FOOD STANDARDS Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

Supporting Document 1

RISK ASSESSMENT REPORT

Executive Summary

FSANZ has previously assessed the toxicological hazard and nutritional adequacy of various irradiated tropical fruits and concluded that there are no public health and safety issues associated with their consumption when irradiated up to a maximum dose of 1 kGy. The current application seeks to extend the existing permission for the irradiation of tropical fruits to include persimmons.

The purpose of this risk assessment was to determine whether persimmons irradiated up to a maximum dose of 1 kGy are as safe as non-irradiated persimmons. FSANZ has approached this assessment by independently evaluating new data relevant to the safety of irradiated food published since its most recent risk assessment. Also taken into consideration were compositional data on irradiated persimmons and the level of consumption of persimmons in Australian and New Zealand.

It is concluded that there are no public health and safety issues associated with the consumption of persimmons, which have been irradiated up to a maximum dose of 1 kGy. This conclusion is based on the following considerations:

- Supplementary data published since 2002 have confirmed that 2-alkylcyclobutanones (2-ACBs) are not genotoxic.
- Available data indicate that the carbohydrate, fat, protein and mineral content of foods are unaffected by irradiation at doses up to 1 kGy. Therefore, irradiation is unlikely to affect the presence of macronutrients and minerals in persimmons.
- While the concentrations of certain vitamins may be decreased as a result of the irradiation process, because persimmons are not widely consumed in Australia and New Zealand they contribute minimally to the total dietary intake of vitamins. Therefore, there are unlikely to be any nutritional disadvantages for Australian and New Zealand consumers from eating irradiated persimmons.
- New data indicates that compounds previously considered to be uniquely formed during food irradiation (i.e. 2-ACBs) are also present in some non-irradiated foods.
- The irradiation of several tropical fruits, are already permitted in Australia and New Zealand. FSANZ has not previously identified any public health and safety issues associated with the consumption of these or other permitted irradiated foods.
- The safety of irradiated food has been extensively assessed by national regulators and international scientific bodies.
- There is a history of safe consumption of irradiated food in many countries.

Abbreviations

Time		**Weight**	
sec	Second	bw	Bodyweight
min	Minute	wt	Weight
d	Day	ng	Nanogram
wk	Week	µg	Microgram
mo	Month	mg	Milligram
yr	Year	kg	Kilogram
Length		**Dosing**	
nm	Nanometre	iv	Intravenous
µm	Micrometre	po	Oral
mm	Millimetre	mg/kg bw/day	mg/kg bodyweight/day
cm	Centimetre	Gy	Gray
m	Metre	kGy	Kilogray
Volume		**Concentration**	
µL	Microlitre	M	Molar
mL	Millilitre	ppb	Parts per billion
L	Litre	ppm	Parts per million
		w/v	Weight per volume
		v/v	Weight per weight

Chemistry	
2-ACBs	2-alkylcyclobutanones
2-HCB	2-hexyl-cyclobutanone
2-OCB	2-octyl-cyclobutanone
2-DCB	2-decyl-cyclobutanone
2-dDCB	2-dodecyl-cyclobutanone
2-dDeCB	2-(dodec-5'-enyl)-cyclobutanone
2-tDCB	2-tetradecyl-cyclobutanone
2-tDeCB	2-(tetradec-5'-enyl)-cyclobutanone
2-tD2eCB	2-(tetradeca-5',8'-dienyl)-cyclobutanone
DMSO	Dimethyl sulfoxide
FISH	Fluorescent-*in-situ*-hybridisation
GC-MS	Gas chromatography-mass spectrometry
SCE	Supercritical CO_2 extraction
SGC	Silica gel chromatography
TLC	Thin layer chromatography
Terminology	
ACF	Aberrant crypt foci
EC	European Commission
FAO	Food & Agricultural Organisation of the United Nations
GIR	Good irradiation practice
GMP	Good manufacturing practice
IAEA	International Atomic Energy Agency
NCS	Australian National Nutrition Survey
NNS	Australian National Children's Nutrition & Physical Activity Survey
NZCS	New Zealand Children's Nutrition Survey
NZS	New Zealand Adult Nutrition Survey
SD	Sprague Dawley (rats)
WHO	World Health Organisation

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1. Introduction

1.1 Background

On the 19th November 2009, Food Standards Australia New Zealand (FSANZ) received an Application from the Queensland Government (Queensland Primary Industries and Fisheries) seeking an amendment to Standard 1.5.3 – Irradiation of Foods of the *Australia New Zealand Food Standards Code* (the Code) to permit the irradiation of persimmons as a quarantine measure. The proposed irradiation dose is a minimum of 150 Gy and a maximum of 1 kGy, which is within the range of the existing permission for the irradiation of selected tropical fruits.

1.2 Risk Assessment Questions & Scope

The irradiation of persimmons would extend the current permission for the irradiation of tropical fruit in the Code. On this basis, an updated risk assessment is appropriate, taking into consideration any new data published since FSANZ's previous (2002) risk assessment prepared in relation to Application A443 – Irradiation of tropical fruits.

For this Application, the risk assessment questions were developed in the context of the Section 18 Objectives of the *Food Standards Australia New Zealand Act 1991*.

The following risk assessment questions are addressed in this Risk and Technical Assessment Report:

- When persimmons are irradiated, are there any new compounds formed that may impact on public health and safety?
- As a form of food processing, what impact does irradiation have on the nutritional composition of persimmons?
- Would a change in the nutrient composition of persimmons from irradiation affect the nutritional adequacy of Australian and New Zealand diets containing persimmons?

This Risk Assessment Report is structured to address the above questions in order and comprises the following components:

(1) Hazard Assessment, which evaluated whether the irradiation of persimmons at the proposed levels could generate hazardous compounds.

(2) Nutrition Assessment, which evaluated whether irradiation at the proposed levels would significantly alter the nutritional composition of persimmons.

(3) Dietary Exposure Assessment, which examined whether there would be any nutritional disadvantages for consumers who consume irradiated persimmons.

Based on these three assessment components, the risk to public health and safety has been characterised.

2. Hazard Assessment

2.1. Background

2.1.1 Previous assessments of irradiated foods by FSANZ

The risk assessments conducted in relation to Applications A413 and A443 concluded that there are no health and safety issues for consumers associated with the irradiation of herbs and spices, and various tropical fruits (breadfruit, carambola, custard apple, lychee, longan, mango, mangosteen, papaya and rambutan) at 2-30 kGy and 50 Gy to 1 kGy, respectively, according to Good Manufacturing/Irradiation Practices (GMP and GRP, respectively).

It is nearly ten years since FSANZ conducted the risk assessments on the safety of irradiated foods for Applications A413[1] and A443[2] (completed in 2001 and 2002, respectively). FSANZ has considered additional evidence on the safety of irradiated foods by examining new scientific publications from the period 2002 to the present day.

2.1.2 Assessments by other agencies & 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). The consensus of scientific opinion is that irradiated food is safe when irradiated at doses necessary to achieve the intended technological function and in accordance with GRP.

2.1.3 Compounds generated in irradiated foods

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) (Sin et al 2006; 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. thermal processing).

Furan can be formed in thermally-processed and irradiated foods, and is derived predominantly from sugars (e.g. glucose, fructose and sucrose) and ascorbic acid (Vranova & Ciesarova 2009). While there are no data specifically on furan levels in irradiated persimmons, Fan and Sokorai (2008a) determined that the irradiation of a variety of cut, fresh fruit at 5 kGy (i.e. 5 times higher than that proposed in the current application) produced only low levels of furan in grape and pineapple, while furan levels were not detectable or < 1 ng/g in apple, banana, strawberry, watermelon, rock melon and honeydew melon. Given the similarity of the sugar and ascorbic acid content of persimmons relative to these fruits, any furan formation in irradiated persimmons is likely to be negligible. In addition, as furan is a highly volatile compound (its boiling point is 31.4ºC) it is likely to rapidly evaporate from fruit.

2-ACBs have to date been considered a novel class of compounds formed as a result of the radiolysis of triglycerides, phospholipids and fatty acids in food (Sommers et al 2007). Table

[1] http://www.foodstandards.gov.au/foodstandards/applications/applicationa413irradiationofherbsandspices/index.cfm
[2] http://www.foodstandards.gov.au/foodstandards/applications/applicationa443irradiationoftropicalfruit/index.cfm

2.1 summarises the different types of 2-ACBs that may be formed and the fatty acids from which they are derived. Obviously the types and concentrations of 2-ACBs in irradiated food would depend on the lipid content and composition of the non-irradiated food in addition to the irradiation dose. Given the linear relationship between the irradiation dose and concentration of 2-ACBs formed, some jurisdictions have adopted the measurement of 2-ACBs as a voluntary standard for the detection of irradiated foods (European Committee for Standardisation 2003).

Perceived health concerns over 2-ACBs stems from their apparently unique formation in irradiated foods and a minority of positive responses in non-standard, *in vitro* genotoxicity assays. It is important to note that the majority of genotoxicity assays conducted using validated protocols have been negative. In addition, the weight-of-evidence from numerous (hundreds) of laboratory animal studies indicates that the long-term consumption of irradiated foodstuffs (that would contain low concentrations of 2-ACBs and other radiolytic compounds) is safe.

Table 2.1: Types of 2-ACBs potentially formed during food irradiation

Fatty Acid		2-ACB
C 10:0	Capric acid	2-hexyl-cyclobutanone (2-HCB)
C 12:0	Lauric acid	2-octyl-cyclobutanone (2-OCB)
C 14:0	Myristic acid	2-decyl-cyclobutanone (2-DCB)
C 16:0	Palmitic acid	2-dodecyl-cyclobutanone (2-dDCB)
C 16:1	Palmitoleic acid	2-(dodec-5'-enyl)-cyclobutanone (2-dDeCB)
C 18:0	Stearic acid	2-tetradecyl-cyclobutanone (2-tDCB)
C 18:1	Oleic acid	2-(tetradec-5'-enyl)-cyclobutanone (2-tDeCB)
C 18:2	Linoleic acid	2-(tetradeca-5',8'-dienyl)-cyclobutanone (2-tD2eCB)
C 18 :2	Linolenic acid	2-(tetradeca-5'8'11'-trienyl)-cyclobutanone (2-tD3eCB)

Adapted from Sommers et al (2007)

Since FSANZ's most recent (2002) consideration of 2-ACBs undertaken as part of Application A443, the European Commission's (EC) Scientific Committee on Food (2002), the WHO (2003) and Health Canada (2008) have evaluated the toxicological significance of 2-ACBs. These evaluations have concluded that, based on the current scientific evidence, 2-ACBs in irradiated foods do not pose a health risk to consumers.

In addition to the above considerations, it is worth noting that due to the very low total lipid content of persimmons (0.2%) (FSANZ 2006), there is only limited potential to generate 2-ACBs during irradiation. The total lipid content of persimmons is also lower than that of custard apple (0.6%) and rambutan (0.4%), and comparable to that of lychee (0.1%), mango (0.2%) and papaya (0.1%) (FSANZ 2006); as mentioned above, these fruits have previously been assessed by FSANZ as safe for consumers when irradiated up to 1 kGy.

2.1.4 Scope of the hazard assessment

The scope of this hazard assessment is to evaluate relevant data published after FSANZ's most recent (2002) evaluation of irradiated foods, paying particular attention to any new data on 2-ACBs.

2.2 Evaluation of Supplementary Data on 2-ACBs

FSANZ has independently evaluated supplementary studies published since 2002. In addition to those studies submitted by the Applicant [marked with an asterix (*)], FSANZ identified a number of additional relevant studies.

2.2.1 Metabolism studies

*Horvatovich P, Raul F, Miesch M, Burnouf D, Delincée H, Hartwig A, Werner D & Marchiono E (2002) Detection of 2-alkylcyclobutanones, markers for irradiated foods, in adipose tissues of animals fed with these substances. Journal of Food Protection **65**(10): 1610-1613.

2-(tetradec-5'-enyl)-cyclobutanone (2-tDeCB) or 2-tetradecyl-cyclobutanone (2-tDCB) (synthesised by the performing laboratory) in 1% ethanol was administered to groups of six, male Wistar rats (260-270 g bodyweight; age and source unspecified) via the drinking water at a concentration of 0 or 0.005% daily for 4 months. All groups, including the control, were offered the same non-irradiated diet *ad libitum*. Drinking water containing the test compounds was prepared 'freshly' (specific details not given). Water consumption was recorded daily; the authors stated that the daily intake of both test compounds was 1 mg/day (~4 mg/kg bw/day). Bodyweight was recorded weekly. Faeces were collected from each rat on the last three days of the experimental period over approximately a 12 h period. Samples from each rat were pooled in order to obtain at least 10 g of material for analysis. Adipose tissue was collected from each rat. Following hexane extraction of samples, 2-tDeCB and 2-tDCB were analysed by gas chromatography-mass spectrometry (GC-MS).

Bodyweight gain to the end of the dosing period was unaffected by treatment. The concentrations of 2-tDeCB and 2-tDCB in faeces and adipose tissue are summarised in Table 2.2 and indicate higher concentrations of 2-tDCB than 2-tDeCB in faeces and fat. The authors stated that the daily recovery of 2-tDCB and 2-tDeCB from faeces was 0.3 and 0.1%, respectively, of the administered dose. This low recovery could indicate extensive metabolism in the digestive tract or absorption and distribution to other tissues. Alternatively, it may be the result of poor extraction efficiency or instability of the test compounds; support for this possibility comes from the study of Hijaz (2010), where poor recovery occurred in spiked control samples using the same extraction solvent (i.e. hexane). Without data on the bioavailability of these compounds, their concentration in blood, bile or other tissues, metabolism, and as the results represent a single, point measurement at the end of a 4-month treatment period, no firm conclusions can be drawn. In particular, it is unclear what proportion of the absorbed dose is actually present in adipose tissue.

Table 2.2: Concentration of 2-tDeCB and 2-tDCB in rat faeces and adipose tissue

Treatment	2-tDeCB (µg/g)		2-tDCB (µg/g)	
	Faeces	Fat	Faeces	Fat
Control	ND	ND	ND	ND
2-tDeCB	0.5±0.2	0.07±0.03	ND	ND
2-tDCB	ND	ND	1.0(0.2)	0.31±0.08

Results are expressed as the mean ± 1 SD (n=3); ND = not detected.

*Gadgil P & Smith JS (2006) Metabolism by rats of 2-dodecylcyclobutanone, a radiolytic compound present in irradiated beef. J. Agric. Food Chem. **54**: 4896-4900.

2-dodecylcyclobutanone (2-DCB) was administered by gavage to 6 female Sprague-Dawley (SD) rats (sourced from Harlan, Indianapolis, USA; 200-250 g bodyweight; age unspecified) in corn oil at 5 mg/day for 5 days (~20-25 mg/kg bw/day). A control group of 6 rats received corn oil only. Urine and faeces were sampled daily and pooled for each rat (day 1-5 urine and day 3-5 faeces). Adipose tissue was sampled following sacrifice and pooled for the treatment and control groups. Hexane extracts of pooled urine, faeces and adipose tissue were analysed for 2-DCB and its metabolites by GC-MS. Urine was treated with or without β-glucuronidase prior to extraction to determine whether 2-DCB had been glucuronidated.

Approximately 3-11% of 2-DCB was recovered in faeces after 5 days and approximately 0.33% in adipose tissue. Analysis of faeces by Hijaz et al (2010) (see below) identified 2-

dodecylcylobutanol as a metabolite. No 2-DCB or metabolites were detectable in urine. The poor recovery in faeces and adipose tissue suggests that 2-DCB is rapidly metabolised and eliminated, or is stored in tissues other that fat. Alternatively, this finding could also be due to poor extraction efficiency or instability of 2-DCB and its metabolites. The absence of the analysis of blood levels of 2-DCB or the analysis of other tissues are limitations to this study.

Hijaz F, Shrestha TB, Bossman SH, Hussain F & Smith JS (2010) *In vitro* and *in vivo* metabolism of radiolytic compound 2-dodecylcyclobutanone. Toxicology & Chemical Food Safety **75**(4): T72-80.

The metabolism of 2-DCB by S9 liver preparations from Aroclor 1254-induced rats was analysed by GC-MS following incubation for 2 hours at 37°C. Metabolites present in rat excreta collected in the preceding study (Gadgil & Smith 2006) were also analysed by GC-MS. Recovery of 2-DCB in the *in vitro* control samples was relatively poor and averaged ~88%, 67% and 50% in three separate experiments. This low recovery suggests instability of 2-DCB or a low extraction efficiency (noting that hexane was used to extract samples). In the corresponding test samples, recovery was ~60%, 12% and 23%, respectively. The difference in recovery between the test and control samples of ~28%, 55% and 27% indicates that 2-DCB was partially metabolised by S9. GC-MS analysis indicated that 2-DCB was metabolised to 2-dodecylcyclobutanol *in vitro* in the presence of NADPH. Analysis of excreta identified 2-dodecylcylobutanol in rat faeces. No metabolites were detected in urine.

2.2.2 Genotoxicity studies

Table 2.3 summarises the results of genotoxicity assays on various 2-ACBs. Positive and negative (vehicle) controls were tested in most studies and gave expected results.

Table 2.3: Summary of genotoxicity studies on 2-ACBs

Test	Test system	Test article	Concentration or dose range	Result	Reference
Bacterial reverse mutation (Ames test)	*Salmonella typhimurium* strains TA98, TA100, TA1535 & TA1537 (±S9)	2-DCB (>95% purity; Sigma-Aldrich) DMSO vehicle	0, 0.25, 0. 5, 1 & 2 mg/mL	Negative No cytotoxicity	*Sommers & Schiestl (2004)
	S. typhimurium strains TA97 TA98, TA100, TA102 & TA1535 (±S9)	2-DCB Sigma-Aldrich) DMSO vehicle	0.25, 0.5, 0.75 & 1.0 mg/plate	Negative Cytotoxicity unreported	*Gadgil & Smith (2004)
	S. typhimurium strains TA97 TA98, TA100 (±S9)	2-DCB 2-dDCB 2-tDCB Ethanol vehicle	4-400 µM	Negative Cytotoxicity with 2-DCB & 2-dDCB	*Hartwig et al (2007)
Bacterial reverse mutation	*E. coli* WP2 (pkM101) WP2 *E. coli* WP2 *uvrA* (pKM 101) (±S9)	2-DCB (>95% purity; Sigma-Aldrich) DMSO	0, 0.05, 0.1, 0.5 & 1 mg/well	Negative No cytotoxicity	Sommers (2003)

Test	Test system	Test article	Concentration or dose range	Result	Reference
		vehicle			
Bacterial forward mutation	*E. coli* SF1 umuDC::lacZ Tn10::tolC (±S9)	2-DCB (Sigma-Aldrich) DMSO vehicle	125-1000 µg/mL	Negative Cytotoxicity at 1000 µg/mL	Sommers & Mackay (2005)
Mammalian mutation	Human TK-6 $tk^{\pm}$ lymphoblasts	2-DCB DMSO vehicle	0, 0.018, 0.036, 0.062 mg/mL	Negative	*Sommers (2006)
Intrachromosomal recombination (DEL assay)	*Saccharomyces cerevisiae* strain RS112	2-DCB (>95% purity; Sigma-Aldrich) DMSO vehicle	0, 0.63, 1.25, 2.5 & 5 mg/mL	Negative Concentration-related cytotoxicity	*Sommers & Schiestl (2004)
Comet Assay	HT29 (human adenocarcinoma cell line) HT29clone19A 0.5, 24 or 48 h incubation time	2-tDCB Ethanol vehicle	25-400 µM	Negative No cytotoxicity after 30 min. Concentration-related cytotoxicity at 24 and 48 h incubation	*Delincée et al (2002)
	HT29clone19A; LT97 (human colorectal adenoma cell line) Human primary colon epithelial cells 30, 60, 90 & 120 min incubation times	2dDCB Ethanol vehicle No positive control	150-2097 µM	Positive in LT97 cells & primary colon epithelial cells Concentration-related cytotoxicity[1]	Knoll et al (2006)
	HT29 cells HT29clone19A 30 min incubation time	2-DCB 2-dDCB 2-tDCB 2-tDeCB Ethanol vehicle	25-400 µM	Negative Cytotoxicity[2]	*Hartwig et al (2007)
In vitro alkaline unwinding assay	HT29 cells (2-DCB only) HeLa cells 24 h incubation time	2-DCB 2-dDCB 2-tDCB 2-tDeCB Ethanol vehicle	48-190 µM 42-168 µM 38-340 µM 95-227 µM	Positive in HeLa cells at cytotoxic levels[3]	*Hartwig et al (2007)
In vitro chromosomal	LT97 human adenoma cell line	2dDCB	30, 75 & 150 µM	Positive at 75 & 150 µM	Knoll et al (2006)

Test	Test system	Test article	Concentration or dose range	Result	Reference
aberration assay - FISH	(-S9 only)	Ethanol vehicle		Concentration-related cytotoxicity[1]	

DMSO = dimethyl sulfoxide; FISH – Fluorescent-*in-situ*-hybridisation; ±S9 study conducted in the presence and absence of an exogenous source of metabolic activation (S9 liver preparations from Aroclor 1254-induced rats).
1 = Concentration and time-dependent cytotoxicity, which occurred at every concentration in LT97 and primary colon cells. The relative sensitivity of the cell lines to 2dDCB-induced cytotoxicity was HT29clone19a<LT97<primary colon cells. Graphically-presented data indicated that 2dDCB was not genotoxic in HT29clone19A cells. In LT97 cells, there was an increase in tail intensity of ~5% following 30 minutes and ~15% following 60 minutes incubations. However, as only two data points were shown on the graph (150 and 300 µM plus the vehicle control) and in the absence of a concentration-related increase in tail intensity, it is not possible to interpret these findings as positive. Further, the absence of a positive control compound limits the validity of the assay. In primary colon cells, there was an increase in tail intensity of approximately 10% relative to the vehicle control but no concentration-related increase from 150 to 300 µM. Again, these results are not interpretable as positive.
2 = Relative cytotoxicity: 2-tDCB<2-dCB<2-DCB (25-400 µM)
3 = Positive generally only at cytotoxic concentrations. 2-tDCB positive in HeLA cells at non-cytotoxic concentrations.

2.2.3 Carcinogenicity study

Raul F, Gossé F, Delincée H, Hartwig A, Marchioni E, Miesch M, Werner D & Burnouf D (2002) Food-borne radiolytic compounds (2-alkylcyclobutanones) may promote experimental colon carcinogenesis. Nutrition & Cancer **44**(2): 188-191.

Experimental

2-tDCB or 2-tDeCB (synthesised by the performing laboratory) in 1% ethanol was administered daily to groups of male Wistar rats (12/group) via the drinking water at a concentration of 0 or 0.005% for up to approximately 6 months. Bodyweight at the commencement of dosing was 260-270 g. The age and source of the rats were unspecified. All groups, including the control, were offered the same non-irradiated diet *ad libitum*. At weeks 3 and 4, all rats were given a single intraperitoneal injection of the carcinogen, azoxymethane, at 15 mg/kg bw (azoxymethane sequentially induces pre-neoplastic then neoplastic lesions in the rat colon). Water consumption was recorded daily; the authors stated that the daily intake of both test compounds was ~1.6 mg/day (~6 mg/kg bw/day). Bodyweight was recorded weekly. Six rats/group were sacrificed 3 and 6 months after the last injection of azoxymethane and their colons examined for pre-neoplastic and neoplastic lesions. The following parameters were recorded: total number of aberrant crypts (i.e. preneoplastic lesions) and aberrant crypt foci (ACF) in the distal colon; number and size of tumours in the colonic mucosa. Results were statistically analysed.

Findings

No signs of toxicity were reported for either test group. Bodyweight gain was unremarkable.

In rats sacrificed 3 months after injection with azoxymethane, there was no difference in the number of ACF/cm colon or in the total number of ACF between the treated and control groups. In rats sacrificed 6 months after injection with azoxymethane, the mean total number of aberrant crypts was increased in the treated groups relative to the control (218±39, 261±42 and 394±37 in the control, 2-tDCB and 2-tDeCB groups, respectively) but only the increase in the 2-tDeCB group was statistically significant ($p<0.05$).

Six months after injection with azoxymethane, the proportion of rats with the number of aberrant foci per ACF >4 was significantly higher ($p<0.05$) in the 2-tDeCB group than the control (22±3 versus 11±2%, respectively) concomitant with a reduction in the proportion of rats with 1 aberrant crypt/ACF (12±2 versus 24.5±4, respectively). There were no differences in the number of aberrant crypts/ACF in the 2-tDCB group compared to the

control or in the proportion of rats with 2, 3 or 4 aberrant crypts/ACF in the 2-tDeCB group.

Tumours were only detected in rats sacrificed 6 months after injection with azoxymethane; there was no significant difference in tumour incidence between treated and control groups. However, the total number of tumours, the occurrence of multiple tumours and of large tumours (i.e. >25 mm^3) was increased in the two treatment groups (Table 2.4).

Table 2.4: Tumour incidence in rats 6 months after azoxymethane treatment

Parameter	Control	2-tDCB	2-tDeCB
N	6	6	6
No. rats with tumours	4	5	4
No. rats with 1 tumour	4	1	1
No. rats with 2-5 tumours	0	4	3
Total tumours	4	14	13
No. rats with 1-4 large tumours (>25 mm^3)	0	3	3

Conclusion

2-tDeCB (but not 2-tDCB) increased the number of pre-neoplastic lesions in an experimental rat model of colon carcinogenesis. In addition, both compounds increased the total number and size of tumours after 6 months of exposure. However, the interpretation of these results is made difficult by the small group sizes, relatively short exposure duration and absence of additional negative controls to allow an assessment of the background incidence of pre-neoplastic and neoplastic lesions (i.e. without 2-tDCB/2-tDeCB and azoxymethane treatment; 2-tDCB/2-tDeCB without azoxymethane treatment). The relevance of this particular rat model to the dietary risk assessment of 2-ACBs is unclear; given the large number of rat studies already conducted on irradiated food indicating a lack of carcinogenicity, this study is considered to have limited regulatory value.

2.2.4 Analysis of 2-ACBs in food

Variyar PS, Chatterjee S, Sajilata MG, Singhal RS & Sharma A (2008) Natural existence of 2-alkylcyclobutanones. Journal of Agriculture & Food Chemistry **56**: 11817-11823.

2-ACBs were analysed in irradiated and non-irradiated cashew nuts and nutmeg by GC-MS. Various extraction methods were used to prepare the samples for analysis including Soxhlet extraction [extraction with hexane followed by silica gel chromatography (SGC)], supercritical CO_2 extraction (SCE) and thin layer chromatography (TLC). 2-dDCB, 2-tDCB and 2-tDeCB were detected in non-irradiated cashews, while 2-DCB and 2-dDCB were detected in non-irradiated nutmeg (Table 2.5). The sample extraction method appears to have determined whether these 2-ACBs were detectable. This is the first published report of the apparently natural occurrence of 2-ACBs in non-irradiated foods.

Table 2.5: Analysis of 2-ACBs in cashew nuts

Sample	Concentration (µg/g)			
	2-DCB	2-dDCB	2-tDCB	2-tDeCB
Cashews				
Non-irradiated; Soxhlet extraction + SGC	-	ND	ND	ND
Irradiated (1 kGy); Soxhlet extraction + SGC	-	0.95±0.4	ND	ND
Non-irradiated; SFE	-	ND	ND	ND
Irradiated (1 kGy); SFE	-	0.30±0.1	0.13±0.06	ND
Non-irradiated; SFE + TLC	-	2.70±1.71	1.0±0.08	0.52±0.01
Irradiated (1 kGy); SFE + TLC	-	6.12±0.82	2.06±0.4	0.8±0.1
Fresh from farm, shelled, non-irradiated; SFE + TLC	-	1.67±0.62	0.9±0.12	ND
Nutmeg				
Non-irradiated; SFE extraction + TLC	2.67±0.21	0.58±0.19	-	-
Irradiated (5 kGy); SFE + TLC	6.79±0.32	1.74±0.23	-	-

Results are expressed as the mean ± 1 SD (n=6); ND = not detected; SGC = silica gel chromatography; SFE = supercritical fluid extraction; TLC = thin layer chromatography

There were a number of other studies that analysed the concentration of 2-ACBs in different foods. The results of these studies are briefly summarised in Table 2.6.

Table 2.6: Summary of other studies

Reference	Findings
Gadgil et al (2002)	• Dose-related increase in the formation of 2-ACBs following irradiation of ground beef patties. • 2-DCB was detected at every target dose (0.5-7.0 kGy). More 2-DCB was produced using electron beam irradiation than γ-irradiation above 2.5 kGy. • 2-TDCB was detected at and above 5.0 kGy. • No 2-ACBs were detected in non-irradiated beef patties.
*Kim et al (2004)	• Various hydrocarbons and 2-ACBs were detected in irradiated (but not non-irradiated) dried shrimp. • The main hydrocarbons were 1-tetradecene and pentadecane, derived from palmitic acid. Other hydrocarbons included heptadecane and 1-hexadecene, derived from stearic acid, and 8-heptadecene and 1,7-hexadecadiene derived from oleic acid. • 2-tDeCB was the main 2-ACB detected. Other 2-ACBs detected included 2-DCB and 2-tDCB. • There was a generally linear relationship between the irradiation dose and concentrations of these hydrocarbons and 2-ACBs.
Gadgil et al (2005)	• There was a dose-related increase in 2-DCB in ground beef patties following electron beam irradiation at 1.0, 2.0, 3.0 or 4.5 kGy. • There was no difference in 2-DCB formation between patties containing 15% or 25% fat. • Non-irradiated beef patties were not analysed.
Horvatovich et al (2005)	• Monounsaturated alkyl side chain 2-ACBs (*cis*-2-dDeCB and *cis*-2-tDeCB) were detected in liquid whole egg, sheep cheese, poultry and avocado samples irradiated at 0.5-5 kGy, 100 kGy, 0.5-10 kGy and 0.1-1 kGy, respectively. • There was a linear relationship between the radioactive dose and the concentration of *cis*-2-dDeCB and *cis*-2-tDeCB. • *cis*-2-dDeCB and *cis*-2-tDeCB were not detectable in non-irradiated samples. • Decomposition (~50%) of *cis*-2-dDeCB, *cis*-2-tDeCB, 2-dDCB and 2-tDCB occurred in poultry meat following storage at 4 or 24°C for up to 28 days.

Obana et al (2005)	• 2-DCB and 2-TCB were detected in samples of γ-irradiated beef, pork, chicken and salmon following accelerated solvent extraction. • There was a generally linear relationship between the concentration of precursor palmitic or stearic acid and the concentration of 2-DCB and 2-TCB, respectively, following irradiation at 3.2-3.9 kGy. • 2-DCB and 2-TCB were not detectable in non-irradiated samples.
Sin et al (2006)	• Cellulose radicals, 2-DCB and 2-TCB were detected in melon, pumpkin and sunflower seeds that had been γ-irradiated at doses of 1, 3, 5 or 10 kGy. • There was a generally linear relationship between the concentrations of these compounds and the radiation dose. • Decomposition of the three compounds occurred following storage: cellulose radicals were undetectable after 60 d at 20-25°C; >85% loss of 2-DCB and 2-TCB after 120 days. • Non-irradiated samples were not analysed.
Lee (2008)	• 2-DCB, 2-TCB and 2-TeCB were detected in irradiated sesame seeds (raw, steamed and roasted) and roasted oil; their respective concentration increased linearly with dose. These compounds were not detectable in non-irradiated samples. • Various hydrocarbons were also detected in irradiated samples but the majority of these were also detected in non-irradiated samples.

2.3 Discussion

Supplementary data evaluated as part of the current application included studies on the metabolism, genotoxicity and carcinogenicity of certain 2-ACBs in addition to the analysis of radiolytic compounds in various irradiated foods (e.g. meat, eggs, nuts and fruit).

Metabolism

The three metabolism studies evaluated as part of the current application had some limitations to their respective designs, which restricts their scientific and thereby their regulatory value. Essentially, very low levels of 2-ACBs were detected in fat when 2-tDeCB or 2-tDCB were administered to rats for 4 months via drinking water (Horvatovich et al 2002) or when 2-DCB was administered by gavage for 5 days (Gadgil et al 2006). No urinary metabolites were detected (Gadgil et al 2006; Hijaz et al 2010), while 2-DCB was metabolised to 2-dodecylcyclobutanol *in vitro* and in rat faeces (Hijaz et al 2010). Beyond these basic observations, no new information is available on the absorption, distribution, metabolism and elimination of these three 2-ACBs.

Genotoxicity

In vitro genotoxicity assays have been conducted on several different 2-ACBs including 2-DCB, 2-dDCB, 2-tDCB and 2-tDeCB. Results were uniformly negative in standard mutation assays conducted using bacteria and human cells (Sommers 2003; Gadgil & Smith; (2004); Sommers & Schiestl 2004; Sommers & Mackay 2005; Sommers 2006; Hartwig et al 2007). In some bacterial assays, 2-DCB and 2-dDCB were cytotoxic (Sommers & Mackay 2005; Hartwig et al 2007).

Results were somewhat variable in non-standard cytogenetic assays. In yeast, 2-DCB was negative in the DEL assay (Sommers & Schiestl 2004). Three separate Comet assays were conducted using human colon cells or cell lines. In two of these assays, results were negative for 2-DCB, 2-dDCB, 2-tDCB and 2-tDeCB (Delincée et al 2002; Hartwig et al 2007). A positive response for 2dDC in primary colon epithelial cells and LT97 cells (a colorectal adenoma cells line) in the third Comet assay (Knoll et al 2006) is considered attributable to the confounding effect of compound-induced cytotoxicity. A positive response in HeLa cells

[but not HT29 cells (a human colon adenocarcinoma cell line)] in an alkaline unwinding assay only occurred at cytotoxic concentrations of 2-DCB, 2-dDCB and 2-tDeCB (Hartwig et al 2007); 2-tDCB was positive at non-cytotoxic concentrations. 2dDCB induced chromosomal aberrations in human LT97 cells (an adenoma cell line) at high µM concentrations. Notwithstanding the uncertainty of these non-standard cytogenetic assays to discern biologically-relevant genotoxicity, collectively they indicate that 2-ACBs have a very low potential to damage genetic material, and even then only at cytotoxic or high concentrations not relevant to dietary exposure.

The weight-of-evidence indicates that 2-ACBs are not mutagenic or clastogenic.

Carcinogenicity

In a rat model of colon carcinogenesis, 2-tDeCB significantly increased the number of pre-neoplastic lesions when administered via the drinking water for 6 months when a known carcinogen was injected during the exposure period (Raul et al 2002). In this same study, the total number and size of tumours in the rat colon were increased after 6 months exposure to 2-tDCB or 2-tDeCB. However, the study is considered to have limited regulatory value because of the questionable relevance of the particular model to dietary risk assessment. In addition, there were a number of deficiencies to the study design (e.g. small group size and a lack of appropriate negative controls).

Analysis of irradiated food

2-ACBs, hydrocarbons or free radicals have been analysed in various irradiated foodstuffs including beef patties (Gadgil et al 2002 & 2005), dried shrimp (Kim et al 2004), egg, cheese, poultry and avocado (Horvatovich et al 2005), pork, chicken and salmon (Obana et al 2005), various edible seeds (Sin et al 2006; Lee 2008), nuts and nutmeg (Variyar et al 2008). In the majority of foods there was a dose-related increase in the formation of these radiolytic compounds.

In the studies where non-irradiated samples were also analysed (Gadgil et al 2002; Kim et al 2004; Obana et al 2005; Lee 2008) 2-ACBs were not detectable, which is consistent with the historical view of the unique formation of these compounds in irradiated food. However, a notable exception is the study of Variyar et al (2008), which detected 2-dDCB, 2-tDCB and 2-tDeCB in non-irradiated cashew nuts (including fresh-farmed cashew nuts), and 2-DCB and 2-dDCB in non-irradiated nutmeg. This is the first published report of the apparently natural occurrence of 2-ACBs in non-irradiated foodstuffs and suggests these may no longer be considered novel radiolytic compounds. Results from this study suggest that the extraction method affects the detection of 2-ACBs, which may explain why these compounds have not previously been found in non-irradiated foodstuffs. This study also showed that irradiation increases the concentration of naturally-occurring 2-ACBs.

Some studies have reported the decomposition of 2-ACBs in irradiated foods following storage. Horvatovich et al (2005) observed a 50% decrease in the concentrations of *cis*-2-dDeCB, *cis*-2-tDeCB, 2-dDCB and 2-tDCB in poultry meat stored at 4 or 24°C for up to 28 days. Sin et al (2006) observed the complete decomposition of cellulose radicals within 60 days at room temperature and a greater than 85% loss of 2-DCB and 2-TCB after 120 days.

Evaluation

Supplementary data evaluated as part of the current application indicates that there are no new public health and safety considerations that need to be addressed as part of this application. Indeed, this supplementary data have confirmed that 2-ACBs are not genotoxic

and that consumers are already exposed to 2-ACBs in some non-irradiated foods.

2.4 Conclusion

It is concluded that persimmons irradiated up to a maximum dose of 1 kGy are as safe to consume as non-irradiated persimmons on the basis of the following considerations:

- An evaluation of supplementary data published since 2002 raised no public health and safety issues associated with the consumption of irradiated foods.
- Compounds formed during food irradiation are found naturally in non-irradiated food.
- The safety of irradiated food has been extensively assessed by national regulators and international scientific bodies.
- The irradiation of a number of tropical fruits are already permitted in Australia and New Zealand. FSANZ has not previously identified any public health and safety issues associated with the consumption of these or other permitted foods when irradiated according to GRP.
- There is a history of safe consumption of irradiated foods in many countries.

3. Nutrition Assessment

3.1 Nutritional implications for irradiated foods

The nutritional impact of the irradiation of several tropical fruits was assessed by FSANZ (2002) in Application A443 (Irradiation of tropical fruits – breadfruit, carambola, custard apple, lychee, longan, mango, mangosteen, papaya and rambutan). It was concluded that, although irradiation may potentially reduce certain vitamins (such as thiamin, vitamin C, folate and the pro-vitamin β-carotene) in these fruit, irradiation is unlikely to have a significant impact on Australian and New Zealand populations as the fruit are minor contributors to the total dietary intakes of these nutrients when considered in the context of the total diet. Refer to the Final Assessment Report for Application A443 for a full description of the nutritional assessment (Attachments 2 and 3).

This assessment will focus, where possible, on research since 2002 of the nutritional impact of irradiation of fruit on the vitamins most likely to be affected (thiamin, vitamin C, vitamin E, folate and β-carotene) at doses up to 1 kGy.

3.2 The impact of irradiation on specific nutrients

Irradiation of food can induce changes to nutrient composition which depend on a variety of factors including irradiation dose, food composition, packaging, temperature, and atmospheric oxygen saturation (Diehl et al 1991; Kilcast 1994; WHO 1994). Consequently, nutrient loss can be minimized by the use of appropriate processing techniques, such as the use of low temperatures and oxygen free conditions (WHO 1994; Diehl 1995).

Some nutrients are insensitive to irradiation while others are more greatly affected, and nutrient loss generally increases with increasing irradiation dose (WHO 1999). Irradiated foods are considered nutritionally equivalent to foods processed by other accepted methods such as thermal heat, smoking, canning, and freezing (Diehl 1991; WHO 1994; Crawford & Ruff 1996).

3.2.1 Macronutrients

There appears to be no significant effect of the irradiation of foods up to 1 kGy on the amount and nutritional quality of carbohydrates, proteins or fats (Diehl et al 1991; WHO 1994).

Some studies have investigated the effect of irradiation on the carbohydrate content of certain fruit compared to non-irradiated controls and found:

- Irradiation of apples and pears at 0.05, 0.3, 0.6 or 0.9 kGy did not influence the total or individual (sucrose, glucose, fructose, and sorbitol) carbohydrate concentrations (Drake et al 2003).
- No significant loss of sugar in three quarter ripe or fully ripe pineapple irradiated at 0.15 kGy (Susheela et al 1997).
- No difference in the total sugar concentrations of Dwarf Brazilian bananas irradiated at 0.2, 0.4, 0.6, or 0.8 kGy. However, irradiation accelerated sucrose hydrolysis in a dose-dependent fashion with a decrease in the sucrose concentration and a corresponding increase in both glucose and fructose concentrations (Wall 2007).

3.2.2 Minerals

There is no evidence to suggest that irradiation used for food processing decreases the amount of minerals in food and it is unlikely that such treatment would adversely affect their bioavailability (Diehl et al 1991; WHO 1994).

3.2.3 Vitamins

Certain vitamins are particularly sensitive to irradiation as well as to other food treatment methods, and each vitamin has a different sensitivity to irradiation (Table 2.1) (Kilcast 1994) The vitamin composition of foods can be affected by variety and season, and multiple studies have found that changes in the nutrient composition of a range of fruit and vegetables were greater due to storage effects than irradiation (Mitchell et al 1992; Boylston et al 2002; Fan & Sokorai 2008b).

Table 2.1: General summary of the radiation sensitivity of vitamins in food (Kilcast 1994)

High sensitivity	Medium sensitivity	Low sensitivity
Vitamin C	β-carotene	Vitamin D
Thiamin	Vitamin K (in meat)	Vitamin K (in vegetables)
Vitamin E (α-tocopherol)		Riboflavin
Vitamin A (retinol)		Vitamin B_6
		Vitamin B_{12}
		Niacin
		Folic acid[a]
		Pantothenic acid
		Biotin
		Choline

[a] The term 'folic acid' was used in the cited article to describe folate naturally present in foods, such as in broccoli, spinach and liver, rather than a synthetic form added to foods.

Water-soluble vitamins

The effects of irradiation on vitamin concentration vary between foods, and the order of sensitivity of water-soluble vitamins in food is generally (from most to least sensitive): Thiamin > Vitamin C > Vitamin B_6 > Riboflavin > Folic acid (folate) > Vitamin B_{12} > Nicotinic acid (niacin) (WHO 1994).

Recent reviews of the effect of irradiation on the vitamin composition of food indicate that most studies investigating irradiation of fruits up to 1 kGy predominantly assessed changes in vitamin C concentration with a small number of studies also assessing carotenoids and α-tocopherol (WHO 1999; Arvanitoyannis et al 2009; Dionisio et al 2009; Arvanitoyannis 2010).

<u>Vitamin C</u>

Vitamin C (ascorbic acid) is one of the most sensitive vitamins to irradiation (Kilcast 1994).

However, its sensitivity is also high in relation to several other factors including exposure to oxygen, temperature elevation, and pH modifications. Irradiation results in some ascorbic acid being converted to dehydroascorbic acid, but both forms are biologically active. Most research studies indicate that vitamin C losses in fruit are minimal when irradiation doses up to 1 kGy are used (Arvanitoyannis et al 2009; Dionisio et al 2009). For example, no statistically significant decrease in vitamin C concentration was found when the following fruit were compared to non-irradiated controls:

- Hawaiian fruit (Kau orange, rambutan and papaya) were irradiated at 0.75 kGy (Boylston et al 2002)
- Papaya and mango were irradiated at 0.5-0.95 kGy (Lacroix et al 1990)
- A variety of fruit (custard apples, lemons, lychees, mandarins, mangoes, nectarines, papayas, peaches, and persimmons) were irradiated at 0.075 or 0.3 kGy (Mitchell et al 1992).

When early season grapefruit were irradiated at 0.07, 0.2, 0.4 or 0.7 kGy, there was no significant change in the vitamin C content compared to non-irradiated controls. However, in the same study, there was a significant decrease in the vitamin C content of late season grapefruit exposed to irradiation doses ≥0.2 kGy (Patil et al 2004).

When several tropical fruits (carambola, mango, papaya, rambutan, and lychee) were irradiated at 0.75 kGy, only carambola (star fruit) showed a significant decrease in vitamin C concentration compared to non-irradiated controls (Moy & Wong 2002).

B vitamins

Thiamin is the most sensitive of the water-soluble vitamins to irradiation, and losses have been observed in irradiated chicken, meat, fish and grains (Kilcast 1994). However, in reviewing the literature for this assessment, no relevant data on the effect of irradiation at doses up to 1 kGy on thiamin or other B vitamins in fruit was found.

Fat-soluble vitamins and associated precursors

In general, the sequence of sensitivities for fat-soluble vitamins to irradiation is described as (from most to least sensitive): Vitamin E > Vitamin A > β-carotene > Vitamin D > Vitamin K (Diehl 1995). In the context of this application, the most relevant fat-soluble vitamins to consider are vitamins A and E as fruits are not a significant source of vitamins D and K.

Vitamin A

Yellow and red fruit, and green and red vegetables contain β-carotene and some other carotenoids, which can be converted to vitamin A in the human body. The results of studies investigating the effect of irradiation on carotenoids vary considerably depending on the food assessed (Diehl et al 1991).

When compared to non-irradiated controls, no significant loss in total carotenoid concentration was observed when sliced tomatoes were irradiated at 1 kGy (Mohacsi-Farkas et al 2006), and papaya was irradiated at 0.75 kGy (Boylston et al 2002).

Vitamin E

Some fruit, including persimmons, contain a small amount of vitamin E. The irradiation of sliced tomatoes at 1 kGy resulted in an approximately 40% decrease in the concentration of α-tocopherol compared to non-irradiated controls (Mohacsi-Farkas et al 2006).

3.3 Nutritional implications specific to the irradiation of persimmons

3.3.1 Micronutrient composition of persimmons

In order to identify any micronutrients that may be affected by irradiation in relation to the dietary intakes of Australian and New Zealand populations, the vitamin composition of raw persimmon was outlined (Table 3.2). When compared to other raw fruit, persimmons are a source of vitamin C and β-carotene (FSANZ 2006; New Zealand Institute for Plant & Food Research 2009).

Table 3.2: Vitamin composition of raw persimmon (*Diospyros kaki*) per 100g edible portion as assessed in Australia, New Zealand and the USA

Vitamin	Australia[a]	New Zealand[b]	USA[c]
Thiamin (mg)	0.01	0.02	0.03
Folate (µg DFE)	NA	8.00	8.00
Vitamin C (mg)	14.00	10.00	7.50
β-carotene (µg β-CE)	825.00	1070.00	NA
Vitamin E (mg α-TE)	NA	NA	0.73

[a] Data from NUTTAB 2006 online version (http://www.foodstandards.gov.au/consumerinformation/nuttab2006/onlineversionintroduction/onlineversion.cfm?&action=getFood&foodID=06D10208)
[b] Data from The Concise New Zealand Food Composition Tables (2009) (http://www.crop.cri.nz/home/products-services/nutrition/foodcompdata/Concise_8_Edition.pdf), species of persimmon not specified
[c] Data from USDA National Nutrient Database (http://www.nal.usda.gov/fnic/foodcomp/cgi-bin/list_nut_edit.pl)
DFE, dietary folate equivalents
β-CE, β-carotene equivalents, which equals the amount (µg) of β-carotene plus half the quantity of other provitamin A carotenoids
α-TE, α-tocopherol equivalents
NA, not analysed

3.3.2 Effects of irradiation on the nutrient composition of persimmons

A study of fruit and vegetables including persimmons (*Diospyros kaki*) which were obtained from commercial suppliers in southeast Queensland and irradiated at either 0.075 kGy or 0.3 kGy were compared to non-irradiated persimmons. No statistically significant differences in total vitamin C activity (ascorbic acid plus dehydroascorbic acid), dehydroascorbic acid, fructose or glucose concentrations were detected between the two irradiation doses and the control both immediately after irradiation or after three weeks of storage at 1-7°C (Mitchell et al 1992).

The experimental doses used in this study were considerably lower than 1 kGy, which is the maximum dose that could potentially be used for the irradiation of persimmons. As vitamin losses generally increase in a dose-dependent fashion, there is the potential that some small nutrient loss may occur in persimmons if doses above 0.3 kGy are used.

3.4 Conclusions

As a form of food processing, irradiation could have some impact on the nutrient composition of persimmons; however, there are few indications from studies of other fruit that these impacts are any greater than other forms of food processing, especially for irradiation doses up to 1 kGy.

The available data indicate that the carbohydrate, fat, protein and mineral content of foods are not affected by irradiation at doses up to 1 kGy. Therefore, irradiation is unlikely to affect the presence of macronutrients and minerals in persimmons. However, there is evidence to indicate that the concentrations of certain vitamins (for example, vitamins C and E) may be decreased in fruit to varying extents as a result of the irradiation process.

As the number of consumers of persimmons in Australia and New Zealand is relatively low and fruit consumption is seasonal, it is likely that persimmons are very minor contributors to the total dietary intakes of vitamins C, E, β-carotene, and certain B vitamins when considered within the context of the overall diet (refer to the Dietary Assessment Report for further detail). Therefore the irradiation of persimmons is unlikely to decrease the adequacy of dietary intakes of these vitamins by the Australian and New Zealand populations.

4. Dietary Intake Assessment

4.1 Dietary intake assessment

FSANZ's dietary modelling computer program, DIAMOND, was used to estimate the number of consumers and average consumption of persimmons. The contribution of 'other fruit', including persimmons, to the total dietary intakes of β-carotene, folate, vitamin C, thiamin (vitamin B_1) and vitamin E was calculated for the Australian and New Zealand populations. Nutrient concentrations of foods in Australia and New Zealand were used in conjunction with food consumption data derived from the most recent National Nutrition Surveys for Australia and New Zealand. These surveys are:

- 1995 Australian National Nutrition Survey (1995 NNS) which surveyed 13,858 people aged 2 years and above. The survey used a 24-hour recall method for all respondents and a second 24-hour recall for 10% of the respondents. Food consumption data from the survey was used to estimate the persimmon and nutrient intake of Australians aged 17 years and over.
- 1997 New Zealand Adult Nutrition Survey (1997 NZS) that surveyed 4,636 people aged 15 years and above. The survey used a 24-hour recall method for all respondents and a second 24-hour recall for 15% of the respondents.
- 2002 New Zealand Children's Nutrition Survey (2002 NZCS) which surveyed 3,275 people aged from 5-14 years. The survey used a 24-hour recall method for all respondents and a second 24 hour recall for 15% of the respondents.
- 2007 Australian National Children's Nutrition & Physical Activity Survey (2007 NCS) which surveyed 4,487 people aged 2-16 years of age. The survey used two 24-hour recalls for all respondents.

Persimmon consumption

The consumption of persimmons from 'raw plus other sources' (where it is an ingredient in a mixed food), were estimated using DIAMOND; the results are shown in Table 4.1. Consumers of persimmons represented less than 1% of all respondents in all 4 surveys.

Table 4.1: Consumption of persimmons as reported in the most recent Australian and New Zealand National Nutrition Surveys

Country (survey)	Age (yrs)	Raw persimmons + other sources	
		Number of consumers (as % of respondents)	Mean consumption (g/day)
Australia (1995 NNS)	2 years & above	4 (<0.1)	420
Australia (2007 NCS)	2-16 years	6 (0.1)	90
New Zealand (1997 NZS)	15 years & above	15 (0.3)	217
New Zealand (2002 NZCS)	5-14 years	19 (0.6)	166

Contribution to total vitamin intake

In each of the four nutrition surveys, persimmon consumption was categorised along with certain other fruits in an 'other fruits' category. Contribution of 'other fruits' to the total intake of irradiation sensitive nutrients can be found in Table 4.2. 'Other fruits' contributed less than

5% of total nutrient intake for all nutrients and population groups assessed. The contribution of persimmons to vitamin intake as part of the 'other fruits' category would be considerably less, as indicated by the very small number of consumers highlighted in Table 4.1. A list of all fruits contained within the 'other fruits' category can be found in Table 4.3.

Table 4.2: Percent contribution of 'other fruits' to mean total nutrient intake in Australia and New Zealand

Nutrient	Australia		New Zealand	
	2-16 yrs	17+ yrs	5-14 yrs	15+ yrs
β-carotene	4%	2%	2%	<1%
Vitamin C	3%	3%	3%	4%
Thiamin	<1%	<1%	<1%	<1%
Folate	<1%	<1%	<1%	<1%
Vitamin E	<1%	<1%	1%	1%

Table 4.3: Fruits in 'other fruits' category

Australia (1995 NNS)	Australia (2007 NCS)	New Zealand (1997 NZS)	New Zealand (2002 NZCS)
Raw fruit, not specified as to type Date Feijoa Fig Grapes Honeydew melon Kiwifruit Loquat Lychee Passionfruit Persimmon Rhubarb Rockmelon or cantaloupe Watermelon	Feijoa Fig Grapes Kiwifruit Lychee Honeydew melon Rockmelon Watermelon Passionfruit Persimmon Pomegranate Rhubarb Fresh fruit, not specified as to type Other fruit, commercially sterile	Feijoa Kiwifruit Persimmon Rhubarb Gooseberry Jackfruit Pepino Raw fruit, not further specified Babaco Breadfruit Avocado	Feijoa Kiwifruit Persimmon Rhubarb Grapes Tamarillo Avocado Olives Raw fruit, not further specified

4.2 Limitations of the dietary intake assessment

There are limitations for any dietary intake assessment associated with estimating habitual dietary nutrient intake where only one or two days of 24-hour dietary recall data are available. These data do not take into consideration the variation in nutrient intake over extended periods of time by the same individual (intra-individual variation). Also, 24-hour data tend to overestimate habitual food consumption amounts for high consumers, and therefore may result in higher estimated nutrient intakes. Thus, predicted high percentile nutrient intakes are likely to be greater than actual high percentile nutrient intakes over a lifetime.

A further limitation is that 'other fruits' rather than persimmons were used to estimate the percentage contribution to total nutrient intake. Therefore, this assessment represents a considerable over-estimate of the contribution of persimmons to total dietary intakes.

4.3 Conclusions

The dietary intake assessment indicates that the category of ‘other fruits’ which includes persimmons, is a minor contributor to the total dietary intakes of β-carotene, folate, vitamin C and thiamin when considered within the context of the overall diet. Therefore, any potential reductions of the β-carotene, folate, vitamin C and thiamine content in persimmons due to irradiation are unlikely to have any impact on dietary intakes of these vitamins by the Australian or New Zealand populations.

5. Conclusions

Persimmons irradiated at a minimum dose of 150 Gy and a maximum of 1 kGy are considered to be as safe and wholesome as non-irradiated persimmons to Australian and New Zealand consumers.

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