

Supporting document 1

Risk and technical assessment report – Application A1115

Irradiation of Blueberries & Raspberries

Executive summary

FSANZ has previously assessed the potential toxicological hazard and nutritional adequacy of various fruits and vegetables irradiated at doses of up to 1 kilogray (kGy) and concluded that there are no public health and safety issues associated with their consumption.

There is a technological (phytosanitary) need to irradiate blueberries and raspberries as a quarantine measure for the control of fruit fly and other insect pests within the dose range of 0.15 to 1 kGy. The purpose of this risk assessment was to determine if blueberries and raspberries irradiated at up to 1 kGy are as safe to consume as non-irradiated blueberries and raspberries.

There are negligible risks to public health and safety associated with the consumption of blueberries and raspberries which have been irradiated at up to 1 kGy. This conclusion is based on the following considerations:

- There is a low potential for the generation of 2-alkylcyclobutanones (2-ACBs) in irradiated blueberries and raspberries because of their low lipid content. The weight-of-evidence, supported by new published data, indicates that 2-ACBs are not genotoxic.
- Furan, a volatile genotoxic carcinogen found in some non-irradiated foods, has been either not detected, or detected at only low levels in a range of other fruits irradiated at 5 kGy, which is five-times higher than the maximum dose sought in this Application. It is likely that furan levels are undetectable in blueberries and raspberries irradiated at doses of up to 1 kGy.
- Irradiation of blueberries and raspberries at doses of up to 1 kGy appears to have no consistent effect on the levels of vitamins or provitamins that are potentially sensitive to irradiation. There is limited and conflicting evidence of some losses of vitamin C in irradiated berries, but these reported reductions fall well within the range of vitamin losses that normally occur during the storage and processing of non-irradiated fruit. There is therefore minimal potential for the consumption of irradiated blueberries and raspberries to affect the nutritional adequacy of the Australian and New Zealand populations.
- The safety of irradiated food has been extensively assessed by national regulators and international scientific bodies. The weight of scientific opinion is that irradiated food is safe for consumption when irradiated at doses necessary to achieve the intended technological function and in accordance with 'Good Practice in Food Irradiation'.

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1 Introduction

Food Standards Australia New Zealand (FSANZ) received an Application from the New South Wales Department of Primary Industries (NSW DPI), a division of NSW Department of Trade and Investment, Regional Infrastructure and Services, to permit the irradiation of raspberries (*Rubus idaeus*) and blueberries (*Vaccinium corymbosum*, *V. strigosus*, *V. augustifolium*, *V. virgatum* v *ashei*) as a phytosanitary measure. Approval for an irradiation dose of up to 1000 Gray (1 kGy) is sought.

Standard 1.5.3 – Irradiation of food prohibits the sale of irradiated foods unless the food is in the Standard. A pre-market assessment is required before irradiated raspberries and blueberries can be sold in Australia or New Zealand.

FSANZ has previously undertaken risk assessments of irradiation of herbs, spices and plant material for herbal infusions (Application A413; ANZFA 2001), a range of tropical fruits (A443 and A1038; FSANZ 2002 and 2011), tomatoes and capsicums (A1069; FSANZ 2013) and 12 additional fruits and vegetables: apples, apricots, cherries, honeydew melons, nectarines, peaches, plums, rockmelons, strawberries, table grapes, zucchini and squash (A1092; FSANZ 2014a).

These assessments concluded that there are no health and safety concerns associated with the consumption of the specific foods when irradiated at the proposed maximum doses. For irradiated and non-irradiated fruits and vegetables, the differences in vitamin concentrations, including vitamin C, are within the range of natural variation that normally occurs with different cultivars, seasons, growing conditions and post-harvest storage and processing.

1.1 Objective of the risk assessment

The objective of this risk assessment is to assess the safety of irradiation of blueberries and raspberries for Australian and New Zealand consumers. The following key questions have been posed:

1. Has the technological purpose for using irradiation as a quarantine measure for fresh blueberries and raspberries been established?
2. Is the dose range requested by the Applicant consistent with quarantine requirements?
3. What is the risk to public health and safety for Australian and New Zealand consumers from any compounds formed following irradiation of fresh blueberries and raspberries?
4. Does irradiation affect the nutrient composition of fresh blueberries and raspberries?
5. If so, how does this compare to effects from other post-harvest and processing procedures?
6. Taking into account potential market share and trade of irradiated fresh blueberries and raspberries, in both Australia and New Zealand, would any changes in the nutrient composition of fresh blueberries and raspberries, following irradiation, have the potential to affect the nutritional adequacy of diets for Australian and New Zealand populations?
7. What are the combined cumulative nutritional effects on the nutritional adequacy of diets for Australian and New Zealand populations from irradiation of both the currently permitted irradiated foods and irradiated fresh blueberries and raspberries?

The Risk Assessment report is structured to address each of these questions:

- Technological need assessment – which assessed whether irradiation at up to 1 kGy is effective as a phytosanitary measure and consistent with quarantine requirements (risk assessment questions 1 and 2)
- Hazard Assessment, which evaluated whether the irradiation of blueberries and raspberries at the proposed level could generate hazardous compounds (risk assessment question 3)
- Nutrition Assessment, which evaluated whether irradiation at the proposed level would significantly alter the nutritional composition of blueberries and raspberries, and examined the effect of other post-harvest and processing procedures on nutrient levels in blueberries and raspberries (risk assessment questions 4 and 5)
- Dietary Intake Assessment, which examined whether there would be any nutritional disadvantages from consumption of irradiated blueberries and raspberries (risk assessment questions 6 and 7).

Based on the hazard, nutrition and dietary intake assessment components, the risk to public health and safety has been characterised.

1.2 Risk Assessments by other agencies and scientific bodies

The safety of irradiated foods has been evaluated by regulatory agencies in other countries and international scientific bodies including the Joint FAO/IAEA/WHO Expert Committee on Food Irradiation (JECFI) (WHO 1977 & 1981), International Consultative Group on Food Irradiation (WHO 1994) and Study Group on High-Dose Irradiation (WHO 1999), Health Canada (2008) and the European Food Safety Authority (EFSA 2011a and 2011b). These reviews have examined the efficacy, safety and nutritional effects of irradiation on a wide range of foods. The weight of scientific opinion is that irradiated food is safe for consumption when irradiated at doses necessary to achieve the intended technological function and in accordance with the International Atomic Energy Agency's Manual of Good Practice in Food Irradiation (IAEA 2015).

2 Technological need and quarantine requirements

2.1 Current status of food irradiation for phytosanitary purposes in Australia and New Zealand

To date, FSANZ has approved the irradiation of herbs, spices and herbal infusions, a range of tropical fruits (mango, breadfruit, carambola, custard apple, litchi, longan, mangosteen, papaya and rambutan), persimmons, tomatoes and capsicums. Specific advice on technological need and appropriate dose ranges for phytosanitary purposes was sought at that time from the then Biosecurity Australia (now the Australian Government Department of Agriculture and Water Resources (Agriculture)) and MAF Biosecurity (now the New Zealand Ministry for Primary Industries (MPI)).

Examples of previous approvals by the Australian and New Zealand authorities of irradiation for quarantine purposes are as follows:

Commodity	Date	Purpose	Dose
Fresh mangoes imported from India (BA)	August 2008	Phytosanitary need for control of fruit flies, mealy bugs, red-banded mango caterpillar and mango weevils	400 Gy
Litchis exported from Australia (Biosecurity NZ)	September 2008	Control of Fruit fly and Hemiptera (bugs)	Minimum of 250 Gy
Mangoes and Papaya exported from Australia (Biosecurity NZ)	2004 and 2006, respectively	Control of Fruit fly and other insect pests	250 Gy
Tomatoes and capsicums exported from Australia to New Zealand (NZ Ministry for Primary Industries)	August 2011	Control of fruit fly	150 Gy to 1 kGy

See below links to approvals by the relevant quarantine agencies:

- Litchi from Australia RA: <http://www.mpi.govt.nz/document-vault/2876>
- SPS notification: <http://www.mpi.govt.nz/importing/overview/access-and-trade-into-new-zealand/world-trade-organization-notifications/>
- IHS Mango Australia: <https://www.mpi.govt.nz/document-vault/1878>
- IHS Capsicum Australia: <https://www.mpi.govt.nz/document-vault/1695>
- IHS Tomato Australia: <https://www.mpi.govt.nz/document-vault/1993>

In 2011, the use of irradiation for phytosanitary purposes for domestic trade was approved by all states and territories in Australia.

This treatment is available to businesses under the national Interstate Certification Assurance (ICA) Scheme as Operational Procedure Number 55 (i.e. ICA 55¹) and conforms to the principles of International Standards for Phytosanitary Measures 18 (*ISPM No. 18*) – *Guidelines for the Use of Irradiation as a Phytosanitary Measure*, International Plant Protection Convention, 2003 (ISPM, 2003) that provides technical guidance on the specific procedures for the application of ionising radiation as a phytosanitary treatment for pests or articles.

ICA 55 also sets the minimum doses required as follows:

- 150 Gy for fruit flies of the family Tephritidae.
- 300 Gy for the mango seed weevil.
- 400 Gy for all pests of the class Insecta except pupae and adults of the order Lepidoptera.

2.2 International evidence to support irradiation against fruit flies and other regulated pests

Irradiation is a known effective treatment for fruit fly infestation. For fruits and vegetables that

¹ www.daff.qld.gov.au/data/assets/pdf_file/0008/69578/ICA-55.pdf

are hosts to the fruit fly, the required treatment is applied in accordance with international requirements (under ISPM 18; 2003). The required treatment would specifically comply with *ISPM 28, Irradiation Treatment for Fruit Flies of the Family Tephritidae* (2007) within the dose range of 150 Gy to 1 kGy for prevention of the emergence of adult fruit flies for all fruits and vegetables. Further support for the efficacy of irradiation as a phytosanitary treatment for fruit fly exists in the US. In 2006, the US Animal and Plant Health Inspection Service (APHIS) approved generic irradiation doses of 150 Gy to reduce fruit fly infestation on specific fruits.

In this application, the minimum dose requested is 150 Gy, which is a generic treatment for fruit fly species. The proposed treatment range of 150 Gy minimum dose and 1 kGy maximum dose will comply with ISPM 18 and 28 requirements and is identical to the current levels approved for tropical fruits, persimmons, tomatoes and capsicums in Standard 1.5.3.

Currently, irradiation is an approved treatment to control quarantine pests in 17 fruits and seven vegetables for export from Hawaii to the USA mainland. There is also ongoing research to look at lower doses for phytosanitary needs, which will assist reducing costs, improving quality and increasing capacity due to shorter treatment times. As an example, the Mediterranean fruit fly is controlled in mandarins with a combination treatment of a radiation dose of 30 Gy and cold treatment (1 degree Celsius for 2 days (Follett and Weinart, 2012)).

2.3 Australian and New Zealand quarantine agencies support for irradiation against fruit flies and other regulated pests

Agriculture has provided a letter of support indicating that irradiating fresh horticultural commodities at doses of 150 Gy to 1 kGy is an effective phytosanitary measure against fruit fly and other quarantine pests.

Similarly, MPI has recommended irradiation as an effective quarantine treatment for fruit fly and other pests of quarantine concern to New Zealand.

2.4 Conclusion

In summary, advice received by FSANZ from the relevant quarantine authorities is that irradiation of fruits and vegetables in general for the purpose of pest disinfection does provide an effective alternative to currently used disinfection methods. The proposed minimum dose of 150 Gy and maximum dose of 1 kGy will provide a dose range in order for quarantine agencies to consider irradiation as a treatment for pest disinfection of raspberries and blueberries. FSANZ understands that irradiation is viewed as an important pest reduction protocol for acceptance of Australian produce for interstate trade and in other countries.

However, Agriculture and MPI will still need to independently perform an import risk assessment (for quarantine purposes) on irradiation of the fruits in question, specifically for food imported into Australia or New Zealand. These assessments are separate from the approval processes in the food regulatory regime.

Response to Question 1: *Has the technological purpose for using irradiation as a quarantine measure for raspberries and blueberries been established?*

Yes. Irradiation is an internationally accepted quarantine measure for control of fruit fly and other insect pests.

Response to Question 2: *Is the dose range requested by the Applicant consistent with quarantine requirements?*

The dose range sought by the applicant (up to 1 kGy) is sufficient to meet domestic and international quarantine requirements.

3 Hazard Assessment

3.1 Introduction

The scope of this hazard assessment was to evaluate supplementary data published since FSANZ's most recent evaluation of irradiated foods (specific fruits and vegetables, in 2014)² covering the safety of food irradiation in general, and specifically, the potential hazard of radiolytic compounds generated by the irradiation of blueberries and raspberries. The conclusion of this previous hazard assessment was that the specific fruits and vegetables irradiated at up to 1 kGy are as safe to consume as the non-irradiated fruits and vegetables on the basis of the following considerations:

- Compounds potentially formed during food irradiation such as 2-alkylcyclobutanones (2-ACBs), are found also naturally in non-irradiated food. There is a low potential to generate 2-ACBs because of the low lipid content of the specified fruits and vegetables.
- Furan, a volatile genotoxic carcinogen, was not detected in rockmelon or honeydew melons irradiated at 5 kGy, and only levels below the limit of quantitation (1 ng/g) were detected in strawberries and apples irradiated with 5 kGy. Low levels of furan (2–3.6 ng/g) were detected in grapes irradiated with 5 kGy, which is five-times higher than the maximum dose sought in the application. This furan level is at the low end of the range commonly found in heat-treated foods, while higher levels are found in coffee.
- Adverse effects have been reported in cats and dogs following exclusive consumption of specific brands of pet foods irradiated at doses from 25 to 50 kGy. At these high doses of irradiation, vitamin A levels were shown to be reduced in pet food, however low levels of irradiation (up to 1 kGy) do not appreciably reduce vitamin levels in the requested fruit and vegetables. Therefore, FSANZ considered that these studies had no implications for the safety of fruits and vegetables irradiated for human consumption at up to 1 kGy.

3.2 Evaluation

3.2.1 Compounds generated in irradiated foods

As summarised in the Risk and Technical Assessment Report for Application A1092 – Irradiation of Specific Fruits & Vegetables (FSANZ 2014a), there are a number of compounds that may be generated during the irradiation of food (so-called radiolytic compounds) including free radicals, various hydrocarbons, formaldehyde, amines, furan and 2-alkylcyclobutanones (2-ACBs) (Sommers et al 2007; Vranova & Ciesarova 2009). However, the majority of these compounds are not unique to irradiated food and are naturally present at low levels in food, or are generated via other processing treatments (e.g. heat treatment).

² <http://www.foodstandards.gov.au/code/applications/Pages/A1092-Irradiation.aspx>

2-ACBs are considered to be uniquely formed during food irradiation at levels dependent on the lipid content of the food. The lipid content of raspberries and blueberries is very low (<0.2%; Golding et al. 2014a), hence there is minimal potential for the generation of 2-ACBs. The lipid content of blueberries and raspberries is lower than that of custard apple (0.6%) and rambutan (0.4%), and comparable to that of capsicum (0.1–0.2%), tomato (0.1%), lychee (0.1%), mango (0.2%) and papaya (0.1%) (FSANZ 2011, 2013). These fruits have previously been assessed by FSANZ as safe for consumers when irradiated at up to 1 kGy.

FSANZ evaluated the genotoxic potential of 2-ACBs as part of the risk assessment prepared for Applications A1038 (persimmons) and A1069 (tomatoes and capsicums). The weight-of-evidence indicated that 2-ACBs are not genotoxic, with numerous laboratory animal studies demonstrating that long-term consumption of irradiated foodstuffs (that would contain low concentrations of 2-ACBs and other radiolytic compounds) is safe. Evaluations conducted by the European Commission's (EC) Scientific Committee on Food (2002), WHO (2003), Health Canada (2008) and EFSA (2011a and 2011b) have concluded that, based on the current scientific evidence, 2-ACBs in irradiated foods do not pose a health risk to consumers.

3.2.2 Supplementary data

A search of the scientific literature published since FSANZ's most recent evaluation of irradiated foods (i.e. from May 2014 to March 2016) was conducted to identify any relevant supplementary data on the safety of irradiated food or on the toxicity of 2-ACBs or other radiolytic compounds. Two relevant papers were located and are summarised below.

Repeat-dose toxicity

Sato et al. (2015) performed a 90-day oral toxicity study and an azoxymethane-primed two-stage carcinogenesis study of 2-tetradecylcyclobutanone (2-tDCB) in Fischer (F344) rats to investigate possible biochemical or histopathological changes. In addition, a 5-week oral toxicity study of 2-tDCB was performed to investigate potential effects observed in the 90-day study. Results of the 90-day and 5-week studies in unprimed rats are reported below. Results of the study in azoxymethane-primed rats are reported under *Carcinogenicity*.

In the 90-day rat feeding study the toxicity of 2-tDCB, a 2-alkylcyclobutanone reported to be a radiolytic product of stearic acid was investigated. Four groups of six-week-old F344 rats (n=7–9/sex per group) were given 2-tDCB at concentrations of 0, 12, 60 or 300 ppm in the diet for 13 weeks. It was not reported if there were any deaths or signs of toxicity during the study. Body weight and food intake in each group were similar throughout the study. There were no treatment-related effects on urinalysis or organ weights. Histopathological examinations, conducted on liver and pancreas of control and high dose animals, were normal. In males, there were statistically significant increases in serum total protein at 60 ppm ($p < 0.05$) and 300 ppm ($p < 0.01$), and albumin at 300 ppm ($p < 0.05$), but the increases were small and not considered to be toxicologically relevant. Non-fasting blood glucose levels showed no treatment-related changes in both sexes; however fasting blood glucose levels of males in the 300 ppm group and females in the 60 and 300 ppm groups were lower than those of controls ($p < 0.05$). There was a dose-dependent increase in white blood cell (WBC) counts in males and females; however none of the increases were statistically significant.

These investigators also reported details of a rat study conducted to investigate the potential effects of 2-tDCB on WBC counts and blood glucose from the 90-day study. Six-week-old male F344 rats (n=9/group) were given a basal diet, or a diet that contained 300 ppm 2-tDCB for 5 weeks. At the end of 4 weeks, an oral glucose tolerance test was performed after 16 h of fasting. Fasting glucose and 30-min post-glucose loading blood glucose were measured. At the end of 5 weeks, WBC counts were measured. 2-tDCB had no effect on

fasting or 30-min post-glucose loading blood glucose levels, and no effect on WBC count. Based on the results of the 90-day study and the subsequent 5-week study, it is concluded that the no observed adverse effect level (NOAEL) for 2-tDCB in this study was the highest tested dietary concentration of 300 ppm for both sexes (stated by Sato et al. to be equivalent to 15.5 mg/kg bw/day in males and 16.5 mg/kg bw/day in females).

Carcinogenicity

Yamakage et al. (2014) investigated the tumour promoting potential of 2-tDCB and 2-dodecylcyclobutanone (2-dDCB) in Bhas 42 cells, a cell line derived from BALB/c 3T3 mouse embryo cells transfected with an activated *ras* oncogene. Incubation of Bhas 42 cells with 2-dDCB (0.003–0.014 mg/mL) resulted in statistically significant ($p < 0.05$) increases compared to solvent control in the number of transformants per well at concentrations of 0.012 and 0.014 mg/mL. The numbers of transformants per well at 0.012 and 0.014 mg/mL were 3.6- and 3.7-times the solvent control value, respectively; however at an intermediate concentration (0.013 mg/mL) the number of transformants per well was 0.5-times the solvent control. Incubation of Bhas 42 cells with 2-tDCB (0.0006–0.01 mg/mL) resulted in statistically significant ($p < 0.05$) increases compared to solvent control in the number of transformants per well at the two highest concentrations: 0.005 mg/mL (2.0-times solvent control) and 0.01 mg/mL (3.6-times solvent control). Based on these results, the authors concluded that 2-dDCB and 2-tDCB showed tumour promoting activity *in vitro*. However, the predictive value of this assay for the identification of potential tumour promoting substances is questionable. It was reported in 2012 that the Bhas 42 assay and similar rodent cell transformation assays had not been sufficiently validated for use in regulatory risk assessment (Schechtman 2012). For example, in an inter-laboratory validation study of the Bhas 42 assay in three laboratories, anthracene tested negative for tumour promotion in two laboratories while one laboratory obtained a positive result (Sakai et al. 2011). Furthermore, a search for subsequent information on the Bhas 42 assay has shown that its use in published studies has been minimal.

In order to investigate whether 2-tDCB shows tumour promoting potential *in vivo*, Sato et al. (2015) performed a study in which rats were first injected subcutaneously (SC) with azoxymethane (AOM), a compound known to induce intestinal tumours in rodents. Six-week-old male F344 rats ($n=30$ /group) were administered AOM (15 mg/kg bw SC) once a week for 3 weeks and then received 2-tDCB at concentrations of 0, 10, 50 and 250 ppm in the diet for 25 weeks. One control and one 10 ppm rat died from unknown causes at weeks 20 and 10, respectively. Body weight and food intake were similar in each group throughout the study. Absolute and relative organ weights (liver, kidneys and spleen) were also similar across groups. The number of rats per group with one or more tumours larger than 2 mm in diameter in the small intestine and cecum/colon were reported. The incidence of small intestine tumours in the control group was 17%, while the incidence in the 2-tDCB groups (10, 50 and 250 ppm) was 10%, 0% and 17%, respectively. The incidence of cecum/colon tumours in the control group was 34%, while the incidence in the 2-tDCB groups declined with dose (45%, 40% and 37%, respectively); however, compared to the control group none of the differences were statistically significant. It is concluded that, under the conditions of the study, 2-tDCB did not affect tumour incidence.

Genotoxicity

Yamakage et al. (2014) also studied the genotoxic potential of 2-dDCB and 2-tDCB. A bacterial reverse mutation assay (Ames test) conducted with four strains of *Salmonella typhimurium* and one strain of *Escherichia coli*, was negative for both substances, both with and without metabolic activation. In an *in vitro* chromosomal aberration test with Chinese hamster lung cells and an *in vivo* micronucleus test in mice, no clastogenic effects were observed. No DNA strand breaks were detected in an *in vitro* comet assay. DNA adducts

derived from 2-dDCB and 2-tDCB were not detected in the colon tissue of mice dosed orally with either substance nor in rats dosed orally with 2-tDCB. Additional details of these genotoxicity studies are shown in Table 3.1.

3.2.3 Other relevant safety matters

The Risk and Technical Assessment Report for A1092 (FSANZ 2014a) provided a summary of publications and reports suggesting that irradiated pet foods are responsible for adverse health effects in cats and dogs, including a United States Food and Drug Administration (USFDA) investigation of the cause of illnesses reported in dogs which may be associated with the consumption of irradiated jerky pet treat products. The most recent update from the US FDA (19 Feb 2015) states that the agency is continuing to investigate the issue.³ As stated in FSANZ (2014a), these pet treat products are irradiated at up to 50 kGy to control microbes. To date, extensive investigation by the US FDA has not identified a cause of the pathology observed in the affected animals. FSANZ does not consider that these reported adverse effects have implications for the safety of food irradiated at up to 1 kGy, and will continue to monitor any developments in this area.

Table 3.1: Summary of genotoxicity studies on 2-dodecylcyclobutanone (2-dDCB) and 2-tetradecylcyclobutanone (2-tDCB) (Yamakage et al. 2014)

Compound	End-point	Test system	Concentration/ dose	Results
2-dDCB	Reverse mutation	<i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA1537, and <i>Escherichia coli</i> WP2 <i>uvrA</i>	4.9–5000 µg/plate (pre-incubation method)	Negative ^{a,b}
	Chromosomal aberration	Chinese hamster lung cells (CHL/IU)	0.03–1 mg/mL	Negative ^{a,c}
	Comet assay	Chinese hamster lung cells (CHL/IU)	0.044–0.1 mg/mL	Negative ^a
	Micronucleus induction	Bone marrow from CD-1 mice following oral administration	500–2000 mg/kg bw/day for two days	Negative
	DNA adduct formation	Colon tissue from CD-1 mice following oral administration	500–2000 mg/kg bw/day for two days	Negative
2-tDCB	Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, and <i>E. coli</i> WP2 <i>uvrA</i>	78.1–5000 µg/plate (pre-incubation method)	Negative ^{a,d}
	Chromosomal aberration	Chinese hamster lung cells (CHL/IU)	0.09–4 mg/mL	Negative ^{a,e}
	Comet assay	Chinese hamster lung cells (CHL/IU)	0.13–1 mg/mL	Negative ^a
	Micronucleus induction	Bone marrow from CD-1 mice following oral administration	500–2000 mg/kg bw/day for two days	Negative
	DNA adduct formation	Colon tissue from CD-1 mice following oral administration	500–2000 mg/kg bw/day for two days	Negative
	DNA adduct formation	Colon tissue from F344 rats following oral administration	0.03% in the diet for 90 days	Negative
	DNA adduct	Colon tissue from F344 rats following	0.025% in the diet	Negative

³ <http://www.fda.gov/AnimalVeterinary/NewsEvents/CVMUpdates/ucm434865.htm>

	formation	oral administration	for 25 weeks	
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^a With and without metabolic activation.

^b Precipitation was observed at 156–5000 µg/plate.

^c Precipitation was observed at 0.44–1 mg/mL.

^d Precipitation was observed at 625–5000 µg/plate.

^e Precipitation was observed at 0.13–4 mg/mL.

3.3 Conclusions

Blueberries and raspberries irradiated at doses of up to 1 kGy are as safe to consume as non-irradiated berries on the basis of the following considerations:

- There is a low potential to generate 2-ACBs because of the low lipid content of blueberries and raspberries. The weight-of-evidence, supported by new published data, indicates that 2-ACBs are not genotoxic.
- Furan, a volatile genotoxic carcinogen found in some non-irradiated foods, has been either not detected, or detected at only low levels in a range of other fruits irradiated at 5 kGy, which is five-times higher than the maximum dose sought in this Application. It is likely that furan levels are undetectable in blueberries and raspberries irradiated at up to 1 kGy.

Response to Question 3: *What is the risk to public health and safety for Australian and New Zealand consumers from any compounds formed following irradiation of blueberries and raspberries?*

The risk posed by consuming irradiated blueberries and raspberries irradiated at up to 1 kGy is considered to be negligible.

4 Nutrition Assessment

4.1 Introduction

4.1.1 Previous FSANZ considerations of the effect of irradiation on nutrients in food

FSANZ has previously evaluated the effect of low-dose irradiation on the nutrient profile of various fruits in relation to A443 (Irradiation of tropical fruits – breadfruit, carambola, custard apple, lychee, longan, mango, mangosteen, papaya and rambutan) and A1038 (Irradiation of persimmons) and also A1069 (Irradiation of tomatoes and capsicums). A1092 considered the phytosanitary irradiation of various fruits and vegetables including apple, apricot, cherry, honeydew, nectarine, peach, plum, rockmelon, strawberry, table grape and zucchini. These evaluations concluded that the macronutrient and mineral content of these foods was unaffected by irradiation up to a dose of 1 kGy, although the concentrations of certain water soluble vitamins (e.g. thiamin, vitamin C, folate or β-carotene) may potentially be reduced. However, any impact on vitamin content would be no greater than that from other forms of food processing. Worst case estimate losses of vitamin A and vitamin C across all fresh tomatoes, capsicums and tropical fruits (where irradiation is already permitted) show mean population declines of no more than 1% and 2%, respectively, with mean intakes of vitamin A and vitamin C remaining above the Estimated Average Requirements.

In addition, in 2014 FSANZ carried out a review to assess the impact of phytosanitary doses of irradiation on the nutritional quality of a wide range of fruits and vegetables in order to

make recommendations to amend data requirements for irradiation of fruits and vegetables. The conclusion was that phytosanitary doses of irradiation do not pose a nutritional risk to the Australian and New Zealand populations. The recommendation was made that the data requirements for applications to irradiate fruits and vegetables can be streamlined to focus on data for vitamin C with requirements for other nutrients to be determined on a case-by-case basis.

4.1.2 Impact of irradiation on nutrients in food

Numerous independent reviews have been published on the effects of irradiation on food (WHO 1981; 1994 & 1999; SCF 2003; Arvanitoyannis 2010; EFSA 2011a and 2011b). These reviews have examined the efficacy, safety and nutritional effects of irradiation on a wide range of foods. Irradiation can induce changes in nutrient content, depending on a variety of factors including the irradiation dose, composition of the food, packaging material, ambient temperature and atmospheric oxygen concentration (Diehl et al. 1991; Kilcast 1994; WHO 1994). A small proportion of nutrients are sensitive to irradiation, with their concentrations decreasing with irradiation dose (WHO 1999). Nutrient loss can be minimised by the use of appropriate processing techniques, such as low temperature and oxygen-free conditions (WHO 1994; Diehl 1995).

There has been no demonstrated effect of irradiation up to 1 kGy on the amount and nutritional quality of carbohydrates, proteins or fats and no evidence to suggest, or reason to believe, that irradiation reduces the mineral content of food (Diehl et al. 1991; WHO 1994). The concentrations of certain vitamins in some fruits and vegetables may be affected by irradiation but it is important to recognise that the natural variation in vitamin content in fruits and vegetables is very large, depending on factors such as the plant variety, growing conditions, maturity of the edible portion, post-harvest handling and storage conditions (WHO 1994). On this basis, changes in the concentrations of vitamins observed in individual studies must be interpreted in the context of this variation. Reductions in the vitamin content of a particular fruit or vegetable that has been irradiated may not be able to be extrapolated to other types of fruits or vegetables that differ in baseline nutrient composition.

Notwithstanding the variable effect that irradiation may have on the vitamin content of fruits and vegetables, experience to date suggests that there is a general hierarchy of vitamin sensitivity (Figure 4.1). Consequently, the majority of studies examining the effect of irradiation on fruit or vegetable quality have focussed on the analysis of vitamin C and the carotenoids because these represent the more sensitive nutrients found in fruits and vegetables.

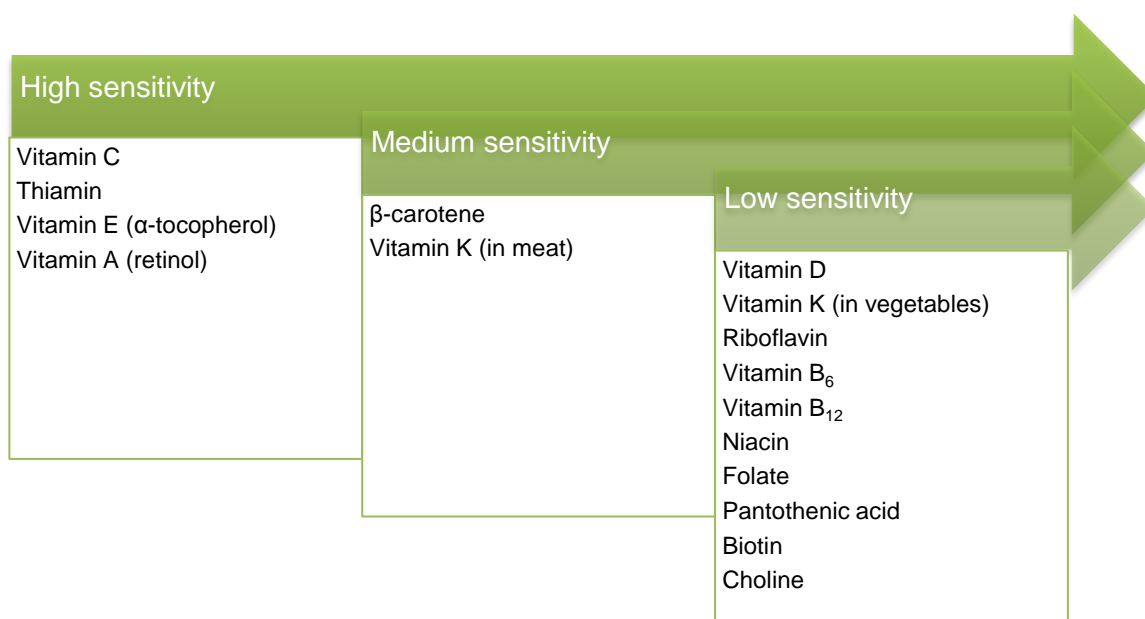


Figure 4.1: General sensitivity of vitamins in food to irradiation (modified from Kilcast 1994)

Ascorbic acid (vitamin C) is one of the most sensitive vitamins to irradiation, although this sensitivity varies due to exposure to oxygen, storage, temperature and pH (Kilcast 1994). Irradiation results in some ascorbic acid being converted to dehydroascorbic acid (Kilcast 1994), however both forms of vitamin C are found in non-irradiated foods and both are biologically active (Tsujiura et al. 2008). Therefore, when reviewing findings of irradiation studies, it is important to consider that losses due to irradiation may be overestimated if only ascorbic acid is reported. Hence, total ascorbic acid content (ascorbic and dehydroascorbic acid) is arguably a more reliable indicator of post-irradiation vitamin C content.

A review by Diehl et al. (1991) concluded that the results of studies investigating the effect of irradiation on carotenoids vary considerably depending on the fruit or vegetable. Several studies assessing the total carotenoid, β-carotene and lycopene content of raw fruit and vegetables show inconsistent effects of irradiation at doses up to 1 kGy. Overall, the carotenoid content of irradiated fruit or vegetables is comparable to non-irradiated fruit or vegetables (Mitchell et al. 1990; Farkas et al. 1997; El-Samahy et al. 2000; Boylston et al. 2002; Patil et al. 2004; Vanamala et al. 2005; Moreno et al. 2007; Reyes & Cisneros-Zevallos 2007; Girenavar et al. 2008; Gomes et al. 2008; Lester et al. 2010).

In some fruit (e.g. mango and papaya), the carotenoid content increases during ripening, and irradiation can delay the ripening process (El-Samahy et al. 2000; D'Innocenzo & Lajolo 2001; Reyes & Cisneros-Zevallos 2007; Singh & Pal 2009). This may account in part for lower total carotenoid concentrations after a period of post-irradiation storage because the comparisons between irradiated and non-irradiated samples are between samples at different stages of ripeness. In some instances, irradiation appears to result in a higher carotenoid content when analysis was conducted near to the time of irradiation. This higher carotenoid concentration post-irradiation may be attributable to increased extraction efficiency or conversion of vitamin precursors to other forms (Diehl et al. 1991, Diehl 1992).

4.1.3 Aim of the nutrition assessment

The aim of this nutrition assessment is to evaluate the potential effect of the proposed

irradiation of blueberries and raspberries up to 1 KGy on the nutrient profile of these fruits.

4.2 Evaluation

4.2.1 Baseline nutrient profiles of blueberries and raspberries

Berries tend to have high sugar content, low fat content and a high amount of dietary fibre. They contain organic acids including citric, malic, tartaric, oxalic and fumaric acids and also certain minerals and vitamins; as well as phytochemicals such as phenolic compounds.

The vitamin content of blueberries and raspberries is highly variable and can depend upon plant factors such as cultivar as well as growing conditions such as geographical location and local weather conditions. Many researchers have shown the considerable influence that genotype has on factors such as ascorbic acid, total phenols and anthocyanins (Beekwilder et al. 2005; Kruger et al. 2011; de Souza et al. 2014). Stage of ripening and maturity and post-harvest storage and handling also influence nutrient content of berries. For example, a recent study showed that ascorbic acid content of raspberries and blueberries grown in subtropical areas of Brazil was much greater than in temperate production zones (de Souza et al. 2014). Song (2015) found considerable differences in the contents of total soluble solids and titratable acids and the ratio of sugars to acids among fifteen tested raspberry cultivars. Vitamin C content ranged from 6.86–10.60 mg/100 g (Skrovankova et al. 2015) found the ascorbic acid content of fresh raspberries generally ranges from 5–40 mg/100 g. Blueberries also contain relatively high and highly variable vitamin C content, ranging from 10–100 mg/100 g fresh fruit (de Souza et al. 2014).

Table 4.1 is taken from the 2014 FSANZ review of the nutritional impact of phytosanitary irradiation of fruits and vegetables and shows the variation in the concentration of a number of vitamins in fruits and vegetables (FSANZ 2014b).

Table 4.1: Concentration range of selected vitamins in fruits and vegetables

	Fruit or vegetable	β-carotene (µg/100 g)	Vitamin C (mg/100 g)	Folates (µg/100 g)	Vitamin E (mg/100 g)
Pome fruit	Apple	0–19	<1–35	0–3	0.1–1.3
	Pear	0–20	3–30	7	0–0.5
Stone fruit	Apricot	197–5170	3–16	6–9	0.9–1.2
	Peach	38–477	4–15	3–4	0.7–1.3
	Nectarine	12–362	4–14	5	0.8
	Cherry	26–56	7–25	4–6	0.1–0.4
	Plum	147–417	3–11	5	0.3–0.8
Berry fruit	Strawberry	0–6	23–185	12–96	0.3–0.4
	Blueberry	8–39	4–13	6–12	0.5–0.9
	Raspberry	0–28	7–41	21–34	0.4–0.9
Citrus fruit	Orange	46–6900	40–63	17–43	0.2–0.5
	Mandarin	56–19800	24–58	0–36	0–0.4
Tropical fruit	Mango	310–3900	12–135	43	0.9–1.3
	Banana	23–75	3–19	10–33	0.1–0.2
	Pineapple	10–60	17–68	5–19	<0.1
	Litchi	0	21–36	NA	NA
	Guava	380	129–248	NA	NA
Other fruit	Kiwifruit	43–54	26–206	25–39	0.9–1.5
	Melon	30–1960	5–50	19–21	<0.1–0.1
	Watermelon	20–427	11–24	0–3	<0.1–0.1
	Grape	0–91	0–7	0–4	0.2–0.5
Vegetables	Tomato	60–3500	1–72	2–42	0.3–0.7
	Capsicum	117–930	24–202	10–85	0–4.0
	Cucurbit	59–2710	2–30	0–41	0–1.4

FSANZ (2014)

4.2.2 Temporal changes in nutrients during ripening

The maturation and ripening of both blueberry and raspberry fruit causes changes in organoleptic properties as well as phytonutrient composition. Ripe fruit tends to have higher water content, decreased starch and increased sugar concentration, reduced acidity and altered pigment profile, as compared to unripe fruit. The effect of ripening on vitamin C levels vary between fruit and cultivar type with reports of increased, decreased or no change in vitamin C content. Similar variations in vitamin C levels have been found in strawberries, with increases reported during fruit development (Shin et al. 2008) or no change (Ferreira et al. 2007).

With respect to blueberry, fruit colour changes rapidly with ripening, with accompanying increases in anthocyanin pigment concentration and decreases in acid content (Forney 2009). Blueberry fruit firmness decreases as fruit matures. Highest total phenolics are found in immature fruit, with total phenolics in blue fruit being 30% higher than in turning fruit (Forney et al. 2012). Glucose and fructose are the main sugars in blueberries and both sugars increase several fold as fruit ripens, accounting for the detectable increase in sweetness (Forney et al. 2012). Antioxidant capacity, as measured by oxygen radical antioxidant capacity (ORAC) values, has been found to decline with fruit maturation and to show positive correlation with total phenolic content (Forney et al. 2012).

Ripening in raspberries is accompanied by a large production of apocarotenoids, which are metabolic breakdown products of carotenoids and important for the flavour characteristics of the fruit (Beekwilder et al. 2008). Kruger et al. (2011) found no effect of enhanced ripening or storage conditions on ascorbic acid content of raspberries. In that study the berries were

stored for one day at 20 °C room temperature or 3 days at 2– 4 °C followed by one day at room temperature. Other researchers have found significant ascorbic acid loss after 3 days at 4 °C (Mullen, 2002).

4.2.3 Impact of other forms of handling and processing on the vitamin content of fruit and vegetables

Fruit and vegetables continue to ripen after harvest. Storage can also affect vitamin levels with vitamin C susceptible to storage associated reduction in some fruits and vegetables. Storage conditions influence vitamin changes with temperature and atmospheric conditions being important considerations. In addition to storage considerations, processing also causes changes in nutrient composition of fruits. Berries are sold in fresh, frozen and processed forms. In Australia raspberries are consumed mainly processed or frozen. It is estimated that 82% of the total domestic supply is used in a processed form (freshlogic 2014b). Blueberries are purchased and consumed fresh, processed pulped or frozen and in manufactured goods such as desserts and baked goods. The majority of processed, pulped and frozen blueberries in Australia are imported and the proportion of locally grown fruit which is processed is decreasing as the domestic demand for fresh berries increases (freshlogic 2014a).

It is important to recognise that all food processing and handling practices are likely to lead to some changes in the vitamin content of fruit and vegetables. The major factors affecting the concentrations of vitamins in food include temperature, moisture, pH and light (Ottaway 2002). Fruits are commonly dried and canned, juiced and made into jams and included in beverages and yoghurts as well as baked goods. Table 4.2 summarises the effect that different processing methods have on the concentrations of vitamin C and carotenoids in berries and other fruits and vegetables. These data indicate that processes other than irradiation (including ripening) can lead to very large changes in vitamin content that outweigh any potential effects of irradiation.

Berries have a short harvest season and can only be stored for short periods under controlled atmospheric conditions. The concentration of ascorbic acid in blueberries decreases during storage depending on the conditions such as oxygen levels, temperature and light. Golding et al. (2014) reported that even short storage times greatly decrease ascorbic acid content and 10 days of refrigerated storage caused ascorbic acid decreases of about 73% compared with fresh blueberries. In frozen berries, vitamin C levels are reduced by about 30%, with greater losses (around 75%) associated with canning (FSANZ 2014b).

Recently another study (Phillips 2016) has reported on the stability of vitamin C in fruit and vegetables, including strawberries. Ascorbic acid can be decreased in raw foods in which endogenous enzymes are active, due to oxidative and enzymatic degradation. Cooking would be expected to denature enzymes and decrease further loss. Phillips et al. (2016) found that the stability of vitamin C was greatest in cooked foods (spinach, potatoes and broccoli) compared to raw foods but the extent of loss varied among foods. In this study no loss of vitamin C was found in any foods after seven days' storage in an ultra-low freezer (-60°C) but there were dramatic differences in the stability of vitamin C in different foods stored at refrigerator and conventional freezer temperatures (Phillips et al. 2016). Vitamin C in strawberries was stable over seven days in a conventional freezer but after seven days of refrigerated storage the amount was reduced by 20%.

A few studies have directly compared the effects of low-dose irradiation on the nutrient profile of fruit and vegetables with other forms of food processing. In one study, the retention of ascorbic acid and carotenoids in mango, papaya and litchi was greater after irradiation at 0.75–2 kGy (83–114%) than freezing (12–100%) or experimental canning (45–102%) (Beyers & Thomas 1979). Similarly in another study, the ascorbic acid, lycopene and β -

carotene content of grapefruit irradiated at 0.3 kGy was similar to control samples, however freeze-dried samples tended to have lower ascorbic acid, lycopene and β -carotene than both irradiated and control samples (Vanamala et al. 2005). Tezotto-Uliana et al. (2013) observed a decrease in ascorbic acid levels in raspberries due to irradiation and storage, with greatest reductions seen in higher irradiation doses (2 kGy) and longer storage times (after 8 days). These researchers concluded that 1 kGy was the optimal dose to reduce fruit decay and weight loss whilst minimising vitamin C loss. Golding et al. (2014) conclude that the length of time in cold storage has a much greater influence upon fruit quality and nutritional content of raspberry and blueberry than any low dose irradiation (1 kGy or less) treatment.

4.2.4 FSANZ review

FSANZ recently reviewed the impact of phytosanitary doses of irradiation on the nutritional quality of a wide range of fruit and vegetables in order to be able to make recommendations to amend data requirements for irradiation of fruits and vegetables (FSANZ 2014b). The conclusion was that doses of irradiation in the typical phytosanitary range from 0.15 to 1 kGy cause no effect on macronutrients or minerals. The report found that phytosanitary doses of irradiation had no effect on carotene levels in fruit and vegetables, did not decrease vitamin C levels in the majority of fruits and vegetables and had little effect on other non-vitamin bioactive compounds. The review recommended that focus be placed on Vitamin C for applications for irradiation of fruits and vegetables with requirements for other nutrients to be determined on a case-by-case basis.

Table 4.2: Changes in vitamin C and carotenoids in capsicums, tomatoes and vegetables generally through a range of processes

Fruit/vegetable	Processing Step	% Change	Reference
<i>Vitamin C</i>			
Capsicum	Ripening from green to red	+44	Martínez et al. (2005)
	Storage at 4 °C for 20 days, green fruit	-11	
	Storage at 4 °C for 20 days, red fruit	-16	
	Storage at 20 °C for 20 days, red fruit	-25	
	Water blanching	-12	
	Freezing	-40	
	Drying	-88	
Capsicum	High Pressure Processing, 200 MPa, 20 minutes, green fruit	-20	Castro et al. (2008)
	High Pressure Processing, 200 MPa, 20 minutes, red fruit	+15	
Tomato	Ripening from pink to red	+60	Periago et al. (2009)
Blueberries	Storage at 4 °C for 10 days	-37	Golding et al. (2014)
Raspberries	Storage for a week	-44	Kalt et al. (1999)
Vegetables (all types)	Frying	-5 to -50	Bell et al. (2006)
	Baking	-5 to -50	
	Boiling	-5 to -80	
<i>Carotenoids</i>			
Tomatoes	Canning	-13	Rickman et al. (2007)
Vegetables (all types)	Frying	-10 to -15	Bell et al. (2006)
	Baking	0 to -20	
	Boiling	-5 to -20	

Adapted from FSANZ (2014b)

4.2.5 Published studies

The applicant also reviewed the existing literature relating to changes in nutrient content due to phytosanitary irradiation of blueberries and raspberries and reported on four major studies, including one carried out by the Department of Primary Industries (Golding et al. 2014). These findings were made available to FSANZ during the writing of the literature review and were incorporated into the analysis and interpretation.

The applicant found that there were few changes in the nutritional content of raspberry fruit following irradiation and storage. Some nutrients, such as sucrose, ascorbic acid and citric and malic acid were affected by irradiation but the measured changes were inconsistent and minor. For ascorbic acid the mean concentration for fruit irradiated at 0.15 kGy was similar to that for untreated fruit. The mean concentration of ascorbic acid for raspberries irradiated at 0.4 kGy and 1 kGy were lower than for the untreated and 0.15 kGy irradiated fruit. No interaction was seen between irradiation treatment and storage time, indicating that irradiation did not alter the storage effects on raspberry fruit nutrient content. Golding et al. (2014) found no effect of phytosanitary irradiation on the nutritional content of northern highbush (cv Brigitta) blueberry. The length of time in storage did have an effect on a number of nutrients, including ascorbic acid, with longer storage times resulting in lower concentration. These researchers draw attention to lack of effect of irradiation on blueberry ascorbic acid and total monomeric anthocyanin levels, and point out that whilst blueberry ascorbic acid concentration did decrease significantly with storage, no interaction with irradiation level was seen.

The literature shows that a number of factors are important in understanding any possible influence of irradiation upon nutritional quality of blueberries and raspberries:

- extensive natural variation exists in individual fruit and vegetable nutrient content due to factors such as cultivar, growing season, location and degree of ripeness. Substantial data documents significant differences being common between cultivars.
- post-harvest storage and processing also influence phytonutrient composition. Vitamin C levels decrease significantly with storage for many fruit.
- whilst blueberries and particularly raspberries are rich in vitamin C they are consumed in relatively small amounts in the Australian and New Zealand diet.
- berries are also often consumed in a frozen or processed form (or after storage), all of which significantly decrease vitamin C levels. Raspberries for example are mainly consumed as a processed fruit and it is not expected that fruit that will undergo processing would first be irradiated. Whilst some studies have shown that low doses of irradiation appeared to increase rate of loss of vitamin C, other studies showed that radiation mitigated or prevented storage related loss.

It would appear that losses of vitamin C due to normal maturation, storage, handling and processing of blueberries and raspberries are generally larger than losses due to phytosanitary doses of irradiation.

4.3 Conclusion

The published literature shows that irradiation up to 1 kGy does not substantially impact the nutritional quality of fruits and vegetables. Whilst vitamin C levels can be reduced by irradiation, the extent of this reduction is generally similar to, or less than that caused by storage or other forms of post-harvest handling and processing.

Response to Question 4: *Does irradiation affect the nutrient composition of blueberries and raspberries?*

Irradiation at doses of up to 1 kGy appears to have no consistent effect on the levels of irradiation potentially sensitive vitamins or provitamins (β -carotene and vitamin C) or the nutrient composition of blueberries and raspberries. There is limited and conflicting evidence of some losses of vitamin C in irradiated berries, but these reported reductions fall well within the range of vitamin losses that normally occur during the storage of non-irradiated fruit.

Response to Question 5: *If so, how does this effect compare to effects from other post-harvest and processing procedures?*

Post-harvest storage, and processing techniques other than irradiation such as cooking, drying or freezing have been demonstrated to have a larger impact on the nutrient composition of fruits and vegetables than irradiation.

5 Dietary Intake Assessment

Based on the literature review conclusions and recommendations and considering the provided data, a full dietary intake assessment was not required.

Response to Question 6: *Taking into account potential market share and trade of the irradiated requested berries, in both Australia and New Zealand, would any changes in the nutrient composition of these fruits, following irradiation, have the potential to affect the nutritional adequacy of diets for Australian and New Zealand populations?*

There is negligible potential for consumption of irradiated berries to affect the nutritional adequacy of the Australian and New Zealand populations.

Response to Question 7: *What are the combined cumulative nutritional effects on the nutritional adequacy of diets for Australian and New Zealand populations from irradiation of both the currently permitted irradiated foods and requested berries?*

Mean population intakes of vitamin A and C are estimated to decrease by 2% or less if all fresh tomatoes, capsicums and tropical fruits for which irradiation is already permitted were to be irradiated such that vitamin concentrations declined by 15%. As not all of these foods would be irradiated, any decrease in intakes would be less than this. Given that consumption of raspberries and blueberries in Australia and New Zealand is reasonably modest and tends to be either seasonal (fresh berries) or to be comprised of processed berries (unlikely to be irradiated) it is not expected that the combined cumulative risk would significantly change.

6 Risk Characterisation

There are negligible risks to public health and safety associated with the consumption of blueberries and raspberries which have been irradiated at doses of up to 1 kGy.

The low lipid content of blueberries and raspberries (<0.2%) means that there is a low potential for the generation of 2-ACBs. The weight-of-evidence, supported by new published data, indicates that 2-ACBs are not genotoxic.

Furan, a volatile genotoxic carcinogen found in some non-irradiated foods, has been either not detected, or detected at only low levels in a range of other fruits irradiated at 5 kGy,

which is five-times higher than the maximum dose sought in this Application. It is likely that furan levels are undetectable in blueberries and raspberries irradiated at doses of up to 1 kGy.

Irradiation of blueberries and raspberries at doses of up to 1 kGy appears to have no consistent effect on the levels of vitamins or provitamins that are potentially sensitive to irradiation. There is limited and conflicting evidence of some losses of vitamin C in irradiated berries, but these reported reductions fall well within the range of vitamin losses that normally occur during the storage and processing of non-irradiated fruit. There is therefore negligible potential for the consumption of irradiated blueberries and raspberries to affect the nutritional adequacy of the Australian and New Zealand populations.

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