especially in developing countries. VAD is prevalent in young children and pregnant women in rice growing areas. The application was filed for so-called “Golden Rice 2” (GR2) (Paine et al., 2005), which is a second generation trait with a supposedly much higher content in carotenoids than the first generation of Golden Rice. For the application, a line of GR2 (called GR2E) was crossed into *indica rice* varieties (BRRI dhan29, IR64 and PSB Rc82). Data were submitted from the green house (which is called screen house by the applicant) and field trials conducted in the Philippines.

Nutritional quality

Data from the application show that the plants grown in field trials produce a much smaller amount of carotenoids (3,5µg/g – 10.9 µg/g) compared to the original GR2 event which is supposed to produce a maximum of more than 30 µg/g. Further, while Paine et al. (2005) identify beta-carotene as having a percentage of around 80 percent of the total carotenoids, the rice in the field trials only reached 59 percent. It is not clear if this much lower content in carotenoids is due to the varietal background or due to environmental conditions. Further reduction in the content of carotenoids has to be expected due to storage, processing and heating of the grain for preparation of food.

Thus, in regard to the nutritional quality of GR2, this application gives the impression that the potential benefits of the rice as, for example, claimed by Paine et al. (2005) are greatly overestimated and cannot be realised under practical conditions.

Further, from existing publications it can be concluded that there are substantial environmental risks, some of which are related to food safety. For example, if the rice spontaneously introgresses regional varieties, fields or seed production or populations of weedy rice, the transgenic construct will be expressed in heterogeneous genetic backgrounds that were never tested. Evidence of adverse effects is available: the crossing of GR2 R1 with the Indian variety *Swarna* resulted in plants showing extensive disturbance in their growth and leaky gene expression. These effects were neither discussed by the applicant nor by FSANZ, even though they are also relevant for food safety.

Findings showing genetic instability of GR2 being crossed with other varieties were not discussed.

Gaps and flaws in the data as presented

- As shown by the applicant, expression of the transgenes is impacted by varietal backgrounds and is dependent on interactions with the environment. Therefore, it would be necessary to gather more data on the range of variability that can be expected under a broader range of genetic backgrounds. In addition, the plants should have been exposed to a wide range of defined biotic and abiotic stressors to assess the functional stability of the transgenes.

- Further, as the data submitted by IRRI show, there are open reading frames that can give rise not only to proteins but also to gene products such as non-coding RNA. However, no attempt was made to assess the structure and the amount of gene products that might show biological activity with specific relevance for food safety.

- No agronomic data for comparison of phenotypical data were provided by IRRI as would have been requested under EU regulation. These data are necessary for assessment of unintended effects that also might affect food safety.

- The ILSI database (International Life Sciences Institute) was used for comparison in the assessment of changes in plant composition; this database is known to be unreliable for the risk assessment of genetically engineered plants. Consequently, the statistical analysis of the relevant data performed by the applicant cannot be regarded as reliable.

Despite the above described flaws in the data, some significant differences were detected e.g. in niacin content and the composition of fatty acids. Even if these changes taken as isolated data might not directly raise safety concerns, the effects should have been taken as a starting point for more detailed investigations.

In any case, based on the data available, no final conclusions can be drawn on the safety of the rice plants.

Surprisingly, in the IRRI application, the line PSB Rc82, which showed lowest concentration in carotenoids, was chosen for field trials and compositional analysis. One possible explanation is that the applicants wanted to establish safety at a low level of transgene activity. Such a low level can decrease the likelihood of unintended changes in plant metabolism and composition. Therefore, such plants might appear to be “safer” compared to plants with a higher level of transgene activity. However, to assess the risks in a realistic manner, food safety should not be assessed on the basis of lines of GR2 with a low carotenoids content if other lines are available with a higher carotenoid content. In general, the lines with higher levels of carotenoids are more likely to be cultivated for food production than those with low levels.

Consequently, the data for risk assessment cannot be regarded as reliable for determining food safety of the GR2 lines with a higher carotenoid content.

Toxicology

According to IRRI, the consumption of this rice is especially beneficial to young children as well as lactating and pregnant women. Nonetheless, it is self-evident that food products with no history of safe use must be subjected to the highest standards of risk assessment before the most vulnerable groups of the population are exposed to it. However, no toxicological studies were performed with the rice.

Many more in-depth toxicological studies would be necessary before any conclusion can be drawn on food safety.

Campaign by industry

An industry campaign was apparently initiated by stakeholders with particular economic interests alongside the filing of the application: Amongst the submissions sent to FSANZ were several letters from companies such as Bayer, Dow and Syngenta.

The expectations raised by this and similar campaigns are in complete contrast to the data filed by IRRI.

Conclusions

If the substantial risks associated with the cultivation of these plants and existing uncertainties in regard to negative health impacts are taken into account, this application indicates a high likelihood of risks without substantial benefits.

In the light of the humanitarian claims made in the context of the Golden Rice project, it is surprising that this application is not based on a full set of data to establish high safety standards and evidence of the actual benefits.

1. Introduction

In 2016, the International Rice Research Institute (IRRI) filed an application at the Food Standards Australia New Zealand (FSANZ) for the market approval of food derived from the so-called Golden Rice (GR2E) for import in food (IRRI, 2016). In December 2017, FSANZ gave the go-ahead for the approval for import and usage as food (FSANZ, 2017).

This rice is genetically engineered to produce to provitamin A carotenoids; and the rice kernels are yellowish in colour. It is intended to be a fortified food with a high content of carotenoids, in particular, beta-carotene in the grains, to help combat vitamin A deficiency (VAD) especially in rice growing areas of developing countries. According to the application, the target groups in the population are young children as well as pregnant or lactating mothers:

> *"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."* (IRRI, 2016, page 18)

The filed application refers to so-called "GR2" (Paine et al., 2005). This second generation trait supposedly has a much higher carotenoid content than the first generation (Ye et al., 2000). The plants used for the IRRI application are identified as lines derived from the GR2E rice event: Kaybonnet (direct line of descent from original transformant as described by Paine et al., 2005), BRRI dhan29, IR64 and PSB Rc82,. Kaybonnet belongs to the *japonica* rice varieties, while the other three lines have the genetic background of *indica* rice which is cultivated more widely than *japonica* (Swamy & Samia, 2016).

The rice is not meant to be grown in New Zealand and Australia. There also seems to be no particular intention to produce food for import into these countries. Thus, the application seems to be driven by more general trade considerations: If the rice enters international markets without being properly segregated from conventionally produced rice products, there is a likelihood that some quantities, especially of milled rice, will be imported into these countries without this being intended. As is stated in the assessment by FSANZ (2017):

> *"Rice containing the GR2E event is not intended for commercialisation in Australia or New Zealand i.e. either for growing or intentional sale in the food supply. The Applicant has however applied for food approval because it is possible the rice could inadvertently enter the food supply via exports from countries that may supply significant quantities of milled rice to Australia or New Zealand."*

In this regard, it has to be emphasised that the application does not allow any conclusions to be drawn on nutritional quality or environmental safety. Further, food safety assessment was performed by assuming low exposure to the population in Australia and New Zealand.

Similar applications for food approval have been filed in the US and Canada. Further, there is an application pending in the Philippines for food and feed (see FSANZ, 2017).

2. Nutritional quality

The context of the nutritional quality of the rice is crucial in assessing the potential benefits claimed in the application. In this context, the basic question has to be answered about whether this rice can be effective in combating VAD. This is a different issue to assessing food safety. But some aspects – such as genetic stability and the expression of the transgenes in the grains – are also directly interlinked with food safety. In addition, other characteristics such as losses of carotenoids through cooking and storage of the grains after harvesting are only indirectly related to food safety. In this backgrounder we have combined some of these issues in order gain a broader perspective.[1]

2.1 What amount of carotenoids does GR2 actually produce?

As the data show, the plants grown in field trials produce a much smaller amount of carotenoids (3,5µg/g – 10.9 µg/g) compared to the original event which is supposed to produce a maximum of more than 30 µg/g (Paine et al., 2005, Swamy & Samia, 2016).

It is not clear if this much lower content is due to the varietal background (higher content of carotenoids in japonica rice and BRRI dhan29, but lower in PSB Rc82) or due to environmental conditions (higher content was measured in the green house, lower in field trials).

There is also some methodological uncertainty in comparing the figures: Some data were provided on the basis of fresh weight (data derived from the green house), others on dry weight (data derived from field trails). Normally, only one method should be used for comparison. In this case, this matter is relevant because the true differences between the data from the green house and those from the fields could be masked.

In any case, these data show a high variability in the content of the carotenoids produced by the grains and indicate a lack of predictability in regard to nutritional quality.

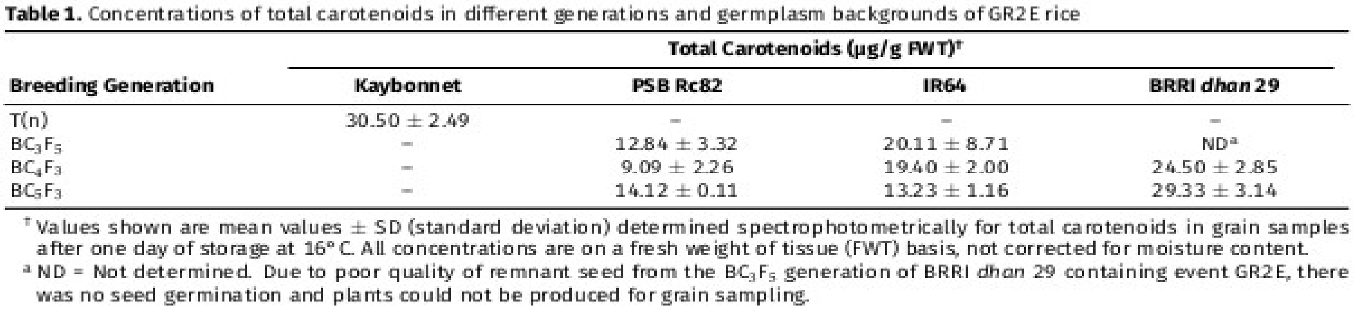

Table 1. Concentrations of total carotenoids in different generations and germplasm backgrounds of GR2E rice

	Total Carotenoids (µg/g FWT)†			
Breeding Generation	**Kaybonnet**	**PSB Rc82**	**IR64**	**BRRI *dhan* 29**
T(n)	30.50 ± 2.49	–	–	–
BC_3F_5	–	12.84 ± 3.32	20.11 ± 8.71	ND[a]
BC_4F_3	–	9.09 ± 2.26	19.40 ± 2.00	24.50 ± 2.85
BC_5F_3	–	14.12 ± 0.11	13.23 ± 1.16	29.33 ± 3.14

† Values shown are mean values ± SD (standard deviation) determined spectrophotometrically for total carotenoids in grain samples after one day of storage at 16° C. All concentrations are on a fresh weight of tissue (FWT) basis, not corrected for moisture content.

[a] ND = Not determined. Due to poor quality of remnant seed from the BC_3F_5 generation of BRRI *dhan* 29 containing event GR2E, there was no seed germination and plants could not be produced for grain sampling.

Table 1 derived from Swamy & Samia, 2016.

2.2 What about the content of beta-carotene?

In relation to the overall content of carotenoids, the proportion of beta-carotene in the grains which is the most relevant carotene in regard to VAD, is reduced: While originally Paine et al. (2005) identify beta-carotene as having a percentage of around 80 percent of the total carotenoids, the rice in the field trials only reached 59 percent (Samia & Swamy, 2016).

As a result, the differences between the original rice (Paine et al., 2005) compared to the results from the field trials are greater than the difference in total carotenoids: In the field trials only a maximum of 7,31 µg/g was reached (Samia & Swamy, 2016), while Paine et al. (2005) reached 29.36 µg/g (based on the assumption that the content of beta-carotene was 80 percent of the total

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[1] We are not dealing here with bioavailability which is discussed elsewhere (Testbiotech 2014). No new data were made available in the context of this application.

carotenoids).

Again, it is not clear if this much lower content in beta-carotene is due to the varietal background (higher in japonica rice and BRRI dhan29, but lower in PSB Rc82), or due to environmental conditions (higher contents measured in the green house, lower in the field trials).

There are also some methodological uncertainties: The data from the field trials show great differences in the content as measured, using different methods for extraction: When standard procedures were applied, the content of beta-carotene was much lower compared to a specific method applied by IRRI and only reached a maximum level of 2.35 µg/g (Swamy et al., 2016a).

Further, data from the green house are difficult to compare with those from field trials because the data from the green house are based on fresh weight of the grains (compared to dry weight from the fields) and no percentage is given for the proportion of beta-carotenes (Swamy & Samia, 2016).

Whatever the case, these data show a high variability in the content of the beta-carotene produced in the grains and indicate a lack of predictability in regard to the nutritional quality of GR2 and its potential to actually combat VAD effectively.

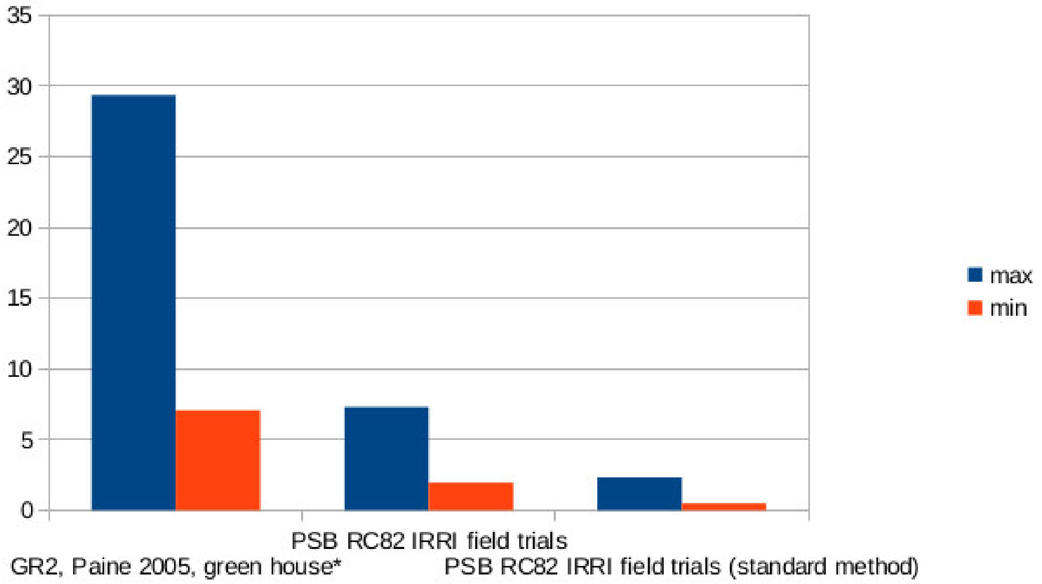

Figure 1: Overview of maximum and minimum content in beta-carotene in lines of GR2 as provided by Paine et al. (2005) and Samia & Swamy (2016). In the grain received from the field trials, different methods for measuring led to different results: A specific method developed by IRRI led to higher results than a standard method showing very low content. **** Data derived from Paine et al. (2005) based on the assumption that the proportion of beta-carotene was 80 percent of the total carotenoids.***

2.3 Losses due to storage and heating

There is another reason to doubt that the GR2 as presented in the application could be effective in combating VAD. These further doubts are related to losses of carotenoids from processing (heating) or storage. No specific data were submitted on these crucial topics. However, general statements were given showing that this problem is very relevant:

> *“More realistic dietary intakes are likely to be even lower as it is unlikely that all rice in the diet will be substituted with GR2E rice, and the known loss of β-carotene over time in all fruits and vegetables where it is found (…) including GR2E rice, due to storage, processing, and cooking.”* (IRRI, 2016, page 75)

In this context, it is remarkable that all the samples used for data generation and for the application were stored frozen at minus 20 C or minus 80 C degrees. After deep freeze storage at minus 80 C, the samples were only kept for “one day” at plus 16 C (Swamy & Samia, 2016). The grains were heated to a maximum of 60 C for 10 mins during the carotenoid extraction process (Swamy & Samia, 2016).

Thus, no conclusion can be drawn from the data presented on losses due to storage or heating under realistic conditions, such as in the countries of rice cultivation. At the same time, it is evident from the application that substantial losses have to be expected. Also Schaub et al (2017) show that quick degradation can be expected from storage of GR2 rice grains; this is in addition to the losses from parboiling.

2.4 Conclusion on nutritional quality

In conclusion, this application gives the impression that the potential benefits of the rice as, for example, presented by Paine et al. (2005) are greatly overestimated and cannot be realised under practical conditions.

3.Environmental risks

As yet, the rice is not being cultivated for food production and no environmental risk assessment for large scale cultivation has been conducted by any authority. However, it can be concluded from existing publications that there are substantial environmental risks some of which are related to food safety.

In particular, there is a high risk of gene flow from GR2 to regional rice varieties and weedy rice. Domesticated grasses (*Poaceae*) still have a high potential for persistence and invasiveness. Rice has a history of double domestication with periods of “de-domestication”, or reversion to a wild form (Vigueira et al., 2013, Kanapeckas et al., 2016). Consequently, gene flow between wild and cultivated rice forms growing in the vicinity (weedy rice) is extensive (Chen et al., 2004). The gene flow between fields and weedy rice can also be circular, reiterative and repetitive (see also Lu & Snow, 2005). Field studies in China (Pu et al., 2014) concluded that insects, in particular, honey bees, are frequently attracted to rice plants and may carry viable pollen over long distances, thus increasing the frequency of transgene flow. Several other publications showed considerable risk of transgene flow to weedy rice. In regions where farm-saved rice seed is dominant, Serrat et al. (2013) found a high risk of genetically engineered red rice weed infestation that is increasing from year to year. Gene flow from herbicide resistant rice to weedy rice was also confirmed by Lu et al. (2014).

As a result, the transgenes might spread and persist uncontrolled throughout the fields and the environment in circular gene flow, between the weedy and the cultivated rice. If the rice spontaneously introgresses regional varieties, the fields or seed production, or populations of weedy rice, the transgenic construct will be expressed in heterogeneous genetic backgrounds that were never tested. This can have detrimental effects: For example Bollinedi et al. (2017) indicate that after crossing GR2R1 with the Indian variety *Swarna*, the resulting plants showed extensive disturbance in their growth. The researchers identified several reasons for this: The new gene constructs interfere with the plant's own gene for producing growth hormones (auxin), and the additional gene constructs were not, as intended, active solely in the kernels, but also in the leaves. This led to a substantial reduction in the content of chlorophyll that is essential for vital functions in the plants.

These findings are alarming in regard to environmental risk assessment: Once released, the transgenes from GR2 could persist in circular gene flow between the fields and populations of weedy rice as described above. Genomic effects not found in the original plants can occur in plant offspring. So far there seems to be no guidance for risk assessment that takes effects into account that occur in subsequent generations, or emerge spontaneously, or might be able to persist and cause further gene flow.

Due to the high potential of circular gene flow between the cultivated and the weedy rice, it might be impossible to remove the transgenes from the environment within the necessary period of time and / or using justifiable methods. Thus, it might be too late to take appropriate measures at the time when adverse effects are finally detected. This problem might also be relevant for the food chain.

These effects were neither discussed by the applicant nor by FSANZ, although they are also relevant for food safety: The Bollinedi et al. (2017) publication showing genetic instability of GR2R1 being crossed into specific varietal backgrounds also raises questions regarding the genetic stability of GR2E being transmitted to further varietal backgrounds.

4. Molecular data and transgene expression data

There are major gaps in the data as presented by IRRI and the way these were assessed by FSANZ:

(1) The genomic flanking regions of the insert in GR2E are only poorly characterised by the applicant (IRRI, 2016). But the Bollinedi et al. (2017) publication also raises questions regarding the genetic stability of GR2E being transmitted to further varietal backgrounds. As shown by the applicant, expression of the transgenes is impacted by varietal backgrounds (Swamy & Samia, 2016). More data, including a broader range of genetic backgrounds would be necessary to conclude on risk assessment.

(2) As shown by the applicant, expression of the transgenes was also impacted by environmental interactions (Samia & Swamy, 2016). Therefore, it would be necessary to gather more data on the range of variability of transgene expression under a broad range of defined biotic and abiotic stressors (see also: Trtikova et al., 2015; Jiang et al., 2017).

(3) Further, as the data as submitted by IRRI show, there are open reading frames that can give rise not only to proteins but also to gene products, such as non-coding RNA. This is relevant, since miRNA is known to survive digestion, to interact with gut bacteria and can be taken up from the intestine (see for example Zhang et al., 2012). If these miRNA enter the blood stream they might show cross-kingdom biological activity with gene regulation in mammalian cells. Since this issue is a matter of great uncertainty and ongoing scientific discussions (see, for example, Del Cornò et al., 2017; Wittwer & Zhang, 2017; Yang et al., 2017), attempts should be made to assess the structure and the amount of gene products that might show biological activity and are therefore are relevant for food safety.

5. Plant composition

There are major gaps in the data presented by IRRI and the way these were assessed by FSANZ:

(1) No agronomic data for comparison of phenotypical and agronomical characteristics were provided by IRRI as would be requested under EU regulation. These data (such as on seedling vigour, stalk lodging, root lodging, stay green, disease incidence, insect damage, seed and pollen viability, plant height, weight and number of grain, time of flowering etc.) are necessary for the assessment of unintended effects that also might affect food safety.

(2) The ILSI database was used for comparison in the assessment of the changes in plant composition. However, these data can hardly be compared to specific data from field trials under specific environmental conditions and specific varieties. In general, ILSI data are generated from a broad range of different varieties grown under different environmental conditions. This creates a high variability within the data. But this variability within the data has nothing to do with the actual field trials. Instead, it has to be regarded as a kind of 'data noise' which can mask the relevant differences. For this reason, the European Food Safety Authority (EFSA) no longer uses this database.

Instead of using the ILSI database, plant composition needs to be compared with varieties with a similar genetic background and grown under the same environmental conditions. Consequently, the statistical analysis of the relevant data performed by the applicant cannot be regarded as reliable.

(3) Surprisingly, in the field trials only the GR2E line was used; this line showed the lowest content in carotenoids (PSB Rc82 – see above). This line was also used to generate the expression data for Phytoene Synthase (ZmPSY1), Phytoene Desaturase (CRTI), and Phosphomannose Isomerase (PMI) as well as compositional analysis (PhilRice & IRRI, 2017). The likelihood that a variety with such a low content of beta-carotene (based on a specific favourable method for extracting beta-carotene, the mean content is around 4,6 µg/g) being used for large scale cultivation seems to be rather low. So why was this line used instead of BRRI dhan29 or IR64 which – at least in the greenhouse – showed a much higher content in carotenoids?

From the perspective of food safety, a low content of carotenoids might be favourable because higher dosages of vitamin A might add to the likelihood of cancer under specific circumstances (FSANZ, 2017). Further, if the genetically engineered rice plants show a low content of carotenoids, this might be caused by a low level gene activity of the transgene. Again, this can be favourable for food safety assessment: A low level of biological activity of the transgene can decrease the likelihood of unintended changes of the plants metabolism and composition. Therefore, genetically engineered rice plants with a lower level of carotenoids might appear “safer” compared to those plants with a higher level of transgene activity.

In general, the lines with a higher level of carotenoids are more likely to be cultivated for food production than those with low levels. Therefore, to realistically assess the risks, food safety should not be assessed on the basis of lines of GR2 that show a low content of carotenoids if there are other lines available with a higher content. The IRRI (2016) application takes the opposite approach: The line PSB Rc82 which showed the lowest concentration in carotenoids was chosen for field trials and compositional analysis.

In conclusion, the data for risk assessment cannot be regarded to be reliable in determining food safety of GR2 lines with a higher carotenoid content.

(4) Despite the flaws in the data as described, some significant differences were detected, such as in the content of niacin and the composition of fatty acids. Even if these changes taken as isolated data might not directly raise safety concerns, the effects should have been taken as a starting point for more detailed investigations. There are clear indications from the data submitted, that GR2E shows effects dependent on interactions with the environment, such as dry or wet seasons. These interactions can not only affect the content of intended compounds (beta-carotene) but also of unintended compounds (such as allergenes).

Several methods, tools and experimental designs are available which should have been used to ensure high safety standards, but were not considered:

- No data from 'Omics' (proteomics, transcriptomics, metabolomics) were used to assist the compositional analysis and the assessment of the phenotypical changes. Indeed, proteome and metabolite analyses performed by Gayen et al. (2016) which was not considered in this risk assessment provides evidence of unintended changes in the metabolic regulation and adaptation of another event of carotenoid rice.
- Field trials were not conducted for more than two seasons (one dry and one rainy season were taken into account). Studies which are performed over several years are essential to assess site-specific effects.
- Further, no data were generated representing more extreme environmental conditions, such as those caused by climate change or specific regions where the rice might be cultivated.
- In addition, more varieties carrying the transgenes and representing a broad range of genetic backgrounds should have been included in the field trials to see how the gene constructs interact with these genetic backgrounds.
- Furthermore, as explained above, varieties carrying the transgenes and showing a higher content in carotenoids should have been included in the field trials.

In any case, based on the data available, no final conclusions can be drawn on the safety of the rice plants.

6. Toxicology

Since, according to the application, the consumption of this rice is meant to be especially beneficial to young children, lactating and pregnant women (IRRI, 2016), it is very surprising the applicant did not provide a feeding study with the whole grains.

It is self-evident that food products with no history of safe use need to be subjected to the highest standards of risk assessment before particularly vulnerable groups of the population are exposed to them. Since this is the first time that an application for the usage of this product as food has been filed, a full set of data, including feeding studies, should have been expected, even if there is no intention to cultivate or market unprocessed grains in Australia or New Zealand. However, no toxicological studies were performed with the rice. Instead, the applicant creates a false impression by saying that feeding studies would not be requested in other regions of the world and by pointing to outdated EFSA guidance for risk assessment (2006) (IRRI, 2016, page 87).

Contrary to the impression given by the applicant, if this application had been filed in the EU, Regulation No 503/2013 would require at least a subchronic rat feeding study as a minimum. Given the vulnerability of the main target group of consumers (children and pregnant women), further investigations such as chronic and multigenerational feeding might be requested under some circumstances. This might also include empirical investigations of carcinogenicity which is known to be an issue under specific circumstances if elevated levels of vitamin A are consumed regularly (FSANZ, 2017).

In any case, many more in-depth toxicological studies are necessary before any conclusion can be drawn on food safety.

7. The submissions

As FSANZ states, during the period of examination:

> “*a total of 33 submissions were received of which 11 were very similarly worded. A campaign (entitled Speak up NOW for Golden Rice) urging positive comments on GR2E to both FSANZ and the Philippines biotechnology regulator (Bureau of Plant Industry), which was also seeking public comments on GR2E at the same time as FSANZ, was initiated by the Cornell Alliance for Science.*” (FSANZ, 2017)

This campaign was apparently initiated by stakeholders with particular economic interests: Amongst the submissions were several letters from companies such as Bayer, Dow and Syngenta.

The expectations raised by this and similar campaigns[2] are in deep contrast to the poor data filed by IRRI and the gaps in risk assessment performed by FSANZ.

8. Conclusions

The data provided to FSANZ are mostly from a specific line of the GR2E event (see Paine et al., 2005) that shows a low level of carotenoids especially beta-carotene. There are several possible explanations why this specific GR2E line was used for the application. One possible explanation is that the applicants wanted to establish safety at a low level of transgene activity.

There is a substantial dilemma for the applicant: If benefits are not evident, there will be no interest in growing the plants. But if there are lines of GR2 available (or are generated in future) with a higher content of carotenoids, FSANZ risk assessment cannot be regarded as conclusive because it is based on data without sufficient reliability.

There are further problems with the data provided by IRRI and the risk assessment performed by FSANZ such as:

- As shown by the applicant, expression of the transgenes is impacted by varietal backgrounds. More data, including a broader range of genetic backgrounds would be necessary to conclude on risk assessment.
- The data from IRRI show that gene expression and plant composition are influenced by the environment. These complex interactions would need further investigations.

Further this application does not offer information on:

- losses in carotenoids during storage and heating of this specific line;
- impact vulnerable groups, such as young children and pregnant women, from consumption of this rice;

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[2] http://supportprecisionagriculture.org/nobel-laureate-gmo-letter_rjr.html

- environmental risks.

Taking into account the substantial risks that go along with the cultivation of these plants and existing uncertainties in regard to negative health impacts, this application indicates significant risks without substantial benefits.

In the light of the humanitarian claims made in the context of the Golden Rice project, it is surprising that this application is not based on a full set of reliable data to establish high safety standards and evidence on the actual benefits.

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