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

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Supporting document 1

Risk and technical assessment report – Application A1158 (at Approval)

Rosemary extract as a food additive

Executive summary

Kalsec Inc has applied to change the Australia New Zealand Food Standards Code to permit the use of rosemary extract as a food additive. The applicant seeks to use the extract as an antioxidant in a range of foods where a maximum permitted level (MPL) is specified for each food.

Rosemary extract is isolated from the leaves of the rosemary plant by ethanol or acetone extraction. The extraction and subsequent processing yields an extract which is enriched in two antioxidant compounds called carnosol and carnosic acid. The compounds, which are well-characterised chemically and as antioxidants, increase stability and extend the shelf-life of foods. FSANZ has determined that rosemary extract performs an antioxidant function. There are internationally accepted specifications for rosemary extract used as an antioxidant.

The submitted data, and information from other sources, were considered adequate to define the hazard of rosemary extract. Oral bioavailability of carnosic acid (which is oxidised to carnosol) is estimated to be ≥65%. Oxidation, glucuronidation, and methylation are the major pathways of metabolism with a range of metabolites detected in the urine and faeces.

The acute toxicity of carnosic acid was low in mice and rats. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) report on rosemary extract described a number of unpublished studies in rats that ranged in duration from 14 to 90 days. The highest no-observed-adverse-effect level (NOAEL) among the 90-day studies, expressed as carnosic acid plus carnosol, was 64 mg/kg bw/day.

No chronic or carcinogenicity studies of rosemary extract, carnosic acid, or carnosol were identified. Rosemary extract was not genotoxic in an *in vitro* or an *in vivo* assay. JECFA found no indication of genotoxic concern for extracts of rosemary extract, or carnosic acid and carnosol, in a range of studies *in vitro* or *in vivo*.

No concerns were identified in published human studies although there is limited information on tolerance in the scientific literature. Rosemary also has a long history of safe use as a culinary herb.

JECFA established a temporary Acceptable Daily Intake (ADI) of 0 - 0.3 mg/kg bw for rosemary extract, expressed as the sum of carnosic acid and carnosol, on the basis of a NOAEL of 64 mg/kg bw/day identified in a 90-day toxicity study in rats, with the application of

a 200-fold uncertainty factor. This uncertainty factor incorporates a factor of 2 to account for the temporary designation of the ADI, pending the submission of studies on the potential developmental and reproductive toxicity of rosemary extract.

Following the Call for Submissions, the Applicant provided FSANZ with a draft final report of a reproductive and development toxicity screening study in rats using rosemary extract conducted according to OECD 421 test guideline. The NOAEL for reproductive parameters of parental (P) generation, and the NOAEL for maternal toxicity, was the highest dose tested, equivalent to 316.2 mg/kg bw/day rosemary extract for males and 401.2 mg/kg bw/day rosemary extract for females. When these values are converted to the sum of carnosic acid and carnosol, they are 149.3 mg/kg bw/day for males and 189.4 mg/kg bw/day for females. The NOAEL for offspring toxicity was 166.7 mg/kg bw/day, equivalent to 78.7 mg/kg bw/day carnosic acid and carnosol, on the basis of low group mean serum thyroxine (T4) and thyroid stimulating hormone (TSH) in pups at higher doses on postnatal day 13. The reproductive, maternal and offspring NOAELs are more than 200-fold greater than the JECFA temporary ADI. Therefore FSANZ considers that the JECFA temporary ADI for rosemary extract remains appropriate for the purposes of this assessment.

FSANZ undertook a dietary exposure assessment for both Australia and New Zealand for carnosic acid plus carnosol from rosemary leaves only (naturally occurring source), from rosemary extract only (at the proposed MPLs and at the usual use levels) and from rosemary leaves and rosemary extract (at the proposed MPLs and at the usual use levels) and compared these exposures to the ADI.

For the *Naturally-occurring* scenario where dietary exposures to carnosic acid plus carnosol from rosemary leaves only were considered, mean and P90 exposures for consumers only did not exceed the ADI, for all population groups assessed.

For the proposed MPL scenarios, mean dietary exposures to carnosic acid plus carnosol for consumers only were 25 – 60% of the ADI and 90th percentile (P90) exposures were 50 – 110% of the ADI depending on the population group being assessed. The dietary exposure estimates based on MPLs are highly conservative and likely to be an overestimate of dietary exposure to carnosic acid plus carnosol due to the following assumptions: that all foods within a category contain rosemary extract at the MPL and all of the foods within the food categories requested to contain rosemary extract will use rosemary extract; and that consumers always eat the products containing rosemary extracts at these concentrations over a lifetime.

The Usual Use scenarios represent a more likely estimate of dietary exposures since estimates are based on probable concentrations of carnosic acid plus carnosol in the foods requested for addition of rosemary extract. For the Usual Use scenarios, mean dietary exposures to carnosic acid plus carnosol for consumers only were 15 – 30% of the ADI and P90 exposures were 30 – 60% of the ADI, depending on the population group being assessed.

The dietary exposures estimated for carnosic acid plus carnosol from naturally occurring sources through the consumption of rosemary leaves contributed minimally to the overall exposure estimated in all MPL and Usual Use scenarios.

Based on the safety and dietary exposure assessments, there is no evidence of a public health and safety concern associated with adding rosemary extract as an antioxidant to the requested foods.

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1 Objectives of the assessment

Kalsec Inc of Michigan in the United States applied to change the Australia New Zealand Food Standards Code (the Code) to permit the use of rosemary extract as a food additive for its antioxidant properties in foods.

Permissions are sought for use of the extract in a range of food categories including edible oils, fruit and vegetable spreads, icings and frostings, breakfast cereals and cereal bars, flour-based snacks, biscuits and cakes, processed meats, sausage meats, sauces and toppings, processed nuts, and potato chips. Maximum permitted levels (MPLs) have been specified for each of these foods.

There are no permissions for rosemary extract as a food additive in the Code. If approved, Standard 1.3.1—4(6) will include rosemary extract calculated as the sum of carnosic acid and carnosol. Rosemary extract will be also added to the following schedules of the Code:

- Schedule 8: Food additive names and code numbers (for statement of ingredients)
- Schedule 15: Substances that may be used as food additives.

The objectives of the risk and technical assessment are to:

- determine whether rosemary extract performs the technological purpose of an antioxidant in the amounts and foods proposed for its use
- evaluate any potential public health and safety concerns that may arise.

2 Food technology assessment

2.1 Description of the substance

Rosemary (*Rosmarinus officinallis L)* is a small evergreen shrub native to European countries along the Mediterranean Sea. Dried or fresh rosemary leaves have a long history of consumption in the human diet as a seasoning. Rosemary extracts are isolated by ethanol or acetone extraction of the dried leaves of the rosemary plant. Extracts have also been used in food preparations for aroma and flavouring properties. Rosemary and rosemary extracts are permitted to be used as a flavouring substance under Standard 1.1.2 of the Code as it is listed as a GRAS substance by the Flavour and Extract Manufacturers' Association of the United States from 1960-2015 (edition 7).

Rosemary extracts contain a number of compounds that have antioxidant properties. Most of these compounds belong to the chemical classes of phenolic acids, flavonoid diterpenoids and triterpenes. The main components of rosemary extracts that impart the antioxidative properties are two phenolic diterpenes called carnosol and carnosic acid. As antioxidants, these compounds help stabilise food products and thus extend shelf life. Rosemary extract is approved as a food additive in the European Union, Japan, Singapore and China.

Rosemary extract has been assigned INS[1] number 392 by the Codex Committee on Food Additives and Contaminants (CCFAC).

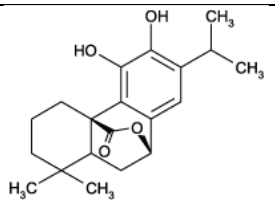
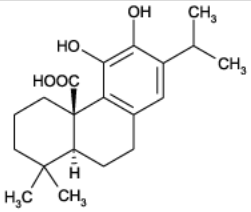
[1] International Numbering System for Food Additives

2.1.1 Identity

Rosemary extract is derived from the dried leaves of the *Rosemarinus officinalis L.* plant. The extract is a mixture of tannins, polyphenols, polysaccharides, triterpenic acids, volatiles, phenolic diterpenes, as well as some protein matter and lipophilic substances. Carnosol and carnosic acid are the two phenolic diterpenes responsible for the main antioxidant activity of rosemary extract. The amount of these two compounds contained in extracts depends on the starting composition of the dried leaves and the isolation method. Other related phenolic antioxidants may be present although in trace amounts and are not considered to contribute significantly to the antioxidant potency (Richheimer et al. 1996). Carnosol and carnosic acid are the components of rosemary extract that are being evaluated in this technical assessment. Details of the identities and structures of carnosol and carnosic acid are provided in Table 2.1.

Rosemary extract is a beige to light brown powder. It is insoluble in water but soluble in oil and can be sold as a liquid in vegetable oil or other compatible carriers. Rosemary extract for use as an antioxidant is commercialised by standardising the carnosol and carnosic acid content to a range from >5% to 25 %w/w by adding appropriate food-grade excipients and carriers. These are listed by the applicant to include silicon dioxide, DATEM (diacetyl tartaric acid ester of mono- and diglycerides), Propylene glycol, Polysorbate 80, monoglycerides of fatty acids, sucroesters of fatty acids, lecithin, glycerol, gum arabic, modified starch, maltodextrin, vegetable oil, or medium chain triglyceride (MCT) oil.[2]

Table 2.1 Chemical names and structures

Chemical name:	2H-9,4a-(Epoxymethano) phenanthren-12-one, 1,3,4,9,10,10ahexahydro-5,6-dihydroxy-1,1-dimethyl-7(1-methylethyl), (4aR-(4aα,9α,10aβ))-	4a(2H)-Phenanthrenecarboxylic acid, 1,3,4,9,10,10ahexahydro-5,6-dihydroxy-1,1-dimethyl-7-(1-methylethyl)-, (4aR-trans)-
Common names:	Carnosol	Carnosic acid
Codex INS number:	392	392
CAS registry number:	5957-80-2	3650-09-7
Chemical formula:	$C_{20}H_{26}O_4$	$C_{20}H_{28}O_4$
Molecular weight:	330.424 g/mol	332.44 g/mol
Chemical structure:		

2.1.2 Technological purpose

The applicant is seeking approval for use of rosemary extract as an antioxidant which means

[2] Noting that only excipients and carriers listed in Schedule 18 of the Code would be permitted for rosemary extract used as an antioxidant in Australia and New Zealand.

it retards or prevents the oxidative deterioration of a food, as defined in Schedule 14 of the Code. Specifically, antioxidants inhibit oxidation of fats/oils (lipid peroxidation) and proteins. Inhibition of these chemical reactions preserves flavours and organoleptic properties, prevents rancidity, and thus extends shelf life (Carocho et al. 2018).

The flavouring and aroma properties of rosemary extract are strong and, unless intentionally removed, would be present in preparations of rosemary extract to be used as an antioxidant. The use of rosemary extract as an antioxidant can be limited if the flavouring properties are not reduced through processing of the extract. Consequently, the processing of rosemary extract is optimised depending on whether the extract is intended to function as a flavour, an antioxidant, or both (Berdahl and McKeague 2015; Raadt et al. 2015; JECFA 2016)

There is no specification available for rosemary extract used as a flavour. Consequently there is no guide to the expected concentrations of carnosic acid plus carnosol in foods where rosemary extract has been used as a flavour. However, flavours are generally used in very small quantities in the food supply and are not expected to be a key source of dietary exposure.

2.1.3 Technological justification

The application cites 31 studies demonstrating the efficacy of rosemary extract as an antioxidant in a variety of foods (Table 2.2). The amounts of rosemary extract, expressed as carnosic acid plus carnosic acid, that was found to be effective in these studies are comparable to the requested permissions in the application.

Table 2.2 Efficacy of rosemary extract used as an antioxidant[1]

Food (no. of studies)	**Outcome**	**Carnosol + carnosic acid**	
		Range used in studies (mg/kg)	Proposed MPL[2] (mg/kg)
Meat (13)	Reduced oxidation, flavour deterioration, microbial growth; improved meat colour	10-200	1.5-50
Nuts/nut oil (4)	Reduced lipid oxidation and/or increased shelf life	10-200	50
Savoury snacks (7)	Inhibited oil oxidation, improved oil quality/sensory quality of snack	10-60 (added to oil used)	(20)[2]
Baked goods/bread (1)	Sensory quality	500-1500 (added to oil used)	(40)[3]
Fats and emulsions (4)	Inhibited oxidation	40-200	50-75
Fish oil (1)	Inhibited oxidation	100-300	50

[1] Summarised from *Table 1: Efficacy Studies* provided in the application (pages 16-19).
[2] Based on the whole food, expressed as the sum of carnosic acid plus carnosol.
[3] Not directly comparable to amount used in experimental study.

Use of rosemary extract may provide an alternative to antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) which are used widely in meats, fats and oils (Carocho et al. 2018). Several efficacy studies cited in the application reported that rosemary extract was more effective than BHA and BHT in stabilising oxygen-sensitive foods.

2.2 Chemical properties

Antioxidants work by reacting with oxygen or oxygen-containing reactive molecules such as radicals formed in lipid peroxidation (Shahidi et al. 1992). In the process the antioxidant becomes oxidised and the degradation of the reactive food component (such as a lipid) is inhibited (Cheng 2016).

Carnosol and carnosic acid are phenol-containing antioxidants for which the mechanism of action is well-characterised. The substances are known to have the strongest potency compared to other phenols in rosemary leaves and have comparable potency to the antioxidants BHT and BHA (Shahidi et al. 1992; Richheimer et al. 1996; Masuda et al. 2001).

The reaction mechanism has been described as a cascade of antioxidant activity whereby carnosol is first produced by oxidation of carnosic acid. Carnosol, in turn, is oxidised to form degradation products that can also have antioxidant activities (Masuda et al. 2001; Razboršek and Ivanović 2016). The antioxidant activity depends on the amounts of carnosol and carnosic acid in the isolated rosemary extract (Schwarz et al. 1992).

2.3 Manufacturing process

2.3.1 Description

There are several solvent-based procedures for preparing rosemary extract for use as a food additive (Berdahl and McKeague 2015). The application specifically refers to the process using food-grade ethanol or acetone. The steps for isolation of the extract are:

1. Rosemary leaves are dried and ground.
2. Dried leaves are extracted with ethanol or acetone.
3. The liquid mixture (containing the active components carnosol and carnosic acid) is separated from the solid residue (leaves) by filtration.
4. Solvents are removed by vacuum-evaporation.
5. Drying and sieving the resulting solid to produce a dry powder.
6. Additional deodorisation and decolourisation are used with approved excipients.
7. The isolated rosemary extract is diluted with approved carriers to the appropriate concentration.

The ethanol and acetone solvent-based extraction method used by the applicant is also described by EFSA (EFSA 2008) and in the Chemical and Technical Assessment for rosemary extract published by JECFA (JECFA 2016).

2.3.2 Product specification

Subsection 1.1.1—15(2) of the Code requires that a substance used as a food additive (paragraph 1.1.1—15(1)(a)) must comply with a relevant specification in Schedule 3 – Identity and purity. United States Pharmacopeial Convention (2017) Food Chemicals Codex (FCC), which is a primary source of specifications, contains a specification for rosemary extract (FCC 2016). Therefore, no specification would be needed to be included in Schedule 3. If this application is approved, the commercial preparation of rosemary extract for use as an antioxidant would need to comply with the identity and purity requirements of the FCC specification.

Table 2.3 shows the proposed specifications for rosemary extract provided in the application. The specifications are based on those listed in Codex (FCC 2016) and in the FAO / WHO Rosemary Extract (Tentative) Monograph (WHO 2017). A final JECFA specification for rosemary extract is expected upon completion of the assessment of the reproductive and

developmental toxicology data (See Section 3.3.1). Specifications for rosemary extract isolated by ethanol or acetone extraction are also set in Commission Regulation (EU) No 231/2012 (European Union 2012).

Table 2.3 Proposed specification for antioxidant rosemary extracts[1]

Parameter	Requirement
Description	Beige to light brown powder
Assay	Not less than 5% of the total carnosic acid plus carnosol
Solubility	Insoluble in water; soluble in oil
Antioxidants/Reference volatiles Ratio	Total % carnosic acid plus carnosol/Total % Reference volatiles ≥ 15 Reference volatiles = (-)-borneol, (-)-bornyl acetate, (-)-camphor, 1,8-Cineole (eucalyptol) and verbenone
Loss on drying	Not more than 5%
Residual solvents	Acetone ≤ 50 mg/kg, Ethanol ≤ 500 mg/kg
Heavy metals	Arsenic ≤ 3 mg/kg, Lead ≤ 2 mg/kg

[1] Reproduced from Table on page 24 of the application.

2.3.3 Product stability

The data in the application and in technical literature indicates that rosemary extract is stable and functions as an antioxidant.

Stability of antioxidant activity in rosemary extract, both as the isolated extract and when present in foods such as oils, is supported by a large body of research (Schwarz et al. 1992; Frankel et al. 1996; Birtić et al. 2015; Razboršek and Ivanović 2016). Overall, studies show that the antioxidative activity depends on the content and ratio of carnosol and carnosic acid in the isolated extract. This, in turn, is affected by drying methods and storage of the plant matter, the solvent extraction method, thermal degradation of the extract at temperatures greater than 100 °C, and the presence of water or light exposure. Thermal degradation products have also been found to be active as antioxidants. The antioxidant activity of rosemary extract is relatively unstable when the extract is stored in solvents but has increased stability in food matrices.

Information on the shelf life of rosemary extract (before use) and the optimum storage conditions was not available. Commercial providers of the product indicate that the product should be stored at ambient temperatures and specify a shelf-life of 12 months.

2.4 Analytical method for detection

The content of carnosol and carnosic acid in isolated rosemary extracts is measured using established chromatographic methods. The ratio of carnosic acid plus carnosol content to reference volatiles[3] is measured by gas chromatography coupled with mass spectrometry detection (GC-MS), and gas chromatography with flame ionisation detection (GC-FID). Methods to assay residual solvents, solubility, loss on drying, and heavy metals are internationally recognised (FCC 2016).

All methods are consistent with those described in FAO/WHO Rosemary Extract (Tentative) Monograph (WHO 2017) and the Food Chemical Codex (FCC) specification which are

[3] Reference volatiles are (-)-borneol, (-)-bornyl acetate, (-)-camphor, 1,8-cineole (eucalyptol) and verbenone. These are the main substances that contribute to the flavouring and aroma properties of rosemary extract.

provided in Appendix 1 and Appendix 2 of the application.

2.5 Food technology conclusion

Rosemary extract containing carnosol and carnosic acid when used as a food additive at the proposed levels performs the technological purpose of an antioxidant. The extract is proposed to be added in a range of foods (see Table 4.3) and evidence has been provided to indicate its efficacy.

3 Hazard Assessment

3.1 Background

3.1.1 Evaluation of the submitted data

FSANZ has assessed the submitted evidence on the safety of rosemary extract and information from other sources. The assessed data on rosemary extract include information on toxicokinetics and metabolism, genotoxicity, toxicity in laboratory animals, and studies in human volunteers. The submitted data, together with the assessment by JECFA (WHO 2017) are considered suitable to assess the hazard of rosemary extract.

3.1.2 Characteristics of rosemary extract

The chemical characteristics of rosemary extract are provided in Section 2. The application concerns rosemary extracts made using solvent extraction with acetone or ethanol, that meet the specifications of the tentative monograph by JECFA (WHO 2017) and the Food Chemicals Codex 10th edition (FCC 2016). Two phenolic diterpenes, carnosol and carnosic acid, are primarily responsible for the antioxidant properties of rosemary extract. The composition of rosemary extract intended for antioxidant use is standardised so that carnosic acid and carnosol are present at between 5 to 25% w/w, by the addition of food-grade excipients and fillers. Rosemary extract also contains several reference volatiles that contribute flavour and odour. The ratio of the carnosic acid and carnosol to the reference volatiles is not less than 15. Other substances that may be present and that are derived from *Rosmarinus officinalis* include triterpenic acids, tannins, polyphenols, polysaccharides, and residues of plant material.

3.2 Toxicological data

Carnosic acid and carnosol are considered to be the most important antioxidants of rosemary extract, and for this reason studies in which purified carnosic acid were used are relevant to this assessment. Studies in which the test article comprised whole rosemary leaves are also relevant, because rosemary leaves would be expected to contain carnosic acid and carnosol as well as a range of other compounds. However, studies of extracts of components of rosemary leaves that are soluble in water are considered to be unlikely to be comparable to the extracts made using ethanol or acetone (which are soluble in oil) and are therefore reviewed under the heading ‘Other studies’ (subsection 3.2.7).

3.2.1 Toxicokinetics and metabolism

Oral gavage study of the kinetics of carnosic acid in rats (Yan et al. *2009) Regulatory status: Non-GLP*

The test article for this study was carnosic acid of >98% purity. The test subjects were young male Sprague Dawley rats weighing 190-220 g at time of receipt. Rats were housed under

standard laboratory conditions and acclimatised for three days prior to the start of the experiment. Water and a standard rat chow were provided *ad libitum*, except overnight prior to dosing, when rats were fasted. Rats were administered carnosic acid by either oral gavage at 90 mg/kg bw, or intravenously via the tail vein at 10 mg/kg. The vehicles and dosing volumes were not stated. The number of rats per group is also not stated, but the plasma concentrations are the means from 8 rats/timepoint. Blood (0.5 mL/timepoint) was collected from the orbital sinus at 5, 15, 30, 60, 120, 240, 360, 480, 600, 720, and 1440 min after gavage administration and at 0, 10, 30, 45, 60, 120, 180, 240 and 360 min after intravenous administration. Pharmacokinetic analysis of the results showed that the oral bioavailability of carnosic acid was 65.09(± 1.422)%. Pharmacokinetic parameters generally had large standard deviations, indicative of considerable variation between individuals; T_{max} was 125.6 (±118.4) min, $t_{1/2}$ was 961.5 (±889.9) min, and clearance was 0.003 (±0.002) L/min/kg. Calculated volume of distribution at steady state (V_{SS}) was 3.228 (±2.628) L/kg. The authors commented that although carnosic acid is readily converted to carnosol by oxidation in air, they did not find carnosol in rat plasma, and therefore they do not consider carnosol to be an *in vivo* metabolite of carnosic acid. They concluded that absorption of carnosic acid from the gastrointestinal tract was slow, but clearance from the plasma was also slow.

A drawback to this study was the high volume of blood removed from small rats, which would be likely to affect xenobiotic kinetics. That mean plasma concentrations were from 8 rats, which indicates that all rats in a cohort were bled at every timepoint. This means that over 24 hours, a total of 5.5 mL was removed from each rat in the gavage cohort, and 4.5 mL was removed from each rat in the intravenous cohort. The short acclimatisation period means that the rats would not have been much heavier than their 190-220 g bodyweight on receipt and would have had normal circulating blood volumes in the range 12 to 14 mL. Therefore blood loss for the gavage cohort would be in the range of 39 to 46% over 24 hours, and blood loss for the intravenous cohort would be in the range of 32 to 38% over 6 hours. These are high levels of blood loss which would have resulted in clinically significant anaemia and hypovolaemia, and would be highly likely to affect the kinetics of any xenobiotic.

Intravenous and oral gavage study of the kinetics of carnosic acid in rats (Doolaege et al. *(2011). Regulatory status: Non-GLP*

The rats used in this study were male Sprague Dawley rats weighing 200-330 g. All rats were fasted overnight prior to dose administration. The test article was carnosic acid purchased from Sigma Aldrich. The purity was not stated by the authors, but carnosic acid currently listed by Sigma Aldrich has a purity of ≥91%. Four rats were administered 4.0 (±0.1) mg carnosic acid intravenously, dissolved in a vehicle of 5% ethanol in water, and five rats were gavaged with 15.4 (± 1.2) mg carnosic acid dissolved in PEG400. Individual animal weights were recorded, and the amount of carnosic acid remaining in syringes after injection was measured by LC-MS, allowing accurate calculation of the dose administered. Blood samples, 0.5 mL, were collected at 7, 15, 30, 60, 120, 180, and 240 minutes after dosing from all rats, and further blood samples were collected from the gavage cohort at 300, 360, 420 and 1440 minutes. Rats were kept in metabolism cages, and urine and faeces were collected, throughout the experiment. Rats in the IV cohort were killed 4 h after dose administration, while rats in the gavage cohort were killed 24 h after dose administration. Liver and intestinal contents were collected from all rats, and muscle tissue from the abdomen and legs was also collected from two rats in the gavage cohort. Samples were subject to extraction and purification of carnosic acid. Preliminary studies of eluents using β-glucuronidase and sulfatase confirmed that carnosic acid was not bound to sulphates or glycosides. Concentrations of carnosic acid in all samples were determined by a validated LC-MS method. Oral absorption was slow, with a T_{max} following gavage of 136.6 ± 151.5 min. Oral bioavailability (0-360 min) was calculated as 40.1%, and bioavailability (0-1440 m) was estimated to be 97%. Only traces of carnosic acid were detected in urine, while 15.6 ± 8.2% was found in the faeces. Only traces of carnosic acid were found in liver, gastrointestinal

contents, and muscle. Analysis of data from the rats dosed intravenously showed that carnosic acid was cleared rapidly from the circulation, and was eliminated within 4 hours of IV administration (Doolaege *et al.* 2011).

It is noted that 3.5 mL blood was removed over 4 hours from all rats, and a total of 5.5 mL of blood was removed from rats in the gavage cohort over 24 hours. Given their bodyweight range of 200-330 g, the approximate blood volumes of these rats would have ranged from 13 to 21 mL. The blood loss in the first 4 hours of sampling would have represented 17 to 27% of their circulating blood volume, and the blood loss of the gavage cohort would have represented 26 to 42% of their total blood volume. These are high levels of blood loss which would have resulted in at least some degree of anaemia and hypovolaemia, and would be very likely to affect the kinetics of any xenobiotic.

Oral gavage study of the kinetics and metabolism of carnosic acid and related compounds in rats (Romo Vaquero et al. *2013). Regulatory status: Non-GLP*

The test article in this study was an ethanolic rosemary extract containing 38.9 ± 1.7% carnosic acid, 6.5 ± 0.1% carnosol, and 6.9 ±0.6% of a methylated derivative of carnosic acid. Other compounds detected but not quantified included rosmarinic acid, rosmanol epirosmanol, epiisorosmanol, epiisorosmanol ethyl ether, rosmadial, caffeic acid hexoside, medioresinol, isorhamnetin 3-O-hexoside, homoplantagin, cirsimaritin and 4'-methoxytectochrysin. In addition, the extract contained carbohydrates (30.0%), fat (7.9%), ash (6.2%), water (2.2%), proteins (<2.5%) and dietary fibre (1%).

The animals used were female Zucker Le (*fa/*+) and Ob (*fa/fa*) rats. The Zucker Ob (*fa/fa*) rat is a spontaneous genetic obesity model. Compared to the lean (*fa*/+) rat, the obese (*fa/fa*) rat exhibits hyperphagia, hyperinsulinemia, and hyperlipidemia, and has a number of differences in expression of hepatic enzymes when compared to the lean rat.

The first experiment in this study was carried out using only the lean Zucker rats. Rats were housed, 3/cage and fed a standard rat chow for three days before being assigned to either the control group (n=6) that remained on the standard chow or the treated group (n=18) that were fed the standard chow supplemented with rosemary extract at 0.5% w/w. After 15 days on their respective diets, rats were fasted overnight before the control group were gavaged with water and the treated group were gavaged with a 100 mg/mL suspension of rosemary extract, but the volume of the suspension administered is not stated, and therefore the dose cannot be determined. At 25, 50, 100, 250, 500 and 800 min after dosing, three rats from the treated group were killed by cardiac exsanguination under ketamine/xylazine anaesthesia, and plasma was processed for analysis. Terminal blood collection from the control rats was performed on three rats at each of 25 and 50 min. Gut content, brain and liver were collected from all rats.

The second experiment in the study was the analysis of plasma, small intestinal content, liver and brain from a previous 64-day dietary study for the main metabolites identified in the acute study. The control group in the 64-day study comprised 7 Zucker Le and 5 Zucker Ob female rats fed standard laboratory chow, while the treatment group comprised the same numbers of Zucker Le and Ob rats fed the standard chow supplemented with 0.5% w/w rosemary extract.

Plasma and tissue samples were processed for analysis by LC-MS/MS. In addition, representative tissue samples were treated with β-glucuronidase and analysis of filtrates by HPLC-DAD-MS was conducted before and after this treatment.

A total of 26 compounds and metabolites were detected in at least some samples. There was evidence that Phase I metabolism included removal of carboxylic groups and water

molecules. Phase II metabolites were detected, principally glucuronides, which included glucuronides of carnosic acid, rosmanol, carnosol, rosmadial and carnosic acid 12-methyl ether. Other metabolites positively or tentatively identified included sulphate derivatives of carnosol, carnosic acid and rosmanol; a glutathione carnosic acid derivative; two quinone derivatives and some methyl ether derivatives.

Following oral gavage, T_{max} for carnosic acid in plasma was 0.4 h and the average time for a molecule to reside in the body (MRT_{last}) was calculated to be 0.6 h. In contrast, T_{max} of carnosol and carnosic acid 12-methyl ether was 13.3 h and MRT_{last} was 8.5 h for carnosol and 7.8 h for carnosic acid 12-methyl ether. T_{max} for the glucuronides of carnosic acid and carnosol were 13.3 h and 8.3 h respectively, and MRT_{last} values were 8.4 and 7.9 h respectively.

Results showed that glucuronidation is the predominant form of conjugation of the diterpenoids in rosemary extract, and glucuronides may appear in the gut lumen as soon as 25 min after dosing, consistent with rapid excretion of glucuronides in the bile. Other major metabolites were a methylated derivative of carnosic acid and a quinone derivative. No major differences in metabolism were found between Zucker Le and Zucker Ob rats.

In vitro and in vivo study of the metabolites of carnosic acid (Song et al. *2014*). *Regulatory status: Non-GLP*

The test article for this study was carnosic acid prepared from rosemary leaves by the study authors. Test systems included human liver microsomes (HLMs), human intestinal microsomes (HIMs), rat liver microsomes (RLMs), two strains of the fungus *Cunninghamella elegans*, and young (approximately 250 g) male Sprague Dawley rats.

Only carnosic acid was used for the *in vivo* part of the study. Rats were housed individually in metabolic cages under standard laboratory environmental conditions, with *ad libitum* access to food and water, with the exception of overnight fasting prior to dose administration. Rats, 3/group, were assigned to either a control group or a treated group. The vehicle/control article was 10% aqueous Tween-80, the dose volume for both groups was 2 mL/rat, and rats in the treated group were administered carnosic acid at 90 mg/kg bw. The dose formulation of carnosic acid was prepared shortly before dose administration. Urine and faeces from 0-24 h postdosing were collected on ice and pooled within groups. Extracts from urine and faeces were analysed using LC-UV-MS/MS. Twelve metabolites were identified in urine, and six in faeces. Oxidation, glucuronidation and methylation were identified as the major pathways of metabolism.

For the *in vitro* investigations with microsomes, carnosic acid at a final concentration of 100 µmol/L was incubated with 1 mg/mL of HLMs, HIMs or RLMs in 0.1 mol/L potassium phosphate buffer at 37ºC for 60 min with continuous shaking. Reactions were initiated by addition of NADPH-generating system (β-$NADP^+$, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, and magnesium dichloride) and quenched by addition of ice-cold acetonitrile. Parallel control systems included incubation with denatured microsomal proteins, absence of incubation, incubation in the absence of the NADPH-generating system, and incubation without carnosic acid. For the *in vitro* investigations with *Cunninghamella elegans*, freshly prepared carnosic acid solution was added to a final concentration of 100 µmol/L to cultures of the fungus, and the cultures were incubated for 5 days. Parallel control systems were cultures without added carnosic acid, and cultures with carnosic acid that were not incubated. For all the *in vitro* experiments, supernatants after centrifugation were analysed by LC-UV-MS/MS. All experiments were conducted in triplicate. A total of ten metabolites and three degradation products of carnosic acid were identified in the *in vitro* experiments. Glucuronidation and oxidation were the major metabolic pathways. The biotransformation pathways observed with human microsomes and rat microsomes were practically identical.

Study of the kinetics of carnosic acid, carnosol and rosmanol in rats (Wang et al*. 2017). Regulatory status: Non-GLP*

The study was conducted using a rosemary extract prepared in the testing laboratory. 100 g of dried leaves of *Rosmarinus officinalis* were extracted under reflux with 1 L ethanol:water, 80:20 v/v. The extraction was conducted three times, for 1 hour each extraction. The extract was then filtered and the combined filtrate evaporated to dryness, then dissolved in water. The final concentration was equivalent to 0.1 g/mL. Male Sprague Dawley rats weighing 220 ±50 g were acclimatised to environmental conditions of 65% relative humidity and 23-27ºC for an unspecified period. Rats were assigned, 6/group, to three dose groups. Rats had *ad libitum* access to water throughout the experiment but were fasted for 12 h before being gavaged with 0.24, 0.82 or 2.45 g/kg bw rosemary extract. Blood, 0.25 mL/timepoint, was collected from the orbital venous plexus at 0.08, 0.25, 0.50, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 h after dose administration. Plasma was analysed for carnosic acid, carnosol and rosmanol. Mean pharmacokinetic values were as follows: For carnosic acid, t_{max} 0.3 to 0.5 h and $t_{1/2}$ 8.02 to 12.84 h; for carnosol, t_{max} 0.4 to 0.7 h and $t_{1/2}$ 12.3 to 12.8 h; for rosmanol, t_{max} 0.25 to 0.55 h and $t_{1/2}$ 8.88 to 15.4 h. The authors remarked on a double-peak phenomenon in the elimination phase of the mean plasma concentration vs. time profiles, particularly of carnosic acid and carnosol in the rats dosed with 2.45 g/kg bw of rosemary extract, which may indicate enterohepatic cycling (Wang *et al*. 2017).

The paper states that each point on the mean plasma concentration vs. time profile, and each mean pharmacokinetic parameter, is based on $n = 6$. It appears that all rats were bled at each timepoint, which in rats weighing 220 g would represent a loss of approximately 21% of their blood volume in 24 hours. This level of blood loss would be very likely to affect xenobiotic kinetics and would cause some degree of anaemia and hypovolaemia in the rats.

Summary of toxicokinetic studies

There have been several studies on the kinetics and metabolism of carnosic acid and other constituents of rosemary extract in rodents, although the value of most of the studies is compromised by the removal of excessive amounts of blood. Estimated oral bioavailability of carnosic acid over 24 hours ranged from 65% (Yan *et al.* 2009) to 97% (Doolaege et al. 2011). Group mean T_{max} following oral gavage ranged from 20 min (Wang *et al.* 2017) to 136.6 min (Doolaege *et al.* 2011), although this may reflect different vehicles because Romo Vaquero *et al*. (2013) and Wang *et al.* (2017) both used water as the vehicle and reported T_{max} of 40 min and 20-30 min respectively, whereas Doolaege *et al.* (2011) used PEG400 as the vehicle. Most of the studies did not investigate distribution, although Doolaege *et al*. (2011) reported that only traces of carnosic acid were found in liver, gastrointestinal contents or muscle. Apparent volume of distribution was 3.228 (±2.628) L/kg (Yan et al.(2009). Yan *et al*. (2009) reported a prolonged $t_{1/2}$ for carnosic acid in plasma of 961.5 (±889.9) min with a slow clearance at 0.003 (±0.002) L/min/kg. In contrast, Doolaege et al. (2011) found that after intravenous dosing, carnosic acid was cleared rapidly from the circulation and eliminated within 4 hours, and Romo Vaquero *et al.* (2013) calculated an MRT_{last} for carnosic acid of only 0.6 h. A wide range of metabolites of carnosic acid have been detected. Oxidation, glucuronidation and methylation are the major pathways of metabolism (Song *et al*. 2014). Glucuronides are detectable in the bile soon after dosing (Romo Vaquero et al. 2013) but may undergo enterohepatic cycling (Wang *et al*. 2017). Metabolites are found in both urine and faeces (Song *et al.* 2014).

There is relatively little information on components of rosemary extract other than carnosic acid. Following a single oral administration of rosemary extract to rats, Wang *et al.* (2017) reported a T_{max} of 0.25 to 0.55 h and $t_{1/2}$ 8.88 to 15.4 h for rosmanol, and for carnosol a T_{max} of 0.27-0.7 h, and $t_{1/2}$ of 12.3-12.8 h. On the other hand, Romo Vaquero *et al.* (2013)

reported that the T_{max} for carnosol was 13.3 h and the MRT_{last} was 8.5 h. Wang *et al.* (2017) provided graphs indicating gradual decline of carnosol over 24 hours with a slight rise at 12 hours, whereas Romo Vaquero *et al.* (2013) reported that carnosol increased over the 13.3 hours of their acute TK study. The reason for these discrepancies between studies is not clear. Wang *et al*. (2017) did not specify their rat strain or the vehicle for administration, either or both of which may have been different to those used by Romo Vaquero *et al*. (2013), and the analytical methods were different.

The feeding of rosemary extract to lambs has been shown to lead to the deposition of rosemary diterpenes and metabolites, including carnosic acid, carnosol, rosmanol, carnosol *p*-quinone, and a metabolite identified as 5,6-dihydroxy-7-isopropyl-1,1-dimethyl-2,3-dihydrophenanthren-9(1H)-one, in their muscles (Jordán *et al.* 2014). Persistence of compounds from rosemary in the muscles of lambs following supplementation of their dams during pregnancy and lactation was investigated by Moñino *et al.* (2008). The dams were supplemented with steam-distilled rosemary leaves at 10 or 20% by weight of the control diet for 240 days, coinciding with gestation and lactation periods. Lambs were weaned at a bodyweight of 13 ± 1 kg and were not exposed to rosemary from weaning to slaughter, which was done when they reached 25 ± 2 kg bw. Levels of carnosic acid, carnosol and rosmarinic acid were elevated in the meat of lambs from treated ewes, when compared to meat from the lambs of control ewes. However the levels of these compounds in the meat of lambs born to ewes in the 20% group were not significantly higher than in the meat of lambs born to ewes in the 10% group, which suggests that muscle as a storage compartment is readily saturable. The time between weaning and slaughter was not stated by Moñino *et al.* (2008), although in a related study with the same breed of sheep, in the same province of Spain, Ortuña *et al*. (2017) reported that the time from weaning to slaughter was 50 ± 8 days. It may be surmised that the study of Moñino *et al.* (2008) demonstrates prolonged storage in muscle of carnosic acid, carnosol and rosmarinic acid. However it is not possible from the study design to determine whether the compounds from rosemary extract crossed the placentas during pregnancy or were transferred to the lambs via milk. Compounds from rosemary extract are transferred into the milk of (Boutoial *et al*. 2013, 2017) and alterations in the properties of milk of ewes fed rosemary leaves (Branciari *et al*. 2015) or rosemary extract (Chiofalo *et al.* 2010) are consistent with compounds from rosemary also being transferred into the milk of ewes.

Exposure to rosemary extract has been found to result in upregulation of some metabolic enzymes. This was first demonstrated by Singletary in 1996, who reported that dietary exposure of female Sprague Dawley rats to rosemary extract at 0.25, 0.5 or 1% w/w for 21 days resulted in significant increases in hepatic levels of glutathione-S-transferase (GST) and NADPH-quinone reductase (QR) in all groups, when compared to untreated controls, although there was not a clear dose-response relationship. However feeding purified carnosol for two weeks did not alter enzyme expression. Similarly, dietary exposure to rosemary extract at 0.3 and 0.6% w/w to mice for 4 weeks resulted in a significant increase in group mean QR expression in both groups, relative to untreated controls, and a significant increase in group mean GST expression in the mice fed the higher dose of 0.6% rosemary extract when compared to untreated controls. Expression of GST, but not QR, was also increased in the stomach of mice fed 0.6% rosemary extract, compared to untreated controls (Singletary and Rokusek, 1997). JECFA (2017) cited an unpublished study report of a 13-week dietary study of rosemary extract in female Sprague Dawley rats. Rosemary extract containing 33% w/w of carnosic acid + carnosol was added to rat chow to achieve an exposure equivalent to 64 mg/kg bw/day of carnosic acid and carnosol combined. As part of this study, total hepatic microsomal cytochrome P450 activity, as well as activities of selected enzymes including CYP1A, CYP2B, CYP2C11, CYP2E, CYP3A and CYP4A were measured in cohorts of rats after 4 weeks of dietary exposure, after 13 weeks of treatment and at the end of a 4-week recovery period after the end of dietary exposure. After 13 weeks of treatment, total hepatic microsomal cytochrome P450 activity was increase 1.5-fold over that

in control animals. Increases occurred in CRP2A, CYP2C11, CYP2E1 and CPY4A, but not in CYP1A, CYP2B or CYP3A. These increases were reversible, in that expression of all enzymes after four weeks of recovery was the same as that in control animals (Covance Laboratories Ltd, as cited by JECFA 2017).

3.2.2 Short term studies in animals

Acute toxicity study of carnosic acid in the mouse (Wang et al. *2012) Regulatory status: Non-GLP*

Kunming mice, 5/sex/group, were maintained under normal laboratory husbandry conditions with *ad libitum* access to water. Mean bodyweights on the day of dosing were 18-22.5 g for females and 18.0 to 25.5 g for males. They were fasted for 24 hours before gavage administration of carnosic acid, 97% pure, in olive oil at a dose volume of 0.2 mL/10 g bw. Dosages were 0, 3500, 4500, 5500, 7500 or 8500 mg/kg bw. After dosing, mice were provided with *ad libitum* access to food and were subject to cageside examination 3-6 h after dosing and twice daily thereafter until scheduled termination on Day 15. Bodyweight and food consumption were measured daily. On Day 15, mice were killed by cervical dislocation and exsanguinated. Heart, intestines, kidney, liver, lungs and stomach were subject to gross examination. The median lethal dose (LD_{50}) was estimated by probit regression model. Clinical signs observed on the day of dosing included shivering, anorexia and diarrhoea. Mortalities increased with dose, although the paper does not indicate on which day or days the mice died, or the sex of the mice that died. All mice in the control group and the group dosed with 3500 mg/kg bw survived to Day 15. Three mice died in each of the groups dosed with 4500 and 5500 mg/kg bw. Five mice died in the 7500 mg/kg bw group, and 7 mice died in the 8500 mg/kg bw group. The LD_{50} was calculated to be 7100 mg/kg bw (95% CI, 6060-8940 mg/kg). Microscopic lesions of lymphoid cell infiltration were found in the kidneys of mice dosed with ≥ 7500 mg/kg bw. The authors also stated that all treated mice exhibited slight hydropic degeneration and single cell foci of necrosis in the liver, and myocardial fibrosis and inflammatory cell infiltration in the heart, but the photomicrographs included in the paper are not convincing.

Acute toxicity study of rosemary extract in rats (Anadón et al. *2008). Regulatory status: Non-GLP*

Two separate rosemary extracts were used for this study, one prepared from leaves collected in spring and the other prepared from leaves collected in autumn. Leaves were dried and stored frozen until the extracts were prepared by supercritical fluid extraction with ethanol as a modifier. The extracts were subject to analysis for diterpene content by HPLC, and were also subject to assay of antioxidant activity. The test subjects were male and female Wistar rats, approximately 8 weeks of age. They were individually housed under standard laboratory environmental conditions. Food and water were provided *ad libitum*, with the exception of the night before dosing, and for 18 hours prior to blood collection on the day of necropsy, when rats were fasted. Rats were assigned to three groups of 6/sex/group. The control group was dosed with corn oil by oral gavage, while the other two groups were dosed with one or other of the rosemary extracts at a dosage of 2,000 mg/kg bw dissolved in corn oil at a concentration of 200 mg/mL. Rats were observed twice daily for mortality and clinical signs, and were subject to a detailed physical examination prior to dosing and daily through to Day 15. Body weight, food consumption and water consumption were measured daily. On Day 15, blood was collected from the retroorbital plexus of fasted rats before they were weighed, killed by CO_2 inhalation, exsanguinated and subject to gross necropsy. Tissues fixed for histopathology were adrenal glands, brain, heart, ileum, jejunum, caecum, colon, duodenum, kidneys, liver, lungs, pancreas, spleen, stomach, tested, thymus, thyroid and parathyroid glands. The method states that organ:bodyweight ratios were calculated, but does not indicate which organs were weighed.

Analysis showed that the total concentration of phenolic components in the extract from leaves collected in spring was approximately twice as high as the concentration in the extract from leaves collected in autumn, and the antioxidant activity was correspondingly higher in the extract from leaves collected in spring.

All rats survived the two-week observation period and there were no treatment-related effects on clinical observations, bodyweights or bodyweight changes, food consumption, water consumption, haematological parameters, clinical chemistry parameters, gross findings, organ weights, organ:body weight ratios, or microscopic findings. It was concluded that 2000 mg/kg bw rosemary extract had no adverse effects on male or female Wistar rats.

Thirty-day oral gavage study of carnosic acid in Wistar rats (Wang et al*. 2012). Regulatory status: Non-GLP*

Carnosic acid, 97% pure, was the test article for this study, and it was administered suspended in olive oil. The test subjects were Wistar rats, 10/sex/group. Rats were housed under standard laboratory environmental conditions, although the paper does not state whether they were group-housed or individually housed. Food and water were supplied *ad libitum*. After 12 days of acclimatization, when they were 8 to 9 weeks old, rats were assigned to four groups. Rats were dosed once daily by oral gavage with 0, 150, 300, or 600 mg/kg bw/day carnosic acid, at a dose volume of 5 mL/kg bw. Clinical observations and bodyweight were recorded daily. Food consumption was measured during the in-life phase but the frequency of measurement is not clear. On day 31 or 32, rats were anaesthetized with CO_2 and blood was collected for routine haematology and clinical chemistry, as well as measurement of thyroid hormones, testosterone and estradiol. Rats were then killed by exsanguination. A complete gross necropsy was conducted and fresh weights were recorded for liver, kidney, spleen, brain, thyroid, thymus, heart, ovary and testis. Motility of spermatozoa from the left epididymis of each male was assessed. A comprehensive list of tissues and organs was preserved in neutral buffered formalin. Sections of brain, heart, kidney, liver, lung, spleen, thyroid gland, prostate and testis from males, and uterus and ovary from females, were processed for microscopic examination.

All rats survived to the end of the study. Mild diarrhoea was reported in one male and two females in the 300 mg/kg bw/day group, and in two males and three females in the 600 mg/kg bw/day group, but the duration of the diarrhoea was not specified. The affected rats in the 600 mg/kg bw/day group also exhibited reduced activity, thin appearance and piloerection. There were no significant treatment-related effects on body weight or food consumption. Group mean aspartate aminotransferase (AST) of male rats treated with ≥300 mg/kg bw/day carnosic acid was slightly higher than that of male controls, and showed an apparent dose-response relationship, 15% and 20% higher than that of male controls in the 300 and 600 mg/kg bw/day groups respectively. Group mean total protein was slightly lower in male rats than in male controls, but a dose-response relationship was not evident and in clinical terms the difference was negligible. No other haematology or clinical chemistry parameters showed statistically significant differences to those of sex-matched controls. Rats of both sexes treated with ≥300 mg/kg bw/day carnosic acid had group mean liver:bodyweight ratios higher than those of sex-matched controls, but a dose-response relationship was evident only in females. Compared to group mean liver:bodyweight ratios of control females, group mean values were approximately 10% and 16% higher in the 300 and 600 mg/kg bw/day females respectively. Group mean kidney:bodyweight ratios of males dosed with 600 mg/kg bw/day carnosic acid were 15% lower than those of sex-matched controls. Microscopic lesions were found only in the 600 mg/kg bw/day group, but were present in both sexes in that group. In hearts, multiple small foci of myocardial fibrosis and inflammatory cell infiltration were observed. Mild multifocal inflammatory infiltration was also present in the liver sinusoids. Minimal multifocal hydropic degeneration was present in the proximal convoluted tubules of the kidneys.

The authors of the study did not identify a NOAEL. Because of lack of detail concerning the clinical observations, and inconsistencies in findings between this study and those summarized by JECFA, FSANZ does not consider this study to be suitable as a definitive study.

JECFA (2017) reviewed a number of unpublished studies of rosemary extract in rats. FSANZ does not have access to those studies. Studies ranged in duration from 14 to 90 days, carnosic acid and carnosol content of the test article ranged from 5 to 33%, and the dosage of carnosic acid and carnosol ranged from 3 to 69 mg/kg bw/day. A consistent finding in the 90 day studies was an increase in liver weight in treated animals, associated with centrilobular hypertrophy, changes in hepatocyte cytoplasm consistent with increased glycogen storage, and increased smooth endoplasmic reticulum. In the absence in increases in circulating liver enzymes, JECFA concluded that these were adaptive changes. Slight bile duct hyperplasia in one four-week study was similarly considered non-adverse. The highest NOAEL among the 90-day studies, expressed as carnosic acid plus carnosol, was 64 mg/kg bw/day.

3.2.3 Chronic and carcinogenicity studies in animals

No chronic or carcinogenicity studies of rosemary extract, carnosic acid or carnosol were submitted in the application or located from other sources. Such studies are not considered to be necessary for hazard assessment of rosemary extract, because genotoxicity assays are negative and there is no evidence of preneoplastic lesions in short-term studies.

3.2.4 Genotoxicity

In vivo *chromosome aberration and micronucleus assay of rosemary extract in bone marrow cells of Wistar rats (Gaiani* et al*. 2006). Regulatory status: Non-GLP*

The rosemary extract used in this study was prepared by the study authors from leaves and stems of rosemary. The extraction was performed using “hydroalcoholic solutions” of unstated alcohol concentration. The test subjects were Wistar rats, obtained at six weeks old and group-housed under standard laboratory environmental conditions. Rats were assigned to three experimental and two control groups, 3 rats/sex/group. The experimental groups were administered a single dose of rosemary extract by oral gavage, at a dose volume of 0.5 mL and a concentration of 6.43, 100 or 200 mg/kg bw of rosemary extract (as dry weight) in water. The negative control group was gavaged with 0.5 mL water. The positive control group was dosed with cyclophosphamide, 30 mg/kg bw. Rats were given an intraperitoneal injection of 0.5 mL 0.16% colchicine 22.5 hours after being dosed by gavage. Ninety minutes after the colchicine injection, rats were killed by CO_2 asphyxiation. Both femurs were excised from each rat, the marrows of both bones removed, and cells processed for examination. Two thousand polychromatic erythrocytes from each rat were examined for presence of micronuclei. One hundred metaphases from each rat were examined for chromosomal aberrations. The mitotic index was determined by counting the number of mitotic cells in 1000 cells per rat. Treatment with rosemary extract had no significant effect on frequency of micronuclei in polychromatic erythrocytes, mitotic index or frequency of any type of chromosomal aberration, when compared to cells from rats in the negative control group. Frequency of chromosomal aberrations was significantly increased in cells from rats in the positive control group, confirming the validity of the assay. It was concluded that the rosemary extract was not cytotoxic or clastogenic at the doses tested.

In vitro *micronucleus assay of rosemary extract (de Oliveira et al. 2017) Regulatory status: Non-GLP*

The test article for this study was a commercial rosemary extract, although the solvent was not specified. The test systems were cultures of murine macrophages (RAW 264.7), human gingival fibroblasts (FMM-1), human breast carcinoma cells (MCF-7) and human cervical carcinoma cells (HeLa). Cells were cultured in 24-well plates at a density of 2 x 10^4/mL in Dulbecco's modified Eagle's medium (DMEM) for 24 h. The supernatant was then discarded and replaced with DMEM containing 0, 25, 50 or 100 mg/mL rosemary extract. Each assay was performed in duplicate. After 24 h incubation, the supernatant was discarded and the cultures were washed with phosphate-buffered saline to remove nonviable cells. Remaining cells were fixed with 10% formaldehyde for 10 min, washed and stained for fluorescence microscopy. The frequency of micronuclei was observed in 1000 cells at each concentration of rosemary extract. Incubation with rosemary extract did not have any significant effect on the frequency of micronuclei in RAW 264.7 cells. In FMM-1 cells and MCF-7 cells, exposure to rosemary extract resulted in a significantly lower frequency of micronuclei than in control cells, and rosemary extract concentration of ≥ 50 mg/mL completely inhibited the production of micronuclei in MCF-7 cells. Micronucleus frequency was low in all HeLa cells, so treatment had no statistically significant effect, but micronucleus production was completely inhibited at concentrations of rosemary extract ≥ 50 mg/mL. It was concluded that rosemary extract is not genotoxic under the conditions of the assay.

No other genotoxicity assays of rosemary extract were submitted or located in the peer-reviewed scientific literature. JECFA (WHO 2017) had access to a number of unpublished reports of genotoxicity assays of rosemary extract. These included a human lymphocyte mutagenicity assay, a TK6 (human lymphoblastoid cell) gene mutation assay, two bacterial reverse mutation assays (Ames tests) and a mouse micronucleus assay. JECFA concluded that the results did not indicate a genotoxic concern.

3.2.5 Developmental and reproductive studies in animals

Reproductive and developmental toxicity study of dietary rosemary extract in Crl:WI(Han) rats (Sequani Ltd. 2018; draft final report) Regulatory status: GLP; conducted according to OECD Guideline 421

The test article in this study was rosemary extract containing 42.98 % carnosic acid and 4.24 % carnosol. The stability of the test article in formulated diets was determined in a preliminary study and diets were maintained and used within the conditions determined. Concentration and homogeneity of the test diets were assayed from diet preparations for the start of dosing, the start of lactation in females, the end of the lactation period (postnatal day 13) in females, and for the final dose preparation for males. Rosemary extract was administered at 0, 2100, 3600 and 5000 ppm in the diet throughout the study, except for P generation females from gestation day (GD) 20 through to termination on postnatal day (PND) 13, when the concentration of test article in the diet was halved for each treatment group in order to adjust for increased food consumption.

The test subjects were Crl:WI(Han) rats. P generation males were eight to nine weeks of age and females were 11 to 12 weeks of age on arrival. Rats were acclimatised for 19 days prior to study start, and then assigned to study groups of 10/sex/group. Through acclimatisation and the in-life phase, rats were kept under standard laboratory environmental conditions. Prior to mating, rats were group-housed as either three males or four females/cage. Rats were kept in pairs of one male and one female for mating. After mating, males were returned to their group cages and females were housed individually through pregnancy and with their pups from parturition. Food and water were provided ad libitum.

P generation rats were dosed for two weeks prior to being pair-housed for mating for two weeks. The P generation rats were subject to twice-daily cageside checks for mortality and

moribundity, daily cageside observations and weekly detailed observations. P generation males were weighed on Study Day 1 and weekly thereafter until they were killed and necropsied, after all the females had been confirmed as pregnant. Bodyweights of P generation females were recorded on the Study Day 1 and then at weekly intervals until the day of mating. Females were also weighed on Days 0, 7, 14 and 20 of gestation and on Days 1, 4, 7, 10 and 13 of lactation. Bodyweights of rats of both sexes in the 5000 ppm dose group were also recorded in the middle of the first week of study. Food consumption of P generation males was recorded weekly prior to mating and after the mating period until termination, but not during the mating period. Food consumption of P generation females was recorded weekly prior to mating, was not recorded during the mating period, but was recorded on an individual basis on Gestation Days (GDs) 0 to 4, 4 to 7, 7 to 10, 10 to 14, 14 to 17 and 17 to 20, and Postnatal Days (PNDs) 1 to 4, 4 to 7, 7 to 10 and 10 to 13.

The number of pups per litter was recorded on the day of birth (PND 1) and anogenital distance of each pup was recorded. On PND 4, litters were culled to not more than eight pups, with as close to an even number of male and female pups as possible. Blood was collected and pooled from culled pups on a per litter basis for T4 determination. Pups were subject to twice-daily mortality and moribundity checks, and daily cage side clinical observations. Bodyweights were recorded for individual pups on PNDs 1, 4, 7, 10 and 13. Male pups were examined for nipples or areolae on PND 12.

P generation males were killed by CO_2 inhalation after all the P generation females had littered. P generation females were killed by CO_2 inhalation on PND 13. All P generation rats were subject to blood collection and gross necropsy, and the number of implantation sites was recorded for each P generation female. The weights of testes, epididymides, prostate, seminal vesicles (including coagulating glands) were recorded for P generation males, and the tissues were then preserved and processed for histopathology, as well as thyroids (including parathyroids) and any gross lesions. Tissues preserved and processed for histopathology from P generation females were thyroids (including parathyroids), any gross lesions, ovaries, vagina, and uterus including cervix and oviducts.

Pups were killed by decapitation on PND 13 with collection of blood from one pup/sex/litter. Pups were subject to gross external examination, and whenever possible, a thyroid gland was collected from one pup/sex/litter and preserved for histopathological examination.

Measured concentrations of carnosic acid confirmed that concentrations of rosemary extract were within 13% of target values and suitable for use in the study. No carnosic acid was detected in control diets. Homogeneous distribution of carnosic acid in the test diets was also confirmed. Mean intakes of rosemary extract by P generation males were 0, 129.5, 218.9 and 316.2 mg/kg bw/day. Mean intakes of rosemary extract by P generation females were 0, 166.7, 275.5 and 401.2 mg/kg bw/day. When expressed as carnosic acid plus carnosol, these values were 0, 61.2, 103.4 and 149.3 mg/kg bw/day for males and 0, 78.7, 130.1 and 189.4 mg/kg bw/day for females.

Administration had no effect on P generation survival, food intake, oestrus cycling, fertility, gestational duration, post-implantation loss, number of pups born alive, pup sex ratio, pup survival, organ weights, or gross necropsy findings. Group mean serum T4, measured in P generation males, did not show any dose-related effects. The group mean bodyweight gain of 5000 ppm males was slightly lower than that of control males, but this is likely to be affected by very poor weight gain in one individual in the two weeks prior to the mating period. Group mean weights of organs of the reproductive tract in P generation males showed no treatment-related effects. Dietary exposure to rosemary extract had no effect on the group mean bodyweight gain of pregnant P generation females, and during lactation their group mean bodyweight gain was slightly increased relative to female controls.

In pups (F generation) there were no treatment-related effects on survival, mean bodyweight or bodyweight gains, clinical signs, anogenital distance at PND 13, retention of nipples or areolae in male pups at PND 13, or external anatomical features including genitalia. At PND 13, but not PND 4, there was a dose-related decrease in group mean serum T4 in pups in all treatment groups. Compared to male F controls, the values for F males were 5%, 18% and 27% lower in the 2100, 3600 and 5000 ppm and high dose groups respectively. The corresponding values in females were 4%, 15% and 25% lower than those of female F controls. The differences for pups of dams in the mid- and high-dose groups were statistically significant ($p \leq 0.001$). There were no test item-related histopathological findings in the thyroid glands in any rats given rosemary extract, and there were no associated changes in developmental rate in the F generation pups.

As a consequence of the findings in group mean T4 values of F generation pups on PND 13, TSH was also determined from the blood collected from the same rats, although the assays were not conducted under GLP. TSH values at PND 13 were highly variable, with the standard deviations around the group means as large as, or larger than, the group means. For males, group mean TSH concentration was higher (approximately +20%) at 2100 ppm when compared with controls, whereas at 3600 and 5000 ppm, group mean TSH concentrations were reduced (approximately -42% and -71% respectively). For females, the group mean TSH value at 2100 ppm was comparable (approximately + 3%) with that of controls but group mean values at the 3600 and 5000 ppm were reduced (approximately 51% and 62% respectively), although there was no clear dose-response relationship.

The NOAEL for reproductive parameters of parental (P) generation, and the NOAEL for maternal toxicity, was the highest dose tested, equivalent to 316.2 mg/kg bw/day rosemary extract for males and 401.2 mg/kg bw/day rosemary extract for females. When these values are converted to the sum of carnosic acid and carnosol, they are 149.3 mg/kg bw/day for males and 189.4 mg/kg bw/day for females. The NOAEL for offspring toxicity was 166.7 mg/kg bw/day, equivalent to 78.7 mg/kg bw/day carnosic acid and carnosol, on the basis of low group mean serum T4 and TSH in pups at higher doses on PND 13. The FSANZ conclusion differs to the study authors who considered that the decreases in T4 concentration were not adverse on the basis that they were not associated with histological changes in the thyroid gland or signs of developmental delay. Triiodothyronine (T3) measurements were not available to FSANZ at the time of review.

3.2.6 Special studies in animals

Replacing the basal diet of Segureña ewes with 10% or 20% w/w distilled rosemary leaves throughout gestation and lactation did not have any significant effect on the mean daily weight gain of their lambs from birth to slaughter, when compared to that of lambs from control ewes fed only the basal diet. The group mean daily weight gain of lambs from control ewes was 0.18 ± 0.087 kg bw/day. The lambs of the ewes in the 10% rosemary extract group had a group mean daily body weight gain of 0.18 ± 0.093 kg/day, while that of lambs from ewes in the 20% group was 0.19± 0.075 kg/day (Moñino *et al*. 2008). Supplementation of the diet of lactating Valle del Belice ewes with 1200 mg/day rosemary extract significantly increased their milk supply. Although the protein and casein content decreased when expressed as a percentage, the increased milk supply meant that there was an overall increase in daily production of casein, total protein, fat and lactose. However rosemary extract fed at 600 mg/day did not have the same effects (Chiafalo *et al*. 2010). Boutoial *et al.* (2013) did not report the daily milk production of Murciana-Granadina goats, but their results show that the percentage of dry matter, fat and lactose decreased in milk of goats fed distilled rosemary leaves as 20% w/w of the diet, while supplementation with ≥ 10% distilled rosemary leaves was associated with an increase in protein content. Changes were also observed in the relative percentages of fatty acids in the milk. At ≥10% rosemary leaves in

the diet, there was a decrease in C_{14} and increases in $C_{18:2}$ and PUFA content, and at 20% rosemary leaves in the diet, there was also a decrease in C_{10} and an increase in C_{17} (Boutoial *et al* 2013). The feeding of Sardinian sheep 10g/day dried rosemary leaves increased the total phenolic content, enhanced antioxidant properties and decreased the lipid oxidation in cheese made from their milk (Branciari *et al*. 2015). In the studies of Chiafalo *et al.* (2010), Boutoial *et al*. (2013) and Branciari *et al.* (2015), the milk was not fed to the offspring of the animals, so the effects, if any, on these compositional changes were not assessed. However Martínez (2013), found that supplementing the diet of Segureña ewes with 10% or 20% w/w distilled rosemary leaves throughout gestation and lactation caused a significant increase in the levels of polyunsaturated fatty acids and unsaturated fatty acids, with a concomitant decrease in the percentage of saturated fatty acids in the meat of the lambs at slaughter. These changes were present even though the lambs had no exposure to rosemary leaves from weaning at 13±1 kg to slaughter at 25±2 kg bodyweight (Martínez, 2013).

3.2.7 Other studies

Reproductive and developmental toxicity study of rosemary extract in Wistar rats (Lemonica et al*. 1996) Regulatory status: Non-GLP*

The test article for this study was an aqueous extract of leaves, flowers and stems of *Rosmarinus offinalis.* A saline solution of unstated concentration was used as the vehicle and was also the control article. Virgin female Wistar rats were maintained under conditions of controlled temperature and light cycle, and provided with food and water *ad libitum*. Housing was not specified. The rats were mated overnight, and assigned to groups. Twelve rats were assigned to be dosed with the test article from Gestational Day (GD) 1 to 6, with a control group of 12 assigned to be treated with the control article over the same days. Fourteen rats were assigned to be dosed with the test article from GD 6 to GD 15, with a control group of 11 rats dosed with the control article on the same days. The test article was administered by gavage of 26 mg/day in a volume of 2.0 mL. All rats on study were weighed on GDs 1, 7, 14 and 21. On GD 21, the rats were killed and their uterine horns were removed. Numbers of implantations, resorptions, dead fetuses and live fetuses were recorded. The number of corpora lutea on the ovaries were also recorded. Rates of pre-implantation and post-implantation loss were calculated. Fetuses were weighed and examined for external abnormalities. Half of the fetuses in each litter were preserved for visceral examination and the other half were processed for examination of skeletal development.

The pregnant dams had no clinical signs of toxicity and maternal weight gain was unaffected by treatment. There were no significant differences between treated rats and controls in number of implantation sites, number of resorptions or number of live fetuses/litter. Fetuses of treated dams had bodyweights comparable to those of fetuses from control dams, and there were no treatment-related effects on fetal development. The rate of postimplantation loss in dams treated from GD1 to GD6 was similar to that of controls gavaged with saline on the same days, but the rate of preimplantation loss was higher in the treated dams (8.5% compared to the control value of 4.0%). However, this difference was not statistically significant. There were no statistically significant differences in any parameter when dams treated from GD 6 to GD15 were compared to control rats dosed with saline on the same days. Post-implantation loss in this group was 8.7%, compared to 6.2% in controls.
The authors concluded that aqueous rosemary extract had no adverse effects after implantation, but may have interfered with implantation. They cited evidence that extracts of rosemary may have an anti-gonadotrophic effect in rats, and also cited evidence that a tea prepared with rosemary and another plant has been used for fertility control in some cultures. FSANZ does not consider that this study is relevant to assessment of the safety of ethanolic or acetone extract of rosemary, because it would be expected that different fractions of the plant would extract into water.

Sixty-three day oral gavage study of rosemary extract in Sprague-Dawley rats (Nusier et al*. 2007). Regulatory status: Non-GLP*

Test article for this study was extracted from fresh rosemary leaves harvested by the authors. The leaves were dried and ground, and then refluxed in 70% ethanol at 60 to 70ºC for 36 hours. The filtered ethanol extract was concentrated by evaporation, weighed and then dissolved in distilled water for administration. Test subjects were adult Sprague Dawley rats, 30 males and 60 females, maintained under controlled environmental conditions and provided with food and water *ad libitum*. Rats were assigned to three groups of 10 males and 20 females/group. Male rats were administered 0, 250 or 500 mg/kg bw rosemary extract by oral gavage, daily for 63 days, at a dose volume of 1 mL. Female rats were brought into oestrus by oestradiol injection and two females were housed with each male for 10 days, a duration that should have included two oestrus cycles. The paper does not make it clear when the females were placed in with the males, relative to the initiation of dosing of the males. One week after the males and females were separated, females were killed and subject to partial necropsy to determine the numbers of pregnancies, implantation sites, viable fetuses and fetal resorptions. The males were killed after 63 days of treatment with rosemary extract, following collection of blood for measurement of serum glucose, cholesterol, triglycerides, bilirubin, AST, ALT, testosterone, FSH and LH. Bodyweights and weights of paired testes, seminal vesicles and preputial glands were measured. Sperm motility and sperm count were assessed using preparations from a cauda epididymis of each rat. The testes, epididymides, accessory sex glands and vasa deferentia were fixed in Bouin's fixative for microscopic examination, which included measurement of diameter of 100 seminiferous tubules, the size of 800 Leydig cell nuclei, and the height in 360 epithelial cells in each of caput epididymis, cauda epididymis and seminal vesicle from each rat. In testis sections from 10 rats/group, counts were made of spermatogonia, spermatocytes, spermatids, and interstitial cell types.

Daily oral gavage with up to 500 mg/kg bw/day rosemary extract had no effect on bodyweight or bodyweight gain. The group mean weights of testes, epididymides, seminal vesicles, ventral prostates and vasa deferentia of the 500 mg/kg bw/day rats, but not the 250 mg/kg bw/day rats, were significantly lower than those of control rats. Other values that were significantly lower in 500 mg/kg bw/day males, when compared to controls, were sperm motility, sperm density, seminiferous tubule diameter, Leydig cell nuclear diameter, and epithelial cell height in all three areas examined. The same group had significantly lower group mean counts of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids, and interstitial cells including fibroblasts, immature Leydig cells, mature Leydig cells, and degenerating cells, compared to controls. Group mean values for glucose, cholesterol, triglycerides, bilirubin, AST and ALT were similar between control and treated rats, but males in the 500 mg/kg bw/day group had significantly lower group mean testosterone, FSH and LH than control males. Female rats bred with males in the 500 mg/kg bw/day rosemary extract group had lower group mean values for implantation sites and viable fetuses in females mated to control males, and a higher ratio of resorptions to total implantations. The authors concluded that rosemary extract decreased fertility in male rats, and suggested that the observed effects on various parts of the male reproductive system are mediated through decreased androgen levels.

FSANZ does not consider this study relevant to the current application. The study used an extract that was soluble in water, whereas commercial rosemary extract is insoluble in water.

Investigation of the diuretic effects of aqueous extract of rosemary on Wistar rats (Haloui et al. *2000). Regulatory status: Non-GLP*

Rosemary is used as a folk remedy for urinary disorders in Morocco, where this study was conducted, and the study was designed to investigate the claimed diuretic properties of

rosemary. The method of preparation of the test article, boiling dried plant material in water, allowing it to stand and filtering, reflected the procedure used to prepare the folk remedy. The test subjects for the study were adult male Wistar rats weighing 280-300 g. Rats had *ad libitum* access to food and water, and were maintained under a 12 hour light/dark cycle. They were assigned to groups of at least 5 rats/group and dosed daily for one week by oral gavage with 0%, 8% or 16% v/v rosemary extract in distilled water, at a dose volume of 10 mL/kg. From immediately after the first dose administration to the end of the in-life phase, rats were housed in individual metabolism cages. Urine was collected daily, and the volume and electrolyte content were measured. Urinary creatinine was measured, and creatinine clearance calculated, on the last day of study. After one week of treatment, rats were anesthetised and blood was collected for measurement of plasma urea, creatinine and electrolytes, and the rats were then killed.

Although statistically significant differences in group mean urine volume, sodium, potassium and chloride, when compared to group mean values of the control group, were observed in the 8% group, they were not observed in the 16% group and are therefore not attributable to rosemary extract. Similarly, a decrease in group mean creatinine clearance in the 8% group, relative to the control group, was not observed in the 16% group and is therefore not related to rosemary extract. Treatment had no effect on plasma urea or potassium, but group mean values for plasma sodium and chloride were significantly lower for the 16% group than for controls.

That authors concluded that aqueous extract of rosemary has a diuretic effect, on the basis of the results from the 8% group, but FSANZ does not agree with this conclusion because the effects were not observed in the 16% group. Furthermore this study is not considered relevant to the safety assessment of ethanolic or d extracts from rosemary, because it is likely that different chemicals would be extracted into water.

3.2.8 Human tolerance studies

Investigation of the effect of rosemary extract on the absorption of non-haeme iron in human volunteers (Samman et al*. 2001) Regulatory status: Non-GLP*

Volunteers for this study were young women between the ages of 19 and 39. Inclusion criteria were a haemoglobin concentration > 7.0 mmol/L, no medications (other than oral contraceptives) or nutritional supplements during the past 2 months, not pregnant or breastfeeding, and no participation in studies involving radioisotopes or donated blood during the past two months. Fourteen of the volunteers were assigned to the rosemary extract cohort of the study. Study results for the other cohort (green tea) are not summarized here. Background radioactivity of each subject was determined prior to the start of the study. The test article was a commercial rosemary extract comprising 2.16% carnosic acid, 3.45% carnosol and 8.18% rosmarinic acid by weight. The extract was diluted (10% w/vol) with ethanol:water (2:1 v/v) solution. Four mL of diluted extract was added to meat sauce which was part of the test meal. Test meals were extrinsically labelled with either ^{55}Fe or ^{59}Fe in random order, and absorption was estimated by whole-body retention and isotope activity in the blood 2 weeks after the test meals. Also 2 weeks after the test meals, a reference dose of iron labelled with ^{59}Fe was given to each subject and whole-body retention was measured after a further 2 weeks. Each subject consumed a control test meal (meal A) or the same meal with extract added (Meal B), either on the previous or the following day. Meals were provided under supervision and consumed between 0800 and 1000, when subjects had fasted for 12 to 16 h. Subjects consumed each meal twice, on four consecutive mornings in the order ABBA or BAAB. Subjects consumed the meals within 30 minutes, with ultrapure water and with bread to wipe residual sauce from the plate. Subjects were asked to refrain from consuming any food or beverage for 4 h after consuming each meal. Total iron content of the meals was determined by atomic absorption spectroscopy, and nonhaeme iron content

was determined by spectrophotometry. Haeme iron content of the meals was calculated as the difference between total and nonhaeme iron content and represented 21% of the total iron content of the meals for the half of the study in which rosemary extract was the test article. Iron absorption was determined two weeks after consumption of the test meals, subjects underwent whole-body counting and haemoglobin, serum ferritin, ^{55}Fe and ^{59}Fe were measured in fasted blood samples. Iron absorption from a radiolabelled reference dose of ferrosulphate was also determined to correct for interindividual differences in iron absorption, with whole-body counting after a further two weeks.

All 14 subjects enrolled in the rosemary extract cohort completed the study. Addition of rosemary extract was found to inhibit non-haeme-iron absorption in 9 of the 14 subjects. The mean absorption of non-haeme iron from Meal B was 6.4 ± 4.7%, in contrast to 7.5 ± 4.0% from Meal A. This is slightly under a 15% decrease in group mean nonhaeme iron absorption, although the standard deviations around the group means were large, and the group sizes were small.

Investigation of the effect of rosemary extract on vascular function in human volunteers (Sinkovic et al. *2011). Regulatory status : non-GLP*

Arterial endothelial dysfunction is an early event in atherogenesis. It can be evaluated by ultrasound measurement of flow-mediated dilatation (FMD) in the brachial artery, and by measuring serum markers such as vascular cell adhesion molecule 1 (VCAM-1) and inter-cellular adhesion molecule 1 (ICAM-1). The aim of this study was to evaluate the effect of 21 day oral supplementation with a rosemary extract on FMD and on serum levels of ICAM-1, VCAM-1, serum plasminogen-activator-inhibitor type 1 (PAI-1), high-sensitivity C-reactive protein (hs-CRP), fibrinogen, superoxide dismutase (SOD), glutathione peroxidase (GPX), tumour necrosis factor-α (TNF-α), total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides in healthy young volunteers.

The test article for this study was rosemary extract which was administered for 21 consecutive days at a daily dose of 77.7 mg, equal to 0.97 mg carnosol, 8.6 mg carnosic acid and 10.30 mg rosmarinic acid. The test subjects were 19 healthy young volunteers; 7 men and 12 women with a mean age of 34.3 ± 7.7 years. Eight were smokers and three had a family history of symptomatic atherosclerosis. Inclusion criteria were being over 18 years of age, written informed consent and for women, a negative urine-β-HCG test. Exclusion criteria were any known chronic, malignant disease, use of any medication, pregnancy, breast-feeding and simultaneous participation in another clinical study. From one week prior to start of rosemary extract consumption to the end of the study, participants were asked to discontinue use of antioxidant supplements, vitamins and alcohol, and they were asked to consume, throughout the study, a well-balanced diet with approximately 30% proteins, 60% carbohydrates and 10% fat, with at least one daily serving of meat but not more than two apples. One week prior to the start of rosemary extract consumption, volunteers underwent clinical examination including recording of pulse, blood pressure and an electrocardiograph (ECG). One week later (Day 7 of study), blood pressure and pulse were again recorded, FMD was measured in the brachial artery, using vascular ultrasound, and fasting blood samples were collected for measurement of serum levels of ICAM-1, VCAM-1, PAI-1, fibrinogen, SOD, GPX, hs-CRP, TNF-α, total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides. On day 7, volunteers commenced taking a daily dose of rosemary extract. Subjects were interviewed on study days 14, 21 and 28 to determine if they experienced any side effects. On day 28, further blood samples were drawn and FMD in the brachial artery was measured again. FMD was defined as the endothelium-dependent dilatation of the brachial artery in response to reactive hyperaemia, and was expressed as a percentage change in diameter relative to the baseline scan. Arterial diameter was measured three times, at the end of diastole through three cardiac cycles, and the results averaged. FMD values of less than 4.5% were defined as endothelial dysfunction.

The rate of endothelial dysfunction decreased significantly as a result of treatment with rosemary extract from a group mean of 68.4% to 15.5% (p = 0.0038). Mean PAI-1 level decreased significantly, from a group mean baseline value of 4.4 ± 1.3 U/ml to 3.3 ±0.7 U/mL (25% decrease; p = 0.0025). Group mean levels of FMD increased from 3.76% to 6.7% (78% increase, p value not reported) and those of PAI-1 decreased from 4.9 U/mL to 3.3 U/mL (33% decrease; p value not reported). FMD increased but the difference was not statistically significant. Consumption of rosemary extract was not associated with significant changes in serum lipid profile or on group mean serum levels of SOD, ICAM-1, VCAM-1, GPX, hs-CRP, TNF-α or fibrinogen. The authors noted that the baseline values for these parameters were within normal limits in all subjects.

This study was not designed as a tolerance study and only one dose of 77.7 mg/day rosemary extract was used, with no adjustment for bodyweight. However it is noted that there were no adverse effects associated with this dose of rosemary extract.

FSANZ notes that the rosemary plant has a long history of human use as a culinary herb and as a folk medicine (Ulbricht *et al* 2010; Begum *et al* 2013; Ribeiro-Santos *et al* 2015; WHO 2017).

3.3 Assessments by other regulatory agencies

3.3.1 Joint FAO/WHO Expert Committee on Food Additives (JECFA)

JECFA assessed rosemary extract at their 82nd meeting and published their conclusions in 2017 (WHO 2017). JECFA's review of the absorption, distribution, metabolism and excretion of rosemary extract was limited to *in vitro* studies and studies in laboratory rodents, and they did not consider the data from studies in sheep or goats. On the other hand, JECFA reviewed a number of unpublished toxicology studies that are not available to FSANZ, including a number of genotoxicity assays, most of which were *in vitro*, and a number of short-term and subchronic toxicity studies in laboratory rats. JECFA reviewed the study of male reproductive toxicity by Nusier *et al*. (2007) also reviewed by FSANZ, but rejected it from consideration because of the inclusion of water at 30% in the extraction solvent, which may have altered in the unstated composition of the extract, and because no similar lesions were observed in the male reproductive tract in the unpublished subchronic studies in rats, which were of longer duration. JECFA also rejected the Lemonica *et al*. (1996) study because the test article was an aqueous extract.

JECFA established a temporary acceptable daily intake (ADI) of 0 - 0.3 mg/kg bw for rosemary extract, expressed as the sum of carnosic acid and carnosol. This ADI was based on a NOAEL of 64 mg/kg bw/day, expressed as carnosic acid plus carnosol, in an unpublished 90-day toxicity study in rats, with application of a 200-fold safety factor. The overall safety factor of 200 includes a factor of two to reflect the temporary designation of the ADI. The ADI is temporary pending the submission of data concerning the reproductive and developmental safety of rosemary extract, and will be withdrawn if such data are not provided by the end of 2018. A safety factor to reflect the lack of a chronic study was not considered necessary, because of the lack of adverse effects in short-term studies at the highest doses tested.

3.3.2 European Food Safety Authority (EFSA)

EFSA published a Scientific Opinion on the use of rosemary extracts as a food additive in 2008 (EFSA 2008). The extracts considered included two ethanolic extracts, an extract prepared using supercritical carbon dioxide, and an extract prepared using a two-step process involving hexane and ethanol. The principal antioxidants in all these extracts are

carnosol and carnosic acid. EFSA concluded that rosemary extracts are not genotoxic, and have low acute and subchronic toxicity in the laboratory rat. A slight, reversible treatment-related increase in relative liver weight in rats in subchronic studies, with no corresponding increase in plasma liver enzymes but with corresponding minimal centrilobular hypertrophy and microsomal enzyme induction, was identified as an adaptive response rather than an adverse effect. The NOAELs of the 90-day studies in rats were in the range 180 to 400 mg extract/kg bw/day, corresponding to combined intake of carnosic acid and carnosol of 20 to 60 mg/kg bw/day. The studies EFSA describes are not referenced but were conducted under GLP, and were submitted by the petitioner, so it appears that they are unpublished study reports. However it is not possible to determine from the information given whether they are the same study reports as those assessed by JECFA. EFSA did not establish a numerical ADI because of the lack of data on reproductive toxicity or chronic toxicity. However EFSA commented that the data they reviewed did not give reason for concern, particularly in light of the negative results on genotoxicity assays and the absence of effects on reproductive organs in the rat subchronic studies. EFSA also noted that the margins between the NOAEL range from the rat subchronic studies and the dietary exposure estimates for European consumers were sufficiently large that EFSA could conclude that dietary exposure was not of safety concern (EFSA 2008).

In 2015, EFSA conducted a refined exposure assessment of rosemary extract in response to a proposed extension of use of rosemary extracts (E392) in fat-based spreads. The Panel concluded that the proposed extension would not result in a significant change to the exposure estimate completed in 2008, and that the conclusions regarding safety that were made in 2008 remained valid. Therefore the Panel considered that it was unlikely that there was any safety concern associated with the extension of use (EFSA 2015).

3.4 Conclusion

The submitted data, together with the recent assessment by JECFA (WHO 2017) are considered suitable to assess the hazard of rosemary extract.

There have been several studies on the kinetics and metabolism of carnosic acid and other constituents of rosemary extract in laboratory rodents, although the value of most of the studies is compromised by the removal of excessive amounts of blood. Oral bioavailability of carnosic acid is estimated to be ≥ 65%. Time to maximum plasma concentration of carnosic acid following oral administration (T_{max}) following oral gavage, with water as the vehicle, is short, between 20 and 40 min. There is relatively little information on the distribution of rosemary extract, carnosic acid or carnosol in rodents, and data on clearance from plasma are inconsistent. Oxidation, glucuronidation and methylation are the major pathways of metabolism, and a wide range of metabolites have been detected. Metabolites are found in both urine and faeces.

The feeding of rosemary extract to lambs has been shown to lead to the presence of rosemary diterpenes and metabolites in their muscles. Persistence of carnosic acid, carnosol and rosmarinic acid in the muscles of lambs has also been reported for approximately 50 days after they were weaned from ewes that were fed rosemary leaves throughout pregnancy and lactation. It is not clear whether these substances cross the placenta, but excretion of rosemary compounds in milk has been demonstrated in ewes and goats. As a storage compartment for these compounds, muscle appears to be saturable, and no adverse effects were observed in the lambs.

Exposure to rosemary extract has been found to result in reversible upregulation of some hepatic microsomal cytochrome P450 enzymes, but there is a lack of evidence of adverse effects of this upregulation.

The acute toxicity of carnosic acid in the mouse is low, estimated at 7100 mg/kg bw (Wang et al. 2010). An acute toxicity study of rosemary extract in rats did not identify any adverse effects at a dose of 2,000 mg/kg bw (Anadón et al. 2008).

JECFA (2017) reviewed a number of unpublished studies of rosemary extract in rats; studies to which FSANZ does not have access. Studies ranged in duration from 14 to 90 days, carnosic acid and carnosol content of the test article ranged from 5 to 33%, and the combined dosage of carnosic acid and carnosol ranged from 3 to 64 mg/kg bw/day. A consistent finding in the 90 day studies was an increase in liver weight in treated animals, associated with centrilobular hypertrophy, changes in hepatocyte cytoplasm consistent with increased glycogen storage, and increased smooth endoplasmic reticulum. In the absence in increases in circulating liver enzymes, JECFA concluded that these were adaptive changes. Slight bile duct hyperplasia in one four-week study was similarly considered non-adverse. The highest NOAEL among the 90-day studies, expressed as carnosic acid plus carnosol, was 64 mg/kg bw/day.

No chronic or carcinogenicity studies of rosemary extract, carnosic acid or carnosol were submitted in the application or located from other sources. No evidence of genotoxicity was found in either of the two genotoxicity assays to which FSANZ has access, and this finding is consistent with the conclusions of JECFA (WHO 2017) who had access to a number of genotoxicity assays of rosemary extract.

Following the Call for Submissions, the Applicant provided FSANZ with a draft final report of a reproductive and development toxicity screening study in rats using rosemary extract conducted according to OECD 421 test guideline . The NOAEL for reproductive parameters of parental (P) generation, and the NOAEL for maternal toxicity, was the highest dose tested, equivalent to 316.2 mg/kg bw/day rosemary extract for males and 401.2 mg/kg bw/day rosemary extract for females. When these values are converted to the sum of carnosic acid and carnosol, they are 149.3 mg/kg bw/day for males and 189.4 mg/kg bw/day for females. The NOAEL for offspring toxicity was 166.7 mg/kg bw/day, equivalent to 78.7 mg/kg bw/day carnosic acid and carnosol, on the basis of low group mean serum T4 and TSH in pups at higher doses on PND 13.

Nusier et al. (2007) concluded from a 63-day oral gavage study in Sprague-Dawley rats that rosemary extract had adverse effects on spermatogenesis in male rats, but JECFA noted that these findings were inconsistent with findings in the unpublished studies they reviewed, and JECFA also noted that the rats were dosed with material that is soluble in water, whereas the material in commercial rosemary extracts is not soluble in water. Similarly, a study by Lemonica et al. 1996, from which the authors concluded that rosemary extract may interfere with implantation of rat pups, is not likely to be relevant to the current assessment because the extract administered was soluble in water.

Supplementation of the diet of pregnant and lactating dairy ewes and goats with rosemary leaves has been shown to alter the composition of their milk (Chiafalo et al. 2010; Boutoial et al 2013), and in one study to decrease the percentage of saturated fatty acids in the meat of their lambs at slaughter (Martínez, 2013). However, these changes are not associated with any adverse effects on weight gain in lambs (Moñino et al. 2008).

No human health and safety concerns were identified for rosemary extract. There is little information on human tolerance in the scientific literature, but FSANZ notes that the rosemary plant has a long history of human use as a culinary herb and as a folk medicine (Ulbricht *et a*l. 2010; Begum *et al*. 2013; Ribeiro-Santos *et al*. 2015; WHO 2017). Sinkovic *et al*. 2011 identified no adverse effects of administration for 21 consecutive days of 77.7 mg rosemary extract, equal to 0.97 mg carnosol, 8.6 mg carnosic acid and 10.30 mg rosmarinic acid, to 19 healthy human volunteers; seven men and 12 women. The study by Samman *et*

al. (2001) suggested that rosemary extract may reduce absorption of non-haeme iron, but the group sizes were small and the standard deviations around the group means were large.

JECFA established a temporary ADI of 0 - 0.3 mg/kg bw for rosemary extract, expressed as the sum of carnosic acid and carnosol, on the basis of a NOAEL of 64 mg/kg bw/day identified in a 90-day toxicity study in rats, with the application of a 200-fold uncertainty factor. This uncertainty factor incorporates a factor of two to account for the temporary designation of the ADI, pending the submission of studies on the potential developmental and reproductive toxicity of rosemary extract. The recently submitted reproductive and developmental screening study in rats using rosemary extract identified NOAELs for reproductive, maternal and offspring toxicity that were more than 200-fold greater than the temporary JECFA ADI. Therefore FSANZ considers that the JECFA temporary ADI for rosemary extract remains appropriate for the purposes of this assessment.

4 Dietary exposure assessment

FSANZ undertook a dietary exposure assessment in order to determine the level of dietary exposure to carnosic acid plus carnosol from both naturally occurring and added sources. An initial dietary exposure estimate was published as part of the Call for Submissions report. In the Call for Submissions process, the applicant updated the proposed MPLs for a number of meat categories as outlined in Table 4.1. Consequently, a new dietary exposure assessment has been conducted. For all details on the dietary exposure assessment conducted at the Call for Submissions stage, please refer to the Call for Submissions SD1 at http://www.foodstandards.gov.au/code/applications/Documents/A1158 SD1.pdf. Only the results for the updated assessment are presented below.

Table 4.1: MPLs for meat categories, including updated concentrations, as requested by the Applicant

Category in application	Description of requested food in application	Carnosic acid plus carnosol concentration (mg/kg)	
		In application	*Revised*
8.2	Processed meat, poultry & game in whole cuts or pieces –fat content ≤10% fat	1.5	15
8.2	Processed meat, poultry & game in whole cuts or pieces –fat content >10% fat	37.5	37.5
8.2.3	Dried meat	37.5	150
8.3.2	Dried sausages (from raw meat)	50	100

4.1 Approach to estimating dietary exposure

Dietary exposure assessments require: data on the concentrations of the chemical of interest in the foods requested, including any naturally-occurring sources and any current permission for additions to food; and consumption data for the foods that have been collected through a national nutrition survey. The dietary exposures to carnosic acid plus carnosol were estimated using: (1) the maximum permitted levels in the requested food categories; (2) usual use levels in the requested food categories, as provided by the applicant; and (3) naturally occurring concentrations in rosemary leaves combined with food consumption data from the most recent Australian and New Zealand national nutrition surveys. Dietary exposures to carnosic acid plus carnosol from flavouring sources were not included (see discussion in Section 4.1.1.2 below). The dietary exposure assessments were undertaken

using FSANZ's dietary modelling computer program, Harvest[4]. Since there is a temporary ADI of 0–0.3 mg/kg body weight set by JECFA for rosemary extract expressed as carnosic acid plus carnosol (FAO/WHO, 2016), dietary exposures as a percentage of the ADI have been estimated in this assessment.

A summary of the general FSANZ approach to conducting the dietary exposure assessment for this application is in Appendix 1. A detailed discussion of the FSANZ methodology and approach to conducting dietary exposure assessments is set out in *Principles and Practices of Dietary Exposure Assessment for Food Regulatory Purposes* (FSANZ 2009).

4.1.1 Concentrations of carnosic acid plus carnosol in foods

4.1.1.1 Naturally-occurring concentrations of carnosic acid plus carnosol in foods

The applicant reported that the concentration of carnosol in dried rosemary leaves is 1-2 mg/gram and that the concentration of carnosic acid is 15-25 mg/gram (see Table 4.2). The concentration of carnosic acid plus carnosol in dried rosemary leaves used in the dietary exposure assessment is the mid-point of this range: 21,500 mg/kg. Using a factor of 0.35, derived using the energy content of dried rosemary leaves and fresh rosemary leaves from the nutrient database AUSNUT (FSANZ 2016), the concentration of carnosic acid plus carnosol used for fresh rosemary leaves is 7,525 mg/kg.

Table 4.2: Concentrations of carnosol and carnosic acid in dried rosemary leaves

Component	Concentration of carnosic acid plus carnosol in dried rosemary leaves	
	mg/g	mg/kg
Carnosol	1 – 2	1,000 – 2,000
Carnosic acid	15 – 25	15,000 – 25,000
Carnosic acid plus carnosol	16 – 27	16,000 – 27,000

4.1.1.2 Current uses of rosemary extract in foods in the Australian and New Zealand food supplies

Currently, rosemary extract may be used as a flavouring in foods sold in Australia and New Zealand (see Section 2.1.2). JECFA did not include non-antioxidant uses of rosemary extract in its assessment of dietary exposures to carnosic acid plus carnosol at its 82nd meeting, citing that flavouring essences are not likely to be used regularly and that dried rosemary and flavouring essences were not likely to significantly affect estimated dietary exposures to carnosic acid plus carnosol (WHO, 2017). FSANZ has considered the contribution of antioxidant uses of rosemary extract with and without the contribution of rosemary leaves to carnosic acid plus carnosol dietary exposures. Dietary exposures from flavours were also not included in the assessment by FSANZ given there is no quantitative data available on where and how much is used, and the likely small contribution to estimates of dietary exposure.

4.1.1.3 Proposed concentrations of carnosic acid plus carnosol as a food additive in foods

The food categories requested in the application to contain Rosemary extract (carnosic acid plus carnosol) as a food additive and their proposed MPLs (including the updated MPLs where relevant as a result of the Call for Submissions) are listed in Table 4.4 The applicant

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

also provided potential Usual Use levels for rosemary extract as an antioxidant in various food categories: 80% MPL in fats and oils and 50% MPL in other food categories. These concentrations are also listed in Table 4.4 Estimates of dietary exposure were calculated using the proposed MPLs and separately using the Usual Use levels.

The food category codes used by the applicant were based on the Australia New Zealand Food Classification System (ANZFCS) in Standard 1.3.1 – Food Additives of the Code and its related Schedules. However, the food classification codes in Harvest can vary and may also be split into sub-groups. To assess the populations' dietary exposures to carnosic acid plus carnosol, the food categories proposed by the applicant and the data provided on naturally-occurring carnosic acid plus carnosol in rosemary leaves were assigned to the relevant Harvest food classification codes. The categories selected reflect the description of the foods requested by the applicant, not the food additive codes.

4.1.1.4 Scenarios for the dietary exposure assessment for carnosic acid plus carnosol

A dietary exposure assessment was conducted for five scenarios (see Figure 4.1):

- Naturally occurring sources only:
    - *'Naturally-occurring'* scenario: includes naturally occurring carnosic acid plus carnosol only (i.e. from dried and fresh rosemary leaves only).
- Rosemary extract used at MPLs. These scenarios represent the most conservative approach. The estimated dietary exposures to carnosic acid plus carnosol from these scenarios are likely to over-estimated dietary exposures for the Australian and New Zealand populations over a period of time:
    - *'Added rosemary extract only_MPL'*: includes only the MPLs requested in the application and the updates as a result of the Call for Submissions, at 100% market penetration into each category requested. This scenario does not include the contribution from naturally occurring levels in rosemary leaves.
    - *'Naturally-occurring plus added rosemary extract_MPL'*: includes *Naturally-occurring* scenario plus all MPLs requested in the application and updated concentrations from the Call for Submissions, at 100% market penetration into each category requested. This scenario includes the contribution from naturally occurring levels in rosemary leaves.
- Rosemary extract used at Usual Use concentrations. Usual Use levels for fats and oils is 80% of the MPL. For all other foods, the Usual Use level is 50% of the MPL. These scenarios reflect a more likely dietary exposure to carnosic acid plus carnosol for the Australian and New Zealand populations over a period of time:
    - *'Added rosemary extract only_Usual Use'*: includes only the Usual Use levels as provided by the applicant, at 100% market penetration into each category requested. This scenario does not include the contribution from naturally occurring levels in rosemary leaves.
    - *'Naturally-occurring plus added rosemary extract_Usual Use'*: includes *Naturally-occurring* scenario plus Usual Use levels as provided by the applicant, at 100% market penetration into each category requested. This scenario includes the contribution from naturally occurring levels in rosemary leaves.

4.1.2 Food consumption data used

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

- **2011-12 Australian National Nutrition and Physical Activity Survey (2011-12 NNPAS)**, one 24-hour food recall survey of 12,153 Australians aged 2 years and above, with a second 24-hour recall undertaken for 64% of respondents (ABS, 2014). Only those respondents who had two days of food consumption data (n=7,735) were used in the assessment of dietary exposures to carnosic acid plus carnosol.
- **2008–09 New Zealand Adult Nutrition Survey (2008 NZ ANS):** a 24-hour recall of 4,721 New Zealanders aged 15 years and above, with a second 24-hour recall undertaken for 25% of respondents. (Ministry of Health 2011a; Ministry of Health 2011b). Only the first day of food consumption data was used in this assessment.
- **2002 New Zealand National Children's Nutrition Survey (2002 NZ CNS)**, one 24-hour food recall covering 3,275 New Zealand school children aged 5-14 years, with 25% of respondents also completing a second 24-hour recall. Only the first day of food consumption data was used in this assessment.

The design of these nutrition surveys and the key attributes, including survey limitations, are set out in Appendix 1.

One day of food consumption data from both of the NZ surveys were used for the dietary exposure assessment whereas the average of two days of data from the 2011-12 NNPAS was used for Australia. The two day average exposures better reflect longer term estimates of dietary exposure and therefore are a better estimate of chronic dietary exposure.

The hazard identification and characterisation did not identify any population sub-groups for which there were specific safety considerations in relation to dietary exposure to carnosic acid plus carnosol. The food categories requested in the application for addition of Rosemary extract are consumed by most of the Australian and New Zealand populations. Therefore, the whole survey population from each of the nutrition surveys were used for the dietary exposure assessment (Table 4.4).

1. Select the type of model

Food additive model in Harvest (best matches food groups requested in application)

2. Select the form of chemical to use in the assessment

Carnosic acid + Carnosol from all sources (rosemary extract & naturally-occurring)

3. Select the national nutrition surveys to use in the dietary exposure assessment

Australia:	*2011-12 National Nutrition and Physical Activity Survey (2011-12 NNPAS) (2 years & above)*
New Zealand:	*2002 National Children's Nutrition Survey (2002 NZ CNS) (5-14 years)*
	2008-09 Adult Nutrition Survey (2008 NZ ANS) (15 years & above)

4. Select the population group(s) to assess

a. Whole population (2 years & above (Au); 15 years & above (NZ); 5-14 years (NZ))

5. Determine the scenarios to model

5a. 'Naturally-occurring'

Exposure to Carnosic acid + Carnosol from naturally-occurring sources (rosemary leaves)

5b. 'Added rosemary extract only_MPL' Scenario

Includes only the MPLs requested in the application, at 100% market penetration into each category requested. Does not include the contribution from naturally occurring levels in rosemary leaves.

5c. 'Naturally-occurring plus added rosemary extract_MPL' Scenario

Includes Naturally-occurring scenario plus all MPLs requested in the application, at 100% market penetration into each category requested. Includes the contribution from naturally occurring levels in rosemary leaves.

5d. 'Added rosemary extract only_Usual Use' Scenario

Includes only the Usual Use levels as provided by the applicant, at 100% market penetration into each category requested. Does not include the contribution from naturally occurring levels in rosemary leaves.

5e. 'Naturally-occurring plus added rosemary extract_Usual Use' Scenario

Includes Naturally-occurring scenario plus Usual Use levels as provided by the applicant, at 100% market penetration into each category requested. Includes the contribution from naturally occurring levels in rosemary leaves.

Figure 4.1: Dietary modelling approach used for assessing dietary exposure to carnosic acid plus carnosol for Australia and New Zealand

Table 4.3: Population groups used in the dietary exposure assessment

Country	Survey	Age group	No. respondents (Day 1 only)	No. respondents (Day 1 and 2)
Australia	2011-12 NNPAS	2 years and above	n/a	7,735
New Zealand	2002 NZ CNS	5 – 14 years	3,275	n/a
	2008 NZ ANS	15 years and above	4,721	n/a

4.2 How were the estimated dietary exposures calculated?

Carnosic acid plus carnosol dietary exposures were calculated for each individual respondent in the national nutrition surveys using their individual food consumption records. The Harvest program multiplied the specified concentrations of carnosic acid plus carnosol for an individual food by the amount of the food that an individual consumed in order to estimate the exposure to carnosic acid plus carnosol from each food. Once this had been completed for all of the foods specified to contain carnosic acid plus carnosol, the total amount of carnosic acid plus carnosol consumed from all foods was summed for each individual. Where results are expressed on a body weight basis, each individuals body weight was used. Mean and 90th percentile (P90) exposures were then derived from the individuals' ranked exposures. Estimated dietary exposures for the population on a body weight basis were compared to the ADI for risk characterisation purposes.

4.2.1 Assumptions and limitations of the dietary exposure assessment

The aim of the dietary exposure assessment was to make the most realistic estimation of dietary exposures to carnosic acid plus carnosol as possible. However, where significant uncertainties in the data existed, conservative assumptions were generally used to ensure that the estimated dietary exposure was not an underestimate of exposure.
Assumptions made in the dietary exposure assessment included:

- Unless otherwise specified, all foods within a category contain carnosic acid plus carnosol at the concentrations listed in Table 4.4
- the request for addition of rosemary extract to '*Margarines (solid & liquid only), <80% oil*' does not include the addition to dairy blends with <80% oil
- the request for addition of rosemary extract to '*Nut butters & nut spreads*' excludes the addition to seed butters and seed spreads (e.g. tahini)
- the request for addition of rosemary extract to '*Grain bars, breakfast bars, breakfast cereals*' includes mueslis, cereals with dried fruit and/or nuts as ingredients, muesli bars and breakfast bars and nut snack bars
- the request for addition of rosemary extract to '*Grain bars, breakfast bars, breakfast cereals*' refers to the dry form of the breakfast cereal only, irrespective of whether it is meant to be cooked with liquid or not (i.e. flavoured porridges mixes etc.)
- the request for addition of rosemary extract to '*Flour based snacks (e.g. pretzels, crackers)*' includes savoury crackers and crispbreads of all grain types, grain-based snack foods (e.g. corn chips, popcorn, extruded grain-based snacks such as Cheezels, "Grain-waves") and pretzels
- the request for addition of rosemary extract to '*Cookies, pancakes, waffles, sweet pastries (rolls, doughnuts, muffins)*' includes all sweet biscuits (excluding fruit/ nut/ chocolate/ filling/ icing/ coating components), pikelets, pancakes, waffles, crumpets, plain sweet buns (excluding fruit/ nut/ chocolate/ filling/ icing/ coating components), Danishes (including filling and toppings), sweet croissants (including filling and

toppings), eclairs and profiteroles (including filling and toppings), baklava and sweet cake-style muffins and muffin bars (excluding fruit/ nut/ chocolate/ filling/ icing/ coating components)

- the request for addition of rosemary extract to '*Sauces & toppings (including mayonnaise & salad dressings)*' includes soy sauce
- the requested concentrations for carnosic acid plus carnosol in 'Sauces & toppings (including mayonnaise and salad dressings)' relates to the product as sold (i.e. MPL of 40 mg/kg in ready-to-eat sauces and dressings and a MPL of 40 mg/kg in dry sauce mixes etc.)
- the request for addition of rosemary extract to 'Processed nuts' includes salted and/or roasted and/or flavoured nuts
- the request for addition of rosemary extract to '*Potato chips including starch based snacks from roots, tubers, pulses and legumes*' excludes grain-based snacks such as corn chips and extruded grain-based snacks such as Cheezels
- the request for addition of rosemary extract to '*Dried sausages (from raw meat)*' includes fermented comminuted meats such as salamis
- dietary exposures to carnosic acid plus carnosol are negligible from flavouring sources
- where a food was not included in the dietary exposure assessment, it was assumed to contain a zero concentration of carnosic acid plus carnosol
- there is 100% market penetration of the use of Rosemary extract into the requested food category markets
- where a concentration is assigned to a food, this concentration is carried over to any mixed dishes where it has been used as an ingredient to capture exposure from all sources of the food in the diet
- there is no contribution to carnosic acid plus carnosol exposures through the use of complementary or other medicines.

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

4.3 Estimated consumer dietary exposures to carnosic acid plus carnosol

In this assessment, dietary exposures have been estimated for 'consumers only' (i.e. consumers of foods containing carnosic acid plus carnosol). Nutrition survey respondents who had no consumption or exposure to carnosic acid plus carnosol were excluded. The proportion of the population who are consumers varies between the different population groups assessed and the proportion changes between different scenarios. The factors should be considered when interpreting the results of the assessment.

4.3.1 Australians aged 2 years and above

4.3.1.1 Naturally-occurring scenario

The estimated mean and P90 consumer dietary exposures to carnosic acid plus carnosol for Australians aged 2 years and above are 0.048 mg/kg bw/day and 0.081 mg/kg bw/day for the *Naturally-occurring* scenario. Approximately 6% of Australians aged 2 years and above are exposed to carnosic acid plus carnosol through consumption of rosemary leaves (either on their own or as a part of mixed dishes such as casseroles). See Table 4.5 for details.

Table 4.4: Concentrations of carnosic acid plus carnosol used in the dietary exposure assessment for all scenarios

Category in application	Description of requested food in application	Harvest food category code	Food category name	Carnosic acid plus carnosol concentration (mg/kg)				
				Naturally-occurring	*Rosemary extract only*		*Naturally-occurring plus rosemary extract*	
					MPL	*Usual use*[ɤ]	*MPL*	*Usual use*[ɤ]
2.1	Fish & algal oils	50.1	Fish oil	0	50	40	50	40
2.2.2	Oil emulsions (<80% oil) – margarines (solid & liquid only)	2.2.2	Oil emulsions (<80% oil), except dairy blends	0	75	60	75	60
Nil	Rosemary	4.1.1.4.1	Fresh rosemary	7,525	0	0	7,525	7,525
		4.3.1.4.1	Dried rosemary	21,500	0	0	21,500	21,500
4.3.4	Nut butters & nut spreads	4.3.6.3	Nut butter	0	50	25	50	25
5.4	Icings & frostings, glazes & fillings	5.4	Icings & frostings	0	20	10	20	10
6.3	Grain bars, breakfast bars, breakfast cereals	6.3.1	Puffed &/or extruded cereals	0	50	25	50	25
		6.3.2	Breakfast biscuits & flakes	0	50	25	50	25
		6.3.3	Processed cereal & meal products, other	0	50	25	50	25
		20.2.2	Grains, cereals & cereal products	0	50	25	50	25
6.4	Flour based snacks (e.g. pretzels, crackers)	7.2.1.2	Biscuits & crackers, savoury	0	10	5	10	5
		20.2.4.2.2.2	Grain based snacks	0	10	5	10	5

Category in application	Description of requested food in application	Harvest food category code	Food category name	Carnosic acid plus carnosol concentration (mg/kg)				
				Naturally-occurring	*Rosemary extract only*		*Naturally-occurring plus rosemary extract*	
					MPL	*Usual use*[ɤ]	*MPL*	*Usual use*[ɤ]
7.2	Cookies, pancakes, waffles, sweet pastries (rolls, doughnuts, muffins)	6.4.3.1	Flour products, batter based products	0	40	20	40	20
		7.2.1.1	Biscuits & crackers, sweet	0	40	20	40	20
		7.1.1.1.2	Bread & related products, wheat, white, yeast, sweet	0	40	20	40	20
		7.2.2.1.0.1	Muffins only	0	40	20	40	20
		20.2.3.1.0.1	Sweet pastries	0	40	20	40	20
8.2	Processed meat, poultry & game in whole cuts or pieces – fat content ≤10% fat	8.2.0.1	Processed meat, poultry, game products in whole cuts or pieces, lower fat	0	15	7.5	15	7.5
8.2	Processed meat, poultry & game in whole cuts or pieces – fat content >10% fat	8.2.0.2	Processed meat, poultry, game products in whole cuts or pieces, higher fat	0	37.5	18.75	37.5	18.75
8.2.3	Dried meat	8.2.3	Dried meat	0	150	75	150	75
		8.2.4	Slow dried cured meat	0	150	75	150	75
8.3.2	Dried sausages (from raw meat)	8.3.1	Fermented, uncooked, processed, comminuted meat products	0	100	50	100	50
12	Salts & condiments	12	Salts & condiments	0	40	20	40	20

Category in application	Description of requested food in application	Harvest food category code	Food category name	Carnosic acid plus carnosol concentration (mg/kg)				
				Naturally-occurring	*Rosemary extract only*		*Naturally-occurring plus rosemary extract*	
					MPL	*Usual use*♂	*MPL*	*Usual use*♂
20.2.04	Sauces & toppings (including mayonnaise and salad dressings)	4.3.7.2	Soy sauce	0	50	25	50	25
		20.2.6.1	Sauces & syrups, sweet	0	50	25	50	25
		20.2.6.2	Gravy, sauces & condiments	0	50	25	50	25
		20.2.7	Mayonnaise & salad dressings	0	50	25	50	25
20.2	Processed nuts	4.1.1.2.1	Untreated fruits & vegetables, nuts, salted/flavoured	0	50	25	50	25
		4.1.1.2.2	Untreated fruits & vegetables, nuts, roasted unsalted	0	50	25	50	25
20.2	Potato chips including starch based snacks from roots, tubers, pulses and legumes	20.2.4.2.2.1	Root vegetable based snacks	0	20	10	20	10

♂ *Usual Use levels for fats and oils is 80% of the MPL. For all other foods, the Usual Use level is 50% of the MPL.*

Table 4.5: Summary of estimated dietary exposures to carnosic acid plus carnosol from all scenarios assessed for Australians and New Zealanders

Country	Age group	Scenario	% cons to resp.♦	Estimated consumer dietary exposure to carnosic acid plus carnosol (mg/kg bw/day)	
				Mean	P90
Australia	2 years and above❖	*Naturally-occurring*	5.8	0.048	0.081
		Added rosemary extract only_MPL	100	0.084	0.18
		Naturally-occurring plus added rosemary extract_MPL	100	0.086	0.18
		Added rosemary extract only_Usual use	100	0.043	0.093
		Naturally-occurring plus added rosemary extract_Usual use	100	0.045	0.096
New Zealand	5 – 14 years▽	*Naturally-occurring*	0.1	0.023	n/a
		Added rosemary extract only_MPL	99	0.17	0.34
		Naturally-occurring plus added rosemary extract_MPL	99	0.17	0.34
		Added rosemary extract only_Usual use	99	0.091	0.18
		Naturally-occurring plus added rosemary extract_Usual use	99	0.091	0.18
New Zealand	15 years and above▽	*Naturally-occurring*	1.1	0.18	0.30
		Added rosemary extract only_MPL	98	0.078	0.15
		Naturally-occurring plus added rosemary extract_MPL	98	0.080	0.16
		Added rosemary extract only_Usual use	98	0.041	0.083
		Naturally-occurring plus added rosemary extract_Usual use	98	0.043	0.085

♦ Consumers as a % of total respondents. A consumer is a respondent in the national nutrition survey who consumes a food containing carnosic acid plus carnosol. A respondent is anyone in a national nutrition survey, irrespective of whether they consume a food that contains carnosic acid plus carnosol or not

❖ Based on consumption data from Day 1 and 2

▽ Based on consumption data from Day 1 respondents only

4.3.1.2 MPL scenarios

Dietary exposures increase from the *Naturally-occurring* scenario under the *Added rosemary extract only_MPL* and *Naturally-occurring plus added rosemary extract_MPL* scenarios, with all Australians aged 2 years and above being exposed to carnosic acid plus carnosol in these scenarios.

For the *Added rosemary extract only_MPL* scenario, mean and P90 consumer dietary exposures are 0.084 mg/kg bw/day and 0.18 mg/kg bw/day respectively. For the *Naturally-occurring plus added rosemary extract_MPL* scenario, mean and P90 consumer dietary exposures are 0.086 mg/kg bw/day and 0.18 mg/kg bw/day respectively. The similarity in the dietary exposures (i.e. with and without the inclusion of the contribution of naturally-occurring carnosic acid plus carnosol from rosemary leaves) confirms the small contribution of rosemary leaves to carnosic acid plus carnosol dietary exposures. See Table 4.5 and Figure 4 2 for details.

4.3.1.3 Usual Use scenarios

Dietary exposures decrease from the MPL scenarios under the *Added rosemary extract only_Usual Use* and *Naturally-occurring plus added rosemary extract_Usual Use* scenarios, with all Australians aged 2 years and above being exposed to carnosic acid plus carnosol in these scenarios.

For the *Added rosemary extract only_Usual Use* scenario, mean and P90 consumer dietary exposures are 0.043 mg/kg bw/day and 0.093 mg/kg bw/day respectively. For the *Naturally-occurring plus added rosemary extract_Usual Use* scenario, mean and P90 consumer dietary exposures are 0.045 mg/kg bw/day and 0.096 mg/kg bw/day respectively. The similarity in the dietary exposures (i.e. with and without the inclusion of the contribution of naturally-occurring carnosic acid plus carnosol from rosemary leaves) confirms the small contribution of rosemary leaves to carnosic acid plus carnosol dietary exposures. See Table 4.5 and Figure 4 2 for details.

4.3.2 New Zealanders aged 15 years and above

4.3.2.1 Naturally-occurring scenario

The estimated mean and P90 consumer dietary exposures to carnosic acid plus carnosol for New Zealanders aged 15 years and above are 0.18 mg/kg bw/day and 0.30 mg/kg bw/day for the *Naturally-occurring* scenario. Approximately 1% of New Zealanders aged 15 years and above are exposed to carnosic acid plus carnosol through consumption of rosemary leaves (either on their own or as a part of mixed dishes). See Table 4.5 for details.

4.3.2.2 MPL scenarios

Dietary exposures decrease from the *Naturally-occurring* scenario under the *Added rosemary extract only_MPL* and *Naturally-occurring plus added rosemary extract_MPL* scenarios. This pattern is different to other population groups. This difference is likely due to only 1% of New Zealanders aged 15 years and above being exposed in the *Naturally-occurring* scenario and 98% being exposed in the MPL scenarios.

For the *Added rosemary extract only_MPL* scenario, mean and P90 consumer dietary exposures are 0.078 mg/kg bw/day and 0.15 mg/kg bw/day respectively. For the *Naturally-occurring plus added rosemary extract_MPL* scenario, mean and P90 consumer dietary exposures are 0.080 mg/kg bw/day and 0.16 mg/kg bw/day respectively. The similarity in the dietary exposures (i.e. with and without the inclusion of the contribution of naturally-occurring carnosic acid plus carnosol from rosemary leaves) confirms the small contribution of rosemary leaves to carnosic acid plus carnosol dietary exposures. See Table 4.5 and Figure

4 2 for details.

4.3.2.3 Usual Use scenarios

Dietary exposures decrease from the MPL scenarios under the *Added rosemary extract only_Usual Use* and *Naturally-occurring plus added rosemary extract_Usual Use* scenarios, with 98% of New Zealanders aged 15 years and above being exposed to carnosic acid plus carnosol in these scenarios.

For the *Added rosemary extract only_Usual Use* scenario, mean and P90 consumer dietary exposures are 0.041 mg/kg bw/day and 0.08 mg/kg bw/day respectively. For the *Naturally-occurring plus added rosemary extract_Usual Use* scenario, mean and P90 consumer dietary exposures are 0.043 mg/kg bw/day and 0.085 mg/kg bw/day respectively. The similarity in the dietary exposures (i.e. with and without the inclusion of the contribution of naturally-occurring carnosic acid plus carnosol from rosemary leaves) confirms the small contribution of rosemary leaves to carnosic acid plus carnosol dietary exposures. See Table 4.5 and Figure 4 2 for details.

4.3.3 New Zealanders aged 5-14 years

4.3.3.1 Naturally-occurring scenario

The estimated mean consumer dietary exposures to carnosic acid plus carnosol for New Zealanders aged 5-14 years are 0.023 mg/kg bw/day for the *Naturally-occurring* scenario. Approximately 0.1% of New Zealanders aged 5-14 years are exposed to carnosic acid plus carnosol through consumption of rosemary leaves (either on their own or as a part of mixed dishes). There were insufficient consumers to be able to derive a P90 dietary exposure to carnosic acid plus carnosol for the *Naturally-occurring* scenario. See Table 4.5 for details.

4.3.3.2 MPL scenarios

Dietary exposures increase from the *Naturally-occurring* scenario under the *Added rosemary extract only_MPL* and *Naturally-occurring plus added rosemary extract_MPL* scenarios, with 99% of New Zealanders aged 5-14 years being exposed to carnosic acid plus carnosol in these scenarios. For the *Added rosemary extract only_MPL* and *Naturally-occurring plus added rosemary extract_MPL* scenarios, mean and P90 consumer dietary exposures are 0.17 mg/kg bw/day and 0.34 mg/kg bw/day respectively. The similarity in the dietary exposures (i.e. with and without the inclusion of the contribution of naturally-occurring carnosic acid plus carnosol from rosemary leaves) confirms the small contribution of rosemary leaves to carnosic acid plus carnosol dietary exposures. See Table 4.5 and Figure 4 2 for details.

4.3.3.3 Usual Use scenarios

Dietary exposures decrease from the MPL scenarios under the *Added rosemary extract only_Usual Use* and *Naturally-occurring plus added rosemary extract_Usual Use* scenarios, with 99% of New Zealanders aged 5-14 years being exposed to carnosic acid plus carnosol in these scenarios.

For the *Added rosemary extract only_MPL* and *Naturally-occurring plus added rosemary extract_MPL* scenarios, mean and P90 consumer dietary exposures are 0.091 mg/kg bw/day and 0.18 mg/kg bw/day respectively. The similarity in the dietary exposures (i.e. with and without the inclusion of the contribution of naturally-occurring carnosic acid plus carnosol from rosemary leaves) confirms the small contribution of rosemary leaves to carnosic acid plus carnosol dietary exposures.

Under the two Usual Use antioxidant use scenarios, New Zealand children aged 5-14 years

have higher mean and P90 dietary exposures on a body weight basis to carnosic acid plus carnosol than for the older group of New Zealanders aged 15 years and above. This is due to their smaller body weight and higher food consumption per kilogram of body weight compared to adults. See Table 4.5 and Figure 4 2 for details.

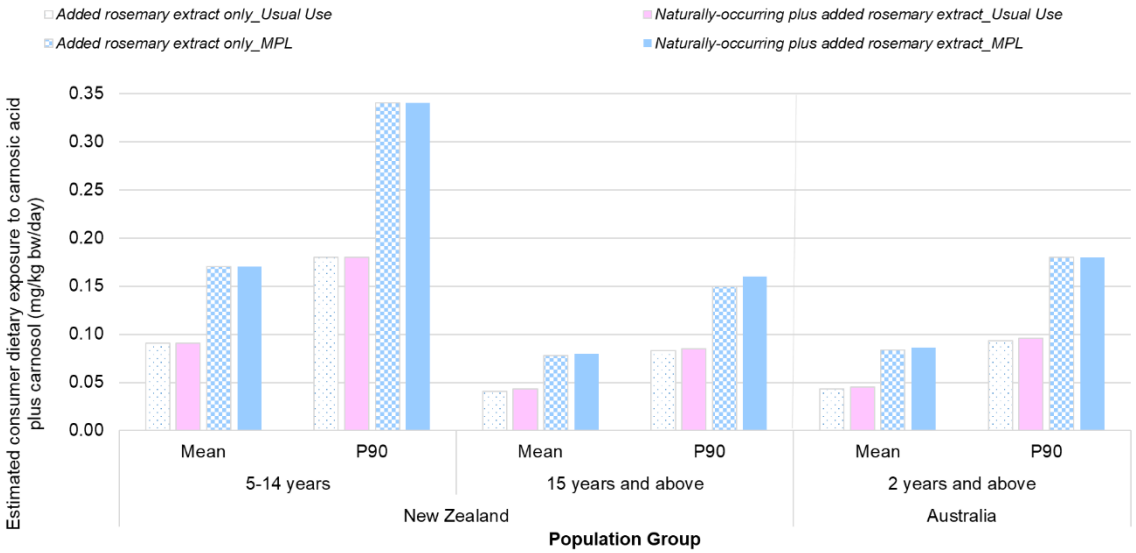


Figure 4 2: Estimated consumer dietary exposures to carnosic acid plus carnosol for Australian and New Zealand population groups for the antioxidant use scenarios, in mg/kg bw/day[5]

4.4 Major contributing foods to carnosic acid plus carnosol dietary exposures

Major contributors to dietary exposures are defined as those that contribute ≥5% of the estimated dietary exposure.

In the *Naturally-occurring* scenario, all of the carnosic acid plus carnosol dietary exposure is from rosemary leaves for all population groups. The contribution of flavouring uses is not considered in this assessment.

In all antioxidant use scenarios, Sauces & toppings (including mayonnaise and salad dressings) is the major contributing food category to carnosic acid plus carnosol dietary exposures for all Australian and New Zealand population groups investigated. Contributing food categories are discussed in more detail below.

[5] For the *Naturally-occurring* scenario, there are insufficient New Zealand consumers aged 5-14 years to derive a P90 dietary exposure. Therefore, no P90 dietary exposure is shown on the figure for this scenario and population group.

4.4.1 Australians aged 2 years and above

4.4.1.1 MPL scenarios

For the MPL scenarios (*Added rosemary extract only_MPL* and *Naturally-occurring plus added rosemary extract_MPL*), the major contributing food groups to dietary exposures for Australians aged 2 years and above are:

- Sauces & toppings (including mayonnaise and salad dressings) (44-45%)
- Grain bars, breakfast bars, breakfast cereals (24%); and
- Cookies, pancakes, waffles, sweet pastries (rolls, doughnuts, muffins) (11%).

Further details can be found in Figure 4.3 and Table 4.6.

4.4.1.2 Usual Use scenarios

For both Usual Use scenarios (*Added rosemary extract only_Usual Use* and *Naturally-occurring plus added rosemary extract_Usual Use*), the major contributing food groups to dietary exposures for Australians aged 2 years and above are:

- Sauces & toppings (including mayonnaise and salad dressings) (42-43%)
- Grain bars, breakfast bars, breakfast cereals (23%)
- Cookies, pancakes, waffles, sweet pastries (rolls, doughnuts, muffins) (10-11%); and
- Margarines (solid & liquid only), <80% fat (6-7%).

Further details can be found in Figure 4.4 and *Table 4.7: Food categories (as listed by applicant) and their contribution to estimated dietary exposures to carnosic acid plus carnosol, for the Usual Use scenarios* Table 4.7.

4.4.2 New Zealanders aged 15 years and above

4.4.2.1 MPL scenarios

For the MPL scenarios, (*Added rosemary extract only_MPL* and the *Naturally-occurring plus added rosemary extract_MPL*), the major contributing food groups to dietary exposures for New Zealanders aged 15 years and above are:

- Sauces & toppings (including mayonnaise and salad dressings) (40-41%)
- Grain bars, breakfast bars, breakfast cereals (20%)
- Margarines (solid & liquid only), <80% fat (11%)
- Cookies, pancakes, waffles, sweet pastries (rolls, doughnuts, muffins) (10-11%); and
- Processed meat, poultry & game in whole cuts or pieces –fat content >10% fat (5%).

Further details can be found in Figure 4.3 and Table 4.6.

4.4.2.2 Usual Use scenarios

For the Usual Use scenarios (*Added rosemary extract only_Usual Use* and *Naturally-occurring plus added rosemary extract_Usual Use*), the major contributing food groups to dietary exposures for New Zealanders aged 15 years and above are:

- Sauces & toppings (including mayonnaise and salad dressings) (37-38%)
- Grain bars, breakfast bars, breakfast cereals (18-19%)
- Margarines (solid & liquid only), <80% fat (16-17%)
- Cookies, pancakes, waffles, sweet pastries (rolls, doughnuts, muffins) (9-10%); and
- Processed meat, poultry & game in whole cuts or pieces –fat content >10% fat (5%).

Further details can be found in Figure 4.4 and *Table 4.7: Food categories (as listed by applicant) and their contribution to estimated dietary exposures to carnosic acid plus carnosol, for the Usual Use scenarios*Table 4.7.

Table 4.6: Food categories (as listed by applicant) and their contribution to estimated dietary exposures to carnosic acid plus carnosol, for the MPL scenarios

Food category as requested by applicant	% contribution								
	Australians 2 years and above❖			New Zealanders 15 years and above▽			New Zealanders 5-14 years▽		
	Naturally-occurring	*Added rosemary extract only_MPL*	*Naturally-occurring plus added rosemary extract_MPL*	*Naturally-occurring*	*Added rosemary extract only_MPL*	*Naturally-occurring plus added rosemary extract_MPL*	*Naturally-occurring*	*Added rosemary extract only_MPL*	*Naturally-occurring plus added rosemary extract_MPL*
Fish & algal oils	0	0	0	0	0	0	0	0	0
Margarines (solid & liquid only), <80% fat	0	4	4	0	11	11	0	9	9
Nut butters & nut spreads	0	<1	<1	0	<1	<1	0	2	2
Icings & frostings, glazes & fillings	0	<1	<1	0	<1	<1	0	<1	<1
Grain bars, breakfast bars, breakfast cereals	0	24	24	0	20	20	0	27	27
Flour based snacks (e.g. pretzels, crackers)	0	1	1	0	1	1	0	2	2
Cookies, pancakes, waffles, sweet pastries (rolls, doughnuts, muffins)	0	11	11	0	11	10	0	19	19
Processed meat, poultry & game in whole cuts or pieces –fat content ≤10% fat	0	3	3	0	3	2	0	1	1
Processed meat, poultry & game in whole cuts or pieces –fat content >10% fat	0	3	2	0	5	5	0	4	4
Dried meat	0	<1	<1	0	<1	<1	0	<1	<1
Dried sausages (from raw meat)	0	3	3	0	1	1	0	1	1
Salts & condiments	0	2	2	0	2	2	0	2	2

Sauces & toppings (including mayonnaise and salad dressings)	0	45	44	0	41	40	0	28	28
Processed nuts	0	2	2	0	2	2	0	1	1
Potato chips including starch based snacks from roots, tubers, pulses and legumes	0	1	1	0	1	1	0	4	4
Rosemary	100	0	2	100	0	2	100	0	<1

❖ Based on food consumption data from Day 1 and 2; ▽ Based on food consumption data from Day 1 only.
Shaded cells indicate major (≥5% contribution to estimated dietary exposures).

Table 4.7: Food categories (as listed by applicant) and their contribution to estimated dietary exposures to carnosic acid plus carnosol, for the Usual Use scenarios

Food category as requested by applicant	% contribution								
	Australians 2 years and above❖			New Zealanders 15 years and above▽			New Zealanders 5-14 years▽		
	Naturally-occurring	*Added rosemary extract only_ Usual Use*	*Naturally-occurring plus added rosemary extract_ Usual Use*	*Naturally-occurring*	*Added rosemary extract only_ Usual Use*	*Naturally-occurring plus added rosemary extract_ Usual Use*	*Naturally-occurring*	*Added rosemary extract only_ Usual Use*	*Naturally-occurring plus added rosemary extract_ Usual Use*
Fish & algal oils	0	0	0	0	0	0	0	0	0
Margarines (solid & liquid only), <80% fat	0	7	6	0	17	16	0	14	14
Nut butters & nut spreads	0	<1	<1	0	<1	<1	0	2	2
Icings & frostings, glazes & fillings	0	<1	<1	0	<1	<1	0	<1	<1
Grain bars, breakfast bars, breakfast cereals	0	23	23	0	19	18	0	25	25
Flour based snacks (e.g. pretzels, crackers)	0	1	1	0	1	<1	0	1	1
Cookies, pancakes, waffles, sweet pastries (rolls, doughnuts, muffins)	0	11	10	0	10	9	0	18	18
Processed meat, poultry & game in whole cuts or pieces –fat content ≤10% fat	0	2	2	0	2	2	0	1	1
Processed meat, poultry & game in whole cuts or pieces –fat content >10% fat	0	2	2	0	5	5	0	4	4
Dried meat	0	<1	<1	0	<1	<1	0	<1	<1
Dried sausages (from raw meat)	0	3	3	0	1	1	0	1	1
Salts & condiments	0	2	2	0	2	2	0	1	1
Sauces & toppings (including mayonnaise and salad dressings)	0	43	42	0	38	37	0	27	27
Processed nuts	0	2	2	0	2	2	0	<1	<1
Potato chips including starch based snacks from roots, tubers, pulses and legumes	0	1	1	0	1	1	0	3	3
Rosemary	100	0	3	100	0	4	100	0	<1

❖ Based food consumption data from Day 1 and 2 respondents; ▽ Based on food consumption data from Day 1 only.
Shaded cells indicate major (≥5% contribution to estimated dietary exposures.

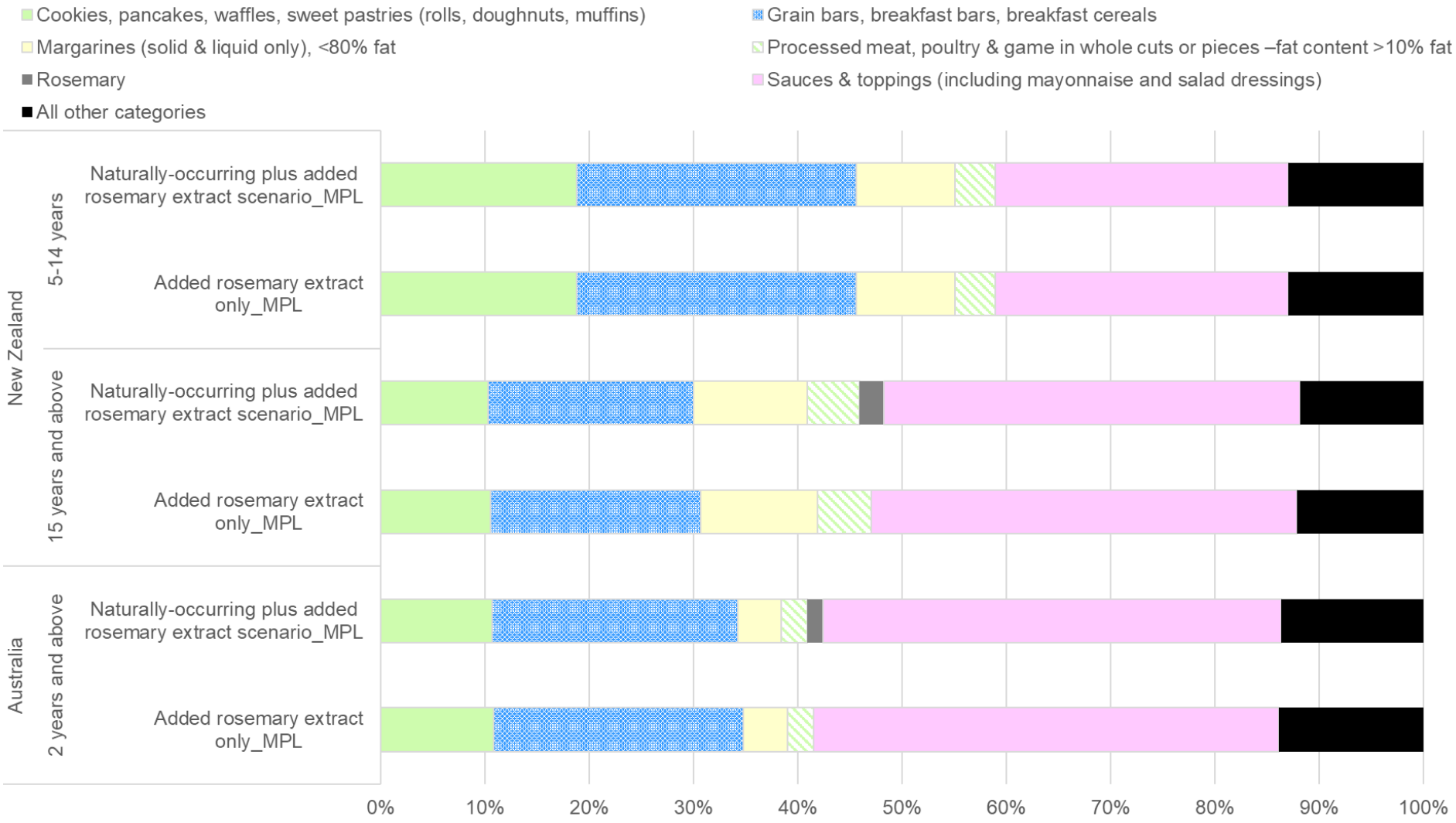


Figure 4.3: Major (≥5%) contributing food categories to carnosic acid plus carnosol dietary exposures for Australian and New Zealand population groups for the MPL antioxidant use scenarios

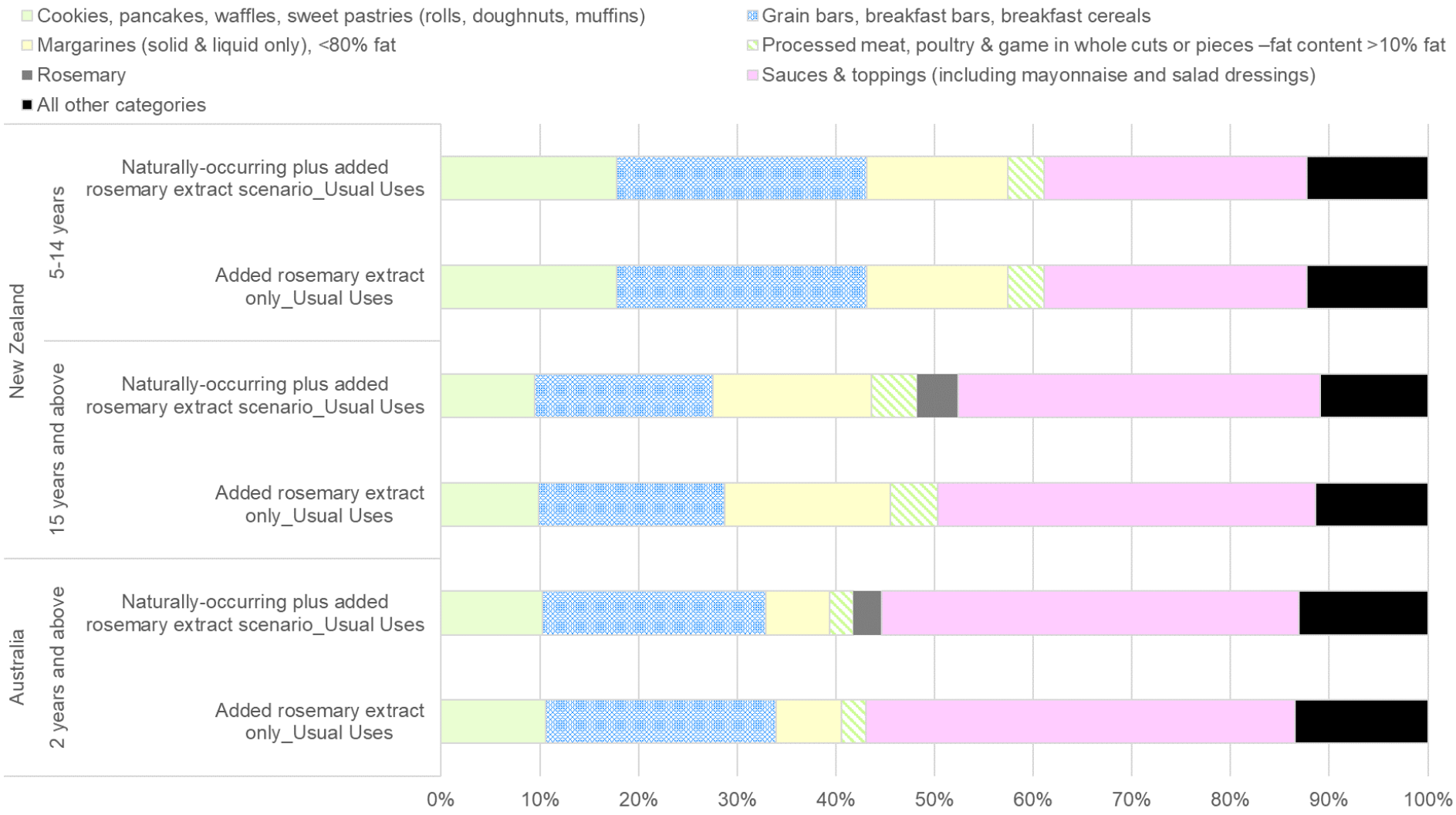


Figure 4.4: Major (≥5%) contributing food categories to carnosic acid plus carnosol dietary exposures for Australian and New Zealand population groups for the Usual Use scenarios

4.4.3 New Zealanders aged 5-14 years

4.4.3.1 MPL scenarios

For the MPL scenarios (*Added rosemary extract only_MPL* and *Naturally-occurring plus added rosemary extract_MPL*), the major contributing food groups to dietary exposures for New Zealanders aged 5-14 years are:

- Sauces & toppings (including mayonnaise and salad dressings) (28%)
- Grain bars, breakfast bars, breakfast cereals (27%)
- Cookies, pancakes, waffles, sweet pastries (rolls, doughnuts, muffins) (19%); and
- Margarines (solid & liquid only), <80% fat (9%).

Further details can be found in Figure 4.3 and Table 4.6.

4.4.3.2 Usual Use scenarios

For the Usual Use scenarios (*Added rosemary extract only_Usual Use* and *Naturally-occurring plus added rosemary extract_Usual Use*), the major contributing food groups to dietary exposures for New Zealanders aged 5-14 years are:

- Sauces & toppings (including mayonnaise and salad dressings) (27%)
- Grain bars, breakfast bars, breakfast cereals (25%)
- Cookies, pancakes, waffles, sweet pastries (rolls, doughnuts, muffins) (18%); and
- Margarines (solid & liquid only), <80% fat (14%).

Further details can be found in Figure 4.4 and *Table 4.7: Food categories (as listed by applicant) and their contribution to estimated dietary exposures to carnosic acid plus carnosol, for the Usual Use scenarios*Table 4.7.

4.5 Risk characterisation

There is a temporary ADI of 0–0.3 mg/kg body weight set by JECFA for rosemary extract, expressed as carnosic acid plus carnosol (FAO/WHO, 2016). Dietary exposures to carnosic acid plus carnosol were compared to the temporary ADI. As discussed in Section 4.3, dietary exposures have been estimated for 'consumers only' (i.e. consumers of foods containing carnosic acid plus carnosol) and the proportion of consumers in each scenario-population group varies.

4.5.1 Australians aged 2 years and above

4.5.1.1 MPL scenarios

Consumer mean and P90 dietary exposures to carnosic acid plus carnosol for Australians aged 2 years and above increased from the Naturally-occurring scenario (15% ADI and 25% ADI, respectively) to 30% ADI and 60% ADI under the two MPL antioxidant use scenarios (Added rosemary extract only_MPL and Naturally-occurring plus added rosemary extract_MPL). See Figure 4.5 and Table 4.8 for further details.

4.5.1.2 Usual Use scenarios

Consumer mean and P90 dietary exposures to carnosic acid plus carnosol for Australians aged 2 years and above increased from the Naturally-occurring scenario (15% ADI and 25% ADI, respectively) to 15% ADI and 30% ADI under the two Usual Use antioxidant use scenarios (Added rosemary extract only_Usual Use and Naturally-occurring plus added rosemary extract_Usual Use). See Figure 4.5 and Table 4.8 for further details.

Table 4.8: Summary of estimated dietary exposures to carnosic acid plus carnosol from all scenarios assessed for Australians and New Zealanders, expressed as %ADI

Country	Age group	Scenario	% cons to resp. ♦	Estimated consumer dietary exposure to carnosic acid plus carnosol (%ADI)	
				Mean	P90
Australia	2 years and above❖	*Naturally-occurring*	5.8	15	25
		Added rosemary extract only_MPL	100	30	60
		Naturally-occurring plus added rosemary extract_MPL	100	30	60
		Added rosemary extract only_Usual use	100	15	30
		Naturally-occurring plus added rosemary extract_Usual use	100	15	30
New Zealand	5 – 14 years▽	*Naturally-occurring*	0.1	8	n/a
		Added rosemary extract only_MPL	99	60	110
		Naturally-occurring plus added rosemary extract_MPL	99	60	110
		Added rosemary extract only_Usual use	99	30	60
		Naturally-occurring plus added rosemary extract_Usual use	99	30	60
New Zealand	15 years and above▽	*Naturally-occurring*	1.1	60	100
		Added rosemary extract only_MPL	98	25	50
		Naturally-occurring plus added rosemary extract_MPL	98	25	55
		Added rosemary extract only_Usual use	98	15	30
		Naturally-occurring plus added rosemary extract_Usual use	98	15	30

♦ Consumers as a % of total respondents. A consumer is a respondent in the national nutrition survey who consumes a food containing carnosic acid plus carnosol. A respondent is anyone in a national nutrition survey, irrespective of whether they consume a food that contains carnosic acid plus carnosol or not.

❖ Based on consumption data from Day 1 and 2

▽ Based on consumption data from Day 1 respondents only

4.5.2 New Zealanders aged 15 years and above

4.5.2.1 MPL scenarios

Consumer mean dietary exposures to carnosic acid plus carnosol for New Zealanders aged 15 years and above decreased from the Naturally-occurring scenario (60% ADI) to 25% ADI under the two MPL antioxidant use scenarios; Added rosemary extract only_MPL and Naturally-occurring plus added rosemary extract_MPL. P90 dietary exposures decreased from 100% ADI for the Naturally-occurring scenario, to 50-55% ADI under the two MPL antioxidant use scenarios. As discussed in Section 4.3.2.2, this pattern is different to other population groups. This difference is likely due to only 1% of New Zealanders aged 15 years and above being exposed in the Naturally-occurring scenario and 98% being exposed in the MPL scenarios. See Figure 4.5 and Table 4.8 for further details.

4.5.2.2 Usual Use scenarios

Consumer mean dietary exposures to carnosic acid plus carnosol for New Zealanders aged 15 years and above decreased from the Naturally-occurring scenario (60% ADI) to 15% ADI under the two Usual Use antioxidant use scenarios (Added rosemary extract only_Usual Use and Naturally-occurring plus added rosemary extract_Usual Use**)**. P90 dietary exposures decreased from 100% ADI for the Naturally-occurring scenario, to 30% ADI under the two Usual Use antioxidant use scenarios. See Figure 4.5 and Table 4.8 for further details.

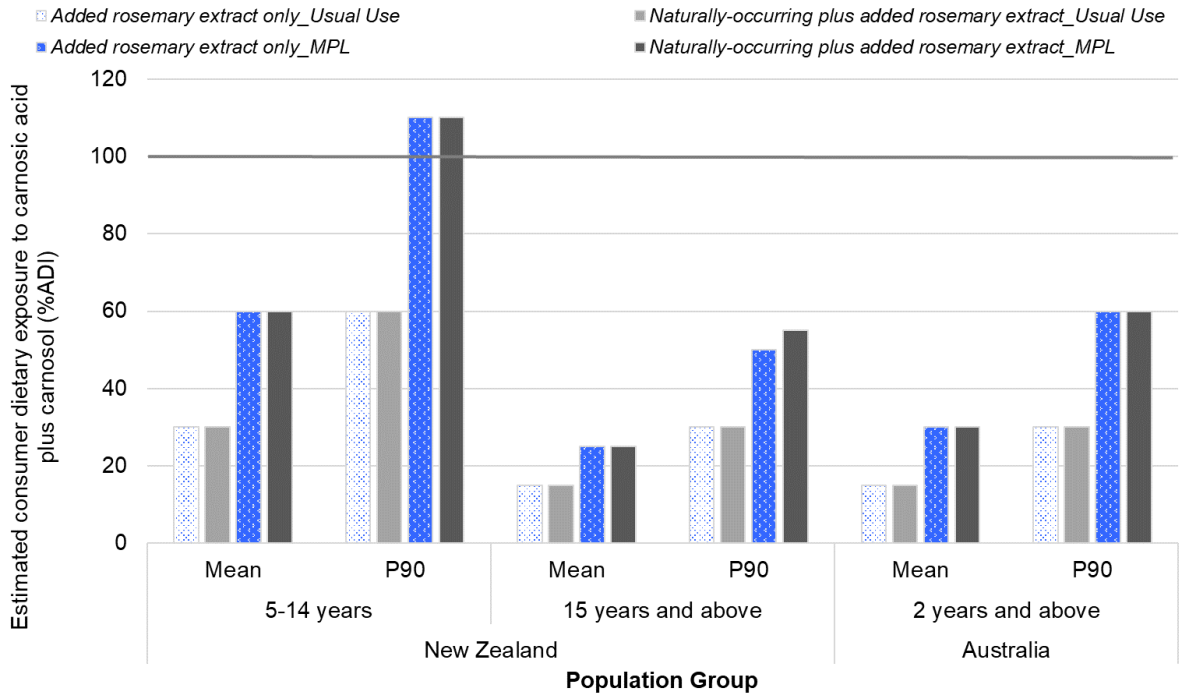


Figure 4.5: Estimated consumer dietary exposures to carnosic acid plus carnosol for Australian and New Zealand population groups for the antioxidant use scenarios, as %ADI[6]

[6] For the *Naturally-occurring* scenario, there are insufficient New Zealand consumers aged 5-14 years to derive a P90 dietary exposure. Therefore, no P90 dietary exposure is shown on the figure for this scenario and population group.

4.5.3 New Zealanders aged 5-14 years

4.5.3.1 MPL scenarios

Consumer mean dietary exposures to carnosic acid plus carnosol for New Zealand children aged 5-14 years increased from the Naturally-occurring scenario (8% ADI) to 60% ADI under the two MPL antioxidant use scenarios (Added rosemary extract only_MPL and Naturally-occurring plus added rosemary extract_MPL). There were insufficient consumers to be able to derive a P90 dietary exposure for the Naturally-occurring scenario. P90 dietary exposures to carnosic acid plus carnosol are 110% ADI for the Added rosemary extract only_MPL and Naturally-occurring plus added rosemary extract_MPL scenarios (see Figure 4.5 and Table 4.8 for further details).

These estimates are highly conservative and are not likely to occur in reality for a number of reasons. Firstly, it is assumed that all foods within a category contain rosemary extract at the proposed MPL and that all of the foods within the food categories requested to contain rosemary extract will use rosemary extract. The Usual Use scenarios represent a more likely estimate of dietary exposures to carnosic acid plus carnosol since the concentrations are what manufacturers are more likely to add to the requested food categories. Secondly, the exposures are based on a single day of food consumption data. The distribution of food consumption amounts for one 24 hour period tends to be much broader than that averaged across two days given that all foods are not consumed on a daily basis. Therefore the dietary exposures when averaged across two days and can result in the tails of the exposure distribution coming in and a lower P90 exposure compared to one day of data only. The Australian two day average exposures for the same age group at the mean and P90 were 45% ADI and 95% ADI, respectively. Thirdly, if the chronic dietary exposures were able to expressed for New Zealand across the relevant time period of the lifetime, including adulthood, the high percentile exposures would be lower.

4.5.3.2 Usual Use scenarios

Consumer mean dietary exposures to carnosic acid plus carnosol for New Zealand children aged 5-14 years increased from the Naturally-occurring scenario (8% ADI) to 30% ADI under the two Usual Use antioxidant use scenarios (Added rosemary extract only_Usual Use and Naturally-occurring plus added rosemary extract_Usual Use). There were insufficient consumers to be able to derive a P90 dietary exposure for the Naturally-occurring scenario. P90 dietary exposures to carnosic acid plus carnosol are 60% ADI for the Added rosemary extract only_Usual Use and Naturally-occurring plus added rosemary extract_Usual Use scenarios. See Figure 4.5 and Table 4.8 for further details.

4.6 Summary of results

For all of the population groups assessed, New Zealanders aged 15 years and above had the highest mean and P90 *Naturally-occurring* consumer dietary exposures to carnosic acid plus carnosol on a body weight basis.

For all of the population groups assessed, New Zealand children aged 5-14 years had the highest mean and P90 antioxidant scenario dietary exposures on a body weight basis for both the Usual Use and the MPL scenarios with and without the inclusion of rosemary leaves in the assessment. Generally, children have higher dietary exposures as they have a higher consumption of food on a body weight basis and therefore are more likely to have higher dietary exposures to food chemicals.

For the *Naturally-occurring* scenario where dietary exposures to carnosic acid plus carnosol from rosemary leaves only were considered, mean dietary exposures were 8 – 60% ADI and P90 exposures were 25 – 100% ADI, depending on the population group being assessed.

For the MPL scenarios, mean dietary exposures to carnosic acid plus carnosol were 25 – 60% ADI and P90 exposures were 50 – 110% ADI, depending on the population group being assessed. The dietary exposure estimates based on MPLs are highly conservative and are not likely to occur in reality due to the following assumptions: all foods within a category contain rosemary extract at the MPL; all of the foods within the food categories requested to contain rosemary extract will use rosemary extract; and consumers always eat the products containing rosemary extracts at these concentrations over a lifetime. The Usual Use scenarios represent a more likely estimate of dietary exposures to carnosic acid plus carnosol.

For the Usual Use scenarios, mean dietary exposures to carnosic acid plus carnosol were 15 – 30% ADI and P90 exposures were 30 – 60% ADI, depending on the population group being assessed.

JECFA didn't include rosemary leaves in conjunction with antioxidant uses in its assessment of dietary exposures to carnosic acid plus carnosol since dried rosemary wasn't likely to significantly affect estimated dietary exposures to carnosic acid plus carnosol (WHO, 2017). The FSANZ assessment confirms the small contribution of rosemary leaves to carnosic acid plus carnosol dietary exposures since there is very little, if any, difference between %ADI for the *Added rosemary extract only* and the *Naturally-occurring plus added rosemary extract* scenarios for Australians aged 2 years and above and New Zealanders aged 15 years and above and aged 5-14 years. In the *Naturally-occurring plus added rosemary extract_Usual Use* scenario, rosemary leaves contribute to <1 – 4% of the dietary exposures. In the *Naturally-occurring plus added rosemary extract_MPL* scenario, rosemary leaves contribute to <1 – 2% of the dietary exposures.

5 Conclusion

FSANZ has found no new evidence around the characterisation of the hazard to suggest that the ADI for rosemary extract should be lower than the temporary ADI established by JECFA (2017).

Dietary exposures to carnosic acid plus carnosol for consumers only solely from use of rosemary leaves resulted in mean dietary exposures that were 8 – 60% of the temporary ADI and P90 exposures of 25 – 100% of the ADI, depending on the population group being assessed. Usual Use scenarios resulted in mean dietary exposures to carnosic acid plus carnosol of 15-30% of the temporary ADI and P90 exposures of 30 – 60% of the ADI, depending on the population group being assessed.

A dietary exposure assessment was also carried out using requested MPLs. For the MPL scenarios, mean dietary exposures to carnosic acid plus carnosol for consumers only were 25 – 60% ADI and P90 exposures were 50 – 110% ADI, depending on the population group being assessed. However, the dietary exposure estimates based on MPLs are highly conservative and are not likely to occur in reality because they are based on the following assumptions: all foods within a category contain rosemary extract at the MPL; all of the foods within the food categories requested to contain rosemary extract will use rosemary extract; and consumers always eat the products containing rosemary extracts at these concentrations over a lifetime. FSANZ considers that the Usual Use scenarios represent a more likely estimate of dietary exposures to carnosic acid plus carnosol.

In estimating dietary exposure to carnosic acid plus carnosol from the use of rosemary extract as an antioxidant, JECFA did not include exposure to carnosic acid plus carnosol through use of rosemary leaves as a culinary herb, because JECFA considered that any

exposure from rosemary leaves was not likely to significantly alter the overall exposure (WHO, 2017). The FSANZ assessment confirmed that there is very little, if any, difference between %ADI for the 'added rosemary extract only' and the 'naturally-occurring plus added rosemary extract' scenarios.

In conclusion, based on the safety and dietary exposure assessments, there is no evidence of a public health and safety concern associated with adding rosemary extract as an antioxidant to the requested foods.

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Appendix 1: Dietary Exposure Assessments at FSANZ

A dietary exposure assessment is the process of estimating how much of a food chemical a population, or population sub group, consumes. Dietary exposure to food chemicals is estimated by combining food consumption data with food chemical concentration data. The process of doing this is called 'dietary modelling', where:

Dietary exposure = food chemical concentration x food consumption

FSANZ's approach to dietary modelling is based on internationally accepted procedures for estimating dietary exposure to food chemicals. Different dietary modelling approaches may be used depending on the assessment, the type of food chemical, the data available and the risk assessment questions to be answered. In the majority of assessments FSANZ uses the food consumption data from each person in the national nutrition surveys to estimate their individual dietary exposure. Population summary statistics such as the mean exposure or a high percentile exposure are derived from the ranked individual person's exposures from the nutrition survey.

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

FSANZ has developed a custom-built computer program 'Harvest' to calculate dietary exposures. Harvest replaces the program 'DIAMOND' that had been used by FSANZ for many years. Harvest has been designed to replicate the calculations that occurred within DIAMOND using a different software package.

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

A1.1 Food consumption data used

The most recent food consumption data available were used to estimate carnosic acid plus carnosol dietary exposures for the Australian and New Zealand populations. The national nutrition survey data used for these assessments were:

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

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

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

The 2011–12 Australian National Nutrition and Physical Activity Survey (2011-12 NNPAS), undertaken by the Australian Bureau of Statistics, is the most recent food consumption data for Australia. This survey includes dietary patterns of a sample of 12,153 Australians aged from 2 years and above. The survey used a 24-hour recall method for all respondents, with 64% of respondents (n=7,735) also completing a second 24-hour recall on a second, non-consecutive day. The data were collected from May 2011 to June 2012 (with no enumeration between August and September 2011 due to the Census). Only those respondents who had two days of food consumption data were used to estimate carnosic acid plus carnosol dietary exposures for this assessment. The Day 1 and 2 average provides the best estimates of carnosic acid plus carnosol dietary exposures for Australians aged 2 years and above. Consumption and respondent data from the survey were incorporated into the Harvest program from the Confidentialised Unit Record Files (CURF) data set (ABS 2014). These data were weighted during the calculations undertaken in Harvest.

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

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

A1.1.3 2008-09 New Zealand Adult Nutrition Survey (2008 NZ ANS)

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

A1.2 Limitations of dietary exposure assessments

Dietary exposure assessments based on 2011-12 NNPAS, 2002 NZ CNS and 2008 NZ ANS food consumption data provide the best estimation of actual consumption of a food and the resulting estimated dietary exposure assessment for the Australian population aged 2 years and above, as well as the New Zealand populations aged 5–14 years and 15 years and above, respectively. However, it should be noted that NNS data do have limitations. Further details of the limitations relating to dietary exposure assessments undertaken by FSANZ are set out in the FSANZ document, *Principles and Practices of Dietary Exposure Assessment for Food Regulatory Purposes* (FSANZ 2009).